ORAL SESSIONS

ORAL-01-01

EVALUATION OF MPTP INDUCED PARKINSON-LIKE CHANGES IN C57BL/6J MICE USING IN VIVO MRI

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Purpose: Currently there is no reliable diagnostic method to assess Parkinson's Disease (PD). The 2006 guidelines from the American Academy of Neurology state that there is insufficient evidence to recommend for or against the use of magnetic resonance imaging (MRI) to diagnose PD. The objective was to develop an imaging protocol, to identify changes between control mice and mice with an methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) induced, Parkinson-like change of the nigrostratial dopamine system. **Method:** The first study used a dosing regime of four hourly i.p. injections of 10mg/kg MPTP, to achieve Dopamine depletion of approximately 70% below control levels, within four days post administration. A second study used a higher dosing regime of 2 subcutaneous 50mg/kg MPTP injections. MRI data was acquired using three diffusion scans with separate diffusion axis directions and a T_2 weighted fast spin echo. **Results:** In the first study, on day 3, there was a statistically significant increase in the apparent diffusion coefficient with diffusion gradients applied in the head-foot direction between control (n = 5) and treated mice (n = 3). On day 4, the high dose study achieved a statistically significant decrease in T_2 intensity between control (n=5) and treated mice (n = 3) and a small increase (p = 0.07) in the diffusion coefficient in the head-foot direction. **Conclusion:** MRI diffusion changes could be detected, at lower MPTP doses then T_2 changes. However, doses were not large enough to induce histological changes, like cell death. This indicates that MRI can be a more sensitive method than histology for evaluating MPTP induced toxicity.

ORAL-01-02

CAPSAICIN PRE-TREATMENT IMPROVES MOTOR FUNCTION IN AN EARLY STAGE RAT MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Although it is known that a loss of dopaminergic neurons occurs in PD, it remains largely unknown what causes these neurons to die. Oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction and inflammation have all been implicated. The neuropeptide substance P (SP) is known to contribute to all of these processes. Our aim was therefore to determine the effect of capsaicin induced neuropeptide (SP) depletion on motor outcome in an experimental model of PD. Animals were pre-treated with capsaicin (150mg/k; n=10) or equal volume vehicle (n=22) over a 3-day period, 14 days prior to the induction of PD by intrastriatal injections of 6-hydroxydopamine (6-OHDA). This method produces a loss of dopamine neurons and striatal dopamine content similar to that seen in the early stage of clinical PD. The sensorimotor function of animals was then assessed using the rotarod test over 21 days post-lesion. 6-OHDA intrastriatal injections produced a profound sensorimotor deficit in vehicle pre-treated animals that showed little evidence of improvement over the assessment period. Conversely, capsaicin pre-treated animals showed improvement over the assessment period. By day 14 post-lesion these animals had significantly ($p<0.001$) better motor function than vehicle animals, and this significance over vehicle pre-treated animals was sustained for the remainder of the assessment period. We conclude that neuropeptide depletion with capsaicin provides protection from motor deficits induced in a 6-OHDA model of PD. Neuropeptides, and in particular SP, which has already been implicated in neuronal cell death in other brain pathologies, may play an integral role in PD pathophysiology.

ORAL-01-03

LOSS OF FUNCTION OF PARKIN COREGULATED GENE (PACRG) CAUSES HYDROCEPHALUS AND INFERTILITY IN THE QUAKING^{VIABLE} MOUSE MUTANT

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Hydrocephalus is a common medical condition (estimated incidence of approximately 1:1500 births) that is characterised by abnormalities in the flow or resorption of cerebrospinal fluid (CSF), resulting in ventricular dilation. Hydrocephalus is a complex and multifactorial disorder, and while a growing body of evidence indicates that genetic factors play a major role in disease pathogenesis, relatively few associated genes have been identified to date. Recent studies have demonstrated that disruption of ependymal cilia function can result in alterations to CSF flow and the development of hydrocephalus. The *Quaking*^{viable} mouse mutant is characterised by dysmyelination, hydrocephalus and male infertility. We recently characterised the genetic defect in this mouse, identifying a deletion affecting *Quaking*, *Parkin* and *Pacrg* (Lockhart et al 2004). **Purpose:** To investigate the molecular function of Pacrg. **Methods:** The temporal and regional expression of Pacrg was investigated in wildtype mice by in situ and immunohistochemical analysis. In addition, *Quaking*^{viable} mice expressing a Pacrg transgene were generated and characterised. **Results:** Pacrg expression in the brain appeared to be localised to the multiciliated ependymal cells that line the CNS ventricular system, including the lateral ventricles, third ventricle, aqueduct, the fourth ventricle and also the choroid plexus (n=6). Transgenic expression of Pacrg was necessary and sufficient to correct the hydrocephalus observed in the *Quaking*^{viable} mutant mouse (average Lateral ventricle area 70±13) to wildtype values (26±9, mean±Std. Dev, n=4). In addition, transgenic Pacrg expression restored sperm production and fertility to wildtype levels in the transgenic mutant mice (n=8) **Conclusions:** Our results demonstrate for the first time that Pacrg is a novel axoneme-associated protein required for both motile ependymal cilia and sperm flagella function in the mouse. We speculate that the highly conserved human homologue may have similar functions, and that mutation of PACRG could potentially result in hydrocephalus and/or male infertility.

ORAL-01-04

A PROGRESSIVE MODEL OF PARKINSON'S DISEASE BY DOPAMINE RECEPTOR KNOCK-OUT

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Purpose: This study addresses the question of whether increased dopamine turnover in nigral neurons leads to formation of Lewy Bodies (LB), the characteristic α-synuclein containing cytoplasmic inclusion of Parkinson's disease. **Methods:** Mice with targeted deletion of the dopamine D2 receptor gene ($D_2R(-/-)$) were studied, primarily using immunohistochemistry. **Results:** $D_2R(-/-)$ mice had higher striatal dopamine turnover and elevated oxidative stress (n=12-15). LB-like cytoplasmic inclusions containing α-synuclein and ubiquitin were detected with increasing frequency in substantia nigra (SN) neurons of older $D_2R(-/-)$ mice. These inclusions displaced the nucleus of affected cells and were eosinophilic. Diffuse cystosolic α-synuclein staining in SN neurons increased with age in both wild type (WT) and $D_2R(-/-)$ mice and but only occasional LB inclusions were also seen in aged WT mice. This accumulation was not driven by increased α-synuclein expression, but instead resulted from a redistribution of α-synuclein from striatal terminals to nigral cell bodies. These changes were accompanied by a loss of dopamine transporter containing terminals in the dorsal striatum, although there was no evidence of progressive cell death in the SN of $D_2R(-/-)$ mice (n=5). **Conclusions:** These data provide support for the argument that increased dopamine turnover leads to the development of LB-like inclusions and axonal degeneration.

ORAL-01-05

PURIFICATION AND ANALYSIS OF ENZYMATIC DOMAINS FROM THE PARKINSON'S DISEASE-ASSOCIATED PROTEIN LEUCINE-RICH REPEAT KINASE 2

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Purpose: Recent genetic analyses of pedigrees segregating familial parkinsonism (PARK8) have identified parkinsonism-associated mutations in the gene encoding leucine rich repeat kinase 2 (LRRK2). These mutations may produce parkinsonism by altering LRRK2 enzymatic activity. **Methods:** To characterise enzymatic domains of LRRK2, the predicted protein kinase and GTPase regions were expressed and purified individually, and with flanking domains, in eukaryotic *Sf9* insect cells and in *E. coli*. Kinase activity was determined by detecting ^{32}P-radiolabelled phosphate incorporated into intact LRRK2 kinase domain and Myelin Basic Protein (MBP), or in tryptic digests resolved by phospho-peptide mapping. GTPase activity was determined by quantification of phosphate released from γ-phosphate-^{32}P-radiolabelled GTP. **Results:** Kinase domain was active when expressed in *Sf9* cells but not *E. coli*. Autophosphorylation occured on at most five sites, and multiple phosphorylation sites were observed in the artificial substrate MBP. The GTPase domain exhibited slow GTP hydrolysis when expressed in *Sf9* cells or *E. coli* (K_{cat} = 0.031 ±0.006 min^{-1}, n=3). **Conclusion:** Inactivity of the kinase domain expressed in *E. coli* suggests unknown factors or signalling events occuring in eukaryotic cells can promote an active conformation. Phosphorylation sites observed within MBP may resemble sites on physiological LRRK2 kinase substrates. GTPase activity is comparable to the unstimulated form of ^{p21}Ras, suggesting full activity requires interaction with flanking LRRK2 domains or regulatory molecules. Further studies will aim to identify LRRK2 protein kinase substrates, and regulators or effectors of the GTPase region. This may reveal new signalling events underlying PARK8 familial parkinsonism.

ORAL-01-06

DETECTION OF NADPH OXIDASE (NOX)-DERIVED REACTIVE OXYGEN SPECIES (ROS) IN A MOUSE MODEL OF PARKINSON'S DISEASE USING THE CHEMILUMINESCENT PROBE L-012

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Purpose: Microglial-mediated inflammation has been implicated in human Parkinson's disease (PD) and is coupled to the production of reactive oxygen species (ROS) through NADPH oxidase (NOX). Previous work from our laboratory identified a time-dependent induction of NOX2 in a mouse model of PD. We aim to further clarify the role of NOX-derived ROS in PD using the chemiluminescent probe L-012. **Methods:** Unilateral injection of 6-hydroxydopamine (6-OHDA; 20µg) or vehicle into the substantia nigra of C57BL/6J (n=8), NOX2$^{-/-}$ (n=8) or matched wildtype mice (n=8) was used to model microglial-mediated neuroinflammation. Brains were removed after 7 days, and tissue containing striatum and substantia nigra were dissected, homogenized and incubated with specific ROS scavengers and L-012 (100µM)-induced luminescence counted. **Results:** The superoxide (O_2^-) scavenger tempol and peroxynitrite/hydrogen peroxide scavenger ebselen significantly decreased basal ROS levels in substantia nigra (P<0.001), indicating the production of peroxynitrite and/or hydrogen peroxide within this tissue. Pharmacological inhibition or genetic deletion of NOX1, NOX2 and NOX4 isoforms had no significant effect on basal ROS levels. 6-OHDA injection caused a significant increase (ranging from 240-450% of control) in ROS production (P<0.001). This stimulated increase was reduced to basal levels (117% of control) in tissue from NOX2$^{-/-}$ mice (P<0.05), suggesting NOX2 is involved in stimulated ROS production under these conditions. **Conclusion:** NOX2-derived O_2^- may be an important contributor to neuroinflammatory neurodegenerative damage and may be a potential target in the treatment of microglial-mediated inflammation in PD.

ORAL-01-07

C-TERMINAL TRUNCATION AND PARKINSON'S DISEASE-ASSOCIATED MUTATIONS DOWN-REGULATE THE PROTEIN SERINE/THREONINE KINASE ACTIVITY OF PINK1

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Purpose: The Parkinson's disease (PD) causative PINK1 gene encodes a mitochondrial protein kinase called PTEN-induced kinase 1 (PINK1). The autosomal recessive pattern of inheritance of PINK1 mutations suggests that PINK1 is neuroprotective and therefore loss of PINK1 function causes PD. Indeed, overexpression of PINK1 protects neuroblastoma against neurotoxin-induced apoptosis. Presumably, PINK1 exerts its neuroprotective effect by phosphorylating specific mitochondrial proteins and in turn modulating their functions. We aimed at elucidating the biochemical basis of PINK1 regulation and how PD-associated mutations modulate PINK1 kinase activity **Methods**: We expressed the recombinant protein consisting of the PINK1 kinase domain either alone (PINK1[KD]) or with the PINK1 C-terminal tail (PINK1[KD+T]) in baculovirus-infected Sf9 cells. Biochemical analyses of their kinase activities and phosphorylation sites specificity were conducted. **Results and Conclusions**: Both recombinant enzymes preferentially phosphorylate the artificial substrate histone H1 exclusively at serine and threonine residues, demonstrating that PINK1 is indeed a protein serine/threonine kinase. Introduction of the PD-associated mutations, G386A and G409V significantly reduces PINK1[KD] kinase activity. Since Gly-386 and Gly-409 reside in the conserved activation segment of the kinase domain, the results suggest that the activation segment is a regulatory switch governing PINK1 kinase activity. We also demonstrate that PINK1[KD+T] is six-fold more active than PINK1[KD]. Thus, the C-terminal tail also contains regulatory motifs capable of governing PINK1 kinase activity. Finally, the availability of active recombinant PINK1 permits future studies to search for PINK1 protein substrates in mitochondria. Identification of PINK1 protein substrates will shed light on the mechanism of pathogenesis of PD. **Reference**: Sim CH, et al (2006) Hum Mol Genet. 15:3251-62.

ORAL-01-08

CHARACTERISATION OF THE FUNCTIONAL ROLE OF PARKIN CO-REGULATED GENE (PACRG)

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PURPOSE: Parkinson's disease (PD) is a neurodegenerative disorder characterised by the selective loss of dopaminergic neurons. Mutations in parkin are the most common cause of early onset autosomal recessive Parkinson's disease (EO-PD). Parkin functions as an E3-ubiquitin ligase in the ubiquitin-proteasomal system (UPS) targeting misfolded or unwanted proteins to the proteasome to be degraded. It was recently shown that PArkin Co-Regulated Gene (PACRG) shares a bi-directional promoter with parkin and that the two genes are co-regulated (West *et al*., 2003). Gene products regulated by bi-directional promoters often interact or function in a common metabolic pathway. Currently, the function of PACRG is unknown however it has been suggested to function as a mediator of neuronal cell death (Imai *et al*., 2003). We have previously shown that PACRG is regulated by the UPS and is a component of the pathological hallmark features of PD and other parkinsonian disorders (Taylor e*t* al., 2007). Therefore, we hypothesise that PACRG and parkin may interact and together play a role in a common pathway. **METHODS:** To investigate the possibility that PACRG and parkin interact, co-immunoprecipitation (co-IP) studies were performed in HEK293 (n=3) and BE(2)-M17 neuroblastoma (n=2) cells. To confirm a direct interaction between the two proteins, *in vitro* binding assays were performed using PACRG and parkin (n=2). **RESULTS:** Co-IP studies identified an interaction between PACRG and parkin in both HEK293 and M17 neuroblastoma cells. The direct binding between PACRG and parkin was confirmed *in vitro*. **CONCLUSION:** Our results demonstrate a direct interaction between PACRG and parkin *in vivo* and *in vitro* and suggest the co-regulation of both genes has functional relevance. Further studies are needed to characterise the interaction between PACRG and parkin and its potential role in the pathogenesis of PD.

ORAL-02-01

LATENT STEM CELLS IN THE HIPPOCAMPUS ARE ACTIVATED BY NEURAL EXCITATION

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The regulated production of neurons in the hippocampus throughout life underpins important brain functions such as learning and memory. Surprisingly, however, studies have so far failed to identify a resident stem cell, which would provide the renewable source of these neurons. We show here that the adult hippocampus contains a large number of latent precursors, including a self-renewing stem cell population, which only become activated following depolarization. We observed a 3-fold increase in the number of neurospheres generated in the presence of depolarizing levels of extracellular KCl (320±36% of control, $P < 0.001$, $n = 6$) compared to those cultures plated in control (4mM) KCl concentrations. Importantly, this population can also be activated in response to disease states such as status epilepticus and Huntington's disease. The discovery of this latent population provides the first direct evidence of a resident stem cell in the adult hippocampus providing a mechanism to combat neuronal loss in ageing and neurodegenerative disorders.

ORAL-02-02

IN VIVO CUPRIZONE CHALLENGE MODULATES THE PROPERTIES OF PRIMARY NEUROSPHERES DERIVED FROM THE SUBVENTRICULAR ZONE

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Purpose: Neural stem and progenitor cells located in the subventricular zone (SVZ) of the adult mouse brain have been postulated to be a potential source of cells for repair of the demyelinated CNS. To assess whether neural precursor cells (NPCs) are responsive to CNS demyelination, we have examined the behaviour of this cell population following cuprizone-induced demyelination. **Methods:** SVZ tissue was isolated from adult C57BL/6 mice after six weeks cuprizone challenge and from age-matched control mice. Dissociated SVZ tissue was cultured in EGF and FGF-2 to generate primary neurospheres or in a semi-solid collagen-based medium at clonal density. **Results:** The number of primary neurospheres that could be derived from cuprizone-challenged mice was 12.2% ($P=0.011$) lower than from controls ($n=4$ per group). Surprisingly, the percentage of β3-tubulin-positive neuronal cells that were generated upon differentiation of primary neurospheres was 2.5-fold ($P=0.020$) higher for neurospheres derived from cuprizone-challenged versus control mice ($n=4$ per group). By contrast, the percentages of O4-positive oligodendroglia were not statistically different. Analysis of primary SVZ tissue using a semi-solid collagen-based neural colony-forming cell assay revealed a bias towards the production of large progenitor-derived colonies at the expense of small colonies. **Conclusion:** These data suggest that cuprizone challenge could modulate the intrinsic differentiation and proliferative potential of NPCs in the SVZ. We are currently assessing whether modulation of cell fate in primary neurospheres derived from cuprizone-challenged mice reflects the potentiation of neuronal fate commitment of NPCs in vivo and whether this is a direct response to cuprizone or a component part of a regenerative response.

ORAL-02-03

HUMAN DENTAL PULP STEM CELLS INDUCE NEUROPLASTICITY IN A HOST DEVELOPING NERVOUS SYSTEM

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Purpose: The human central nervous system has limited capacity for regeneration. Novel cellular therapies may overcome this clinical challenge either through direct or via a 'bystander' effect on the endogenous tissue. A readily accessible cell population to investigate these mechanisms are human dental pulp stem/progenitor cells (DPSC) that exhibit neural crest properties, with the capacity to contribute to neuronal survival following injury. We hypothesized that implanted DPSC produce factors that induce neuroplastic changes within a host nervous system. **Methods:** An avian model system was adapted to investigate DPSC mediated axonal development using a human-chick xeno-transplantation model. **Results:** In ovo transplanted human DPSC were found to induce neuroplastic changes to avian trigeminal ganglion (TG) development (30/43 embryos; n=3 human donors) which was abrogated in the presence of the CXCL12 inhibitor peptide, T140, which competitively binds to the CXCL12 receptor, CXCR4 (3/9). Futhermore, retroviral transduced human foreskin fibroblasts (HFF) engineered to overexpress CXCL12, were found to alter avian TG development to the extent seen in human DPSC-chick chimeric embryos (9/11). In comparison, low CXCL12 expressing HFF vector controls lacked the capacity to induce neuroplastic change (1/10). **Conclusion:** The present study provides evidence that the cellular and molecular mechanisms of neuroplasticity induced by human adult stem/precursor cells are mediated, in part, by CXCL12/CXCR4 interactions. It strongly supports the idea that transplanted stem/progenitor cells exhibit neuroplastic activity as a mechanism for future neuro-regenerative therapies.

ORAL-02-04

NEOGENIN AND RGMA CONTROL NEURAL TUBE CLOSURE AND NEUROEPITHELIAL MORPHOLOGY BY REGULATING CELL POLARITY

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Purpose: In humans, neural tube closure defects occur in 1/1000 pregnancies. Therefore, understanding the molecular events underlying neurulation is essential to designing new strategies for the prevention of neural tube defects. Neural fold elevation is a key morphological process that acts throughout neurulation to drive neural tube closure. Previously we have shown in zebrafish that Neogenin, an axon guidance receptor closely related to the Netrin receptor, DCC, is required for successful neurulation and that loss of Neogenin results in a severe disruption of neural tube morphology. However, to date, the molecular pathways underpinning neural fold elevation have not been elucidated. **Methods:** In this study, we investigated the role of Neogenin and its ligand RGMa in Xenopus embryos using morpholino knock-down technology. Morpholinos targeting either Neogenin (n=200) or RGMa (n=100) were administered using microinjection techniques and the phenotypes analysed by wholemount in situ hybridization and immunohistochemistry. **Results:** The results show that RGMa-Neogenin interactions are essential for effective neural fold elevation during Xenopus neurulation and that loss of these molecules results in failure of neural tube closure. In addition we demonstrate that Neogenin and RGMa are required for establishing and maintaining the apicobasal orientation of deep layer cells in the neural plate throughout neurulation via their ability to regulate the microtubule network. **Conclusion:** We demonstrate that RGMa and Neogenin are essential for efficient Neural Fold elevation and neural tube morphogenesis. Furthermore, these results suggest that perturbations in the RGMa-Neogenin signaling pathway may be responsible for some forms of human neural tube closure defects.

ORAL-02-05

THE ROLE OF NPAS4 IN BRAIN DEVELOPMENT

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Purpose: To elucidate the developmental role a NPAS4 which is a member of the neural transcription factor bHLH / PAS family. These factors have key roles during development and in the mature organism. NPAS4 expression is restricted to brain, with minor levels detected in testes. NPAS4 expression is specifically expressed in the neurogenic regions of the brain. In adults NPAS4 expression is induced in response to environmental stresses such as ischaemia and seizure. The interest of this study is the role of NPAS4 in the developing embryo. **Methods:** Zebrafish embryos (n=100s) were injected with three different NPAS4 antisense morpholino oligonucleotides at the single cell stage to reduce expression. Markers of brain development were analysed by whole-mount *in-situ* hybridisation. **Results:** Firstly, *NPAS4* expression was detected in the developing brain of *Danio rerio*. Zebrafish embryos at 24hpf displayed reduced brain size and organisation. Morphogens responsible for patterning and neural cell fates had altered expression levels as a result of NPAS4 knock-down. The change in expression patterns of shh and dlx molecules supported the observed morphant phenotype of a reduction in development of the diencephalon. **Conclusion:** NPAS4 does have a developmental role in forebrain.

ORAL-02-06

NFIB REGULATES RADIAL GLIAL DEVELOPMENT AND HIPPOCAMPAL MORPHOGENESIS

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Mice deficient in the transcription factor Nuclear Factor I B (Nfib) display abnormalities in lung maturation and brain development including callosal agenesis and defects in hippocampal formation particularly the loss of the upper blade of the dentate gyrus (Steele-Perkins et al., 2005). Nfib is highly expressed in the developing hippocampus. **Purpose:** To investigate how Nfib regulates hippocampal morphogenesis by examining hippocampal formation in Nfib-deficient mice. **Methods:** BrdU birth dating, activated caspase-3 labelling, and immunohistochemistry with cell-type specific markers, and dissociated hippocampal cell cultures were used (n=3 for each genotype in each experiment). **Results:** No significant differences in proliferation or apoptosis in the hippocampus of Nfib-deficient mice were observed. No differences in Tbr-1 and calretinin labeling were observed at E13 and E14. However, at E15 lamination defects in the hippocampus were evident and formation of the dentate gyrus was most affected. In Nfib-deficient mice, Prox1-positive granule cells migrating from a specialised region of the VZ did not reach the dentate gyrus and instead stalled near the fimbria. Since radial glia form a scaffold for the migration of these neurons we examined their development with a range of different markers. Nestin-positive radial glia were detected in the hippocampal region during development, but the more mature GFAP-positive glia were absent in the dentate gyrus and hippocampal ventricular zone of the Nfib-deficient mice. Defects in radial glial morphology were also observed in dissociated cell cultures from E16 embryos. **Conclusion:** Defects in the differentiation of a subset of radial glia prevent the migration of neurons in the dentate gyrus, profoundly disrupting the morphogenesis of the hippocampus.

ORAL-02-07

PAX7 REGULATES THE TIMING OF DEVELOPMENT OF SUPERIOR COLLICULAR NEURONS

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The primary goal of this research is to elucidate the role of the transcription factor Pax7 in the formation of the superior colliculus, a key brain region within the mammalian visual system. To this end, we analysed the superior colliculus of *Pax7* mutant mice relative to those of wildtype littermates at a variety of developmental stages. Immunohistochemistry, Optimas Digital Image Analysis and cellular quantification techniques were used to assess *Pax7* expression profiles and markers of superior collicular development, including diencephalic/mesencephalic boundary formation (Pax6), polarity and retinotopic mapping (En-1/Ephrin-A2), neuronal formation (NeuN), as well as expression of the paralogous *Pax3* gene suspected of redundancy. Immunofluorescence and confocal microscopy were used to colocalise with various markers for characterisation of the cellular status of *Pax7*-expressing cells. Here we report that *Pax7* is requisite, in a dosage-sensitive manner, for a subpopulation of neurons within the dorsalmost, retinorecipient laminae as evidenced by perturbed *Pax7*, *ephrin-A2* and *NeuN* expression profiles. Furthermore, *Pax3* expression profiles are perturbed during mid-embryonic development in *Pax7* mutant mice. Our results concur with previous findings that *Pax7* plays a pivotal role in the specification of sub-populations of neurons, and sheds further light on the synergistic relationship between *Pax7* and *Pax3*.

ORAL-02-08

BOUNDARY CAP CELLS CONSTRAIN SPINAL MOTOR NEURON SOMAL MIGRATION AT MOTOR EXIT POINTS BY A SEMAPHORIN - PLEXIN MECHANISM

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Background: In developing neurons, somal migration and initiation of axon outgrowth often occur simultaneously and are regulated in part by similar classes of molecules. When neurons reach their final destinations, however, somal translocation and axon extension are uncoupled. Insights into the mechanisms underlying this process of disengagement came from our study of the behaviour of embryonic spinal motor neurons following ablation of boundary cap (BC) cells, neural crest derivatives that transiently reside at motor exit points, CNS: PNS interfaces where motor axons leave the CNS. In the absence of BC cells, motor neuron cell bodies migrate along their axons into the periphery, suggesting that repellent signals from these cells regulate the selective gating of somal migration and axon outgrowth at the motor exit point. **Purpose:** Identify BC cell expressed ligands and motor neuron expressed receptors and downstream signalling molecules involved in the selective gating of motor axons and somata at the motor exit point, by a combination of expression and loss-of-function studies in chick and mouse. **Results:** Developing motor neurons express the semaphorin receptors Npn-1 and -2, Plexin-A1, -A2 and -A4. BC cells express Sema3B, -3G and -6A. RNA interference in the chick, specifically targeted at motor neurons, implicated the receptor Npn2 (n=67) and Plexin-A2 (n=35) and the downstream signalling molecule MICAL3 (n=27). RNAi in neural crest demonstrated a role for BC cell expressed Sema6A (n=18). Studies in mutant mice corroborated the involvement of Npn-2 (n=4) and Sema6A (n=4). **Conclusions:** The data support a model in which BC cell semaphorins signal through Npn-2 and/or Plexin-A2 receptors on motor neurons via a cytoplasmic effector, MICAL3, to trigger cytoskeletal reorganisation. This leads to the disengagement of somal migration from axon extension and the confinement of motor neuron cell bodies to the spinal cord.

ORAL-03-01

A ROLE FOR EPHRINS IN DEFINITION OF THE FOVEAL AVASCULAR ZONE

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Purpose The fovea has number of specializations that serve high acuity vision, including a peak density of cones, a predominance of 'midget' circuits and a local absence of vessels – the foveal avascular zone (FAZ). In macaques blood vessels and their guiding astrocytes migrate across the retina between foetal day (Fd) 70 and 120, following a stereotypical course; neither astrocytes nor blood vessels enter the FAZ. Here we investigate expression of ephrins and their receptors to assess their role in vascular guidance and definition of the FAZ. **Methods** RNA was extracted from human foetal retinas and probed for expression of Eph receptors using RT-PCR. PCR products were sequenced and riboprobes generated to investigate expression patterns of EphR by in situ hybridization. Quantitative PCR was used to assess levels of Eph receptors and ephrins in central *vs.* peripheral retina. Immunohistochemistry was used to localize ephrin proteins. **Results** EphA5, A6 & B1 and ephrins A1, A3, A4, & B2 were identified by PCR in human foetal retina. We detect a high-to-low, centre-to-periphery gradient of EphA6 expression in the ganglion cell layer (GCL) of macaque retina after Fd 70. Generally, lower levels of ephrins are detected in central *vs.* peripheral retina, and ephrin protein is localized to astrocytes in the inner retina. **Conclusion** Interaction between EphA6 in the GCL and ephrin A in astrocytes is consistent with a role in guidance of retinal vessels. Peak expression of EphA6 in the developing foveal region is likely to inhibit the migration of astrocytes, thereby endothelial cells, defining the FAZ.

ORAL-03-02

CHARACTERISATION OF VISUAL FUNCTION IN MICE LACKING CONE PHOTORECEPTORS REVEALS TWO ROD-PATHWAYS OF DIFFERING SENSITIVITY

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Purpose: Rod photoreceptors mediate vision under dim lighting conditions, however when lighting conditions are bright the rod response saturates and cones mediate vision. The purpose of this study was to assess retinal function in the absence of cone input. **Method:** Action potentials from retinal ganglion cells (RGCs) in the optic nerve of coneless (n=8) and C57B6/J wildtype (WT; n=10) mice were recorded, *in vivo*, with light- (20 cd/m^2, LA20; 50 cd/m^2, LA50) or dark-adapting (0 cd/m^2, DA) backgrounds. RGC receptive field (RF) organisation was assessed using a series of bright spots, 100 cd/m^2 (LA20 & LA50 conditions) and 3 cd/m^2 (DA condition), of varying diameter (4.5-52° of visual angle). **Results:** At DA levels, the RF organisation of coneless and WT RGCs was similar. The RFs of RGCs in coneless mice showed: center summation, with a clear preference for spots ~14° in diameter, and surround antagonism, whereby large spots reduced RGC responses. Under the LA20 condition, two groups of coneless ON-center RGCs were identified. One group of coneless RGCs failed to respond above spontaneous activity. The other group continued to respond and showed RF organisation similar to WT RGCs. Under the LA50 condition, the distinction between the two groups of coneless RGCs was less apparent, as responses of the second group were reduced. **Conclusion:** When vision is mediated by rod input only, use of an intermediate light-adapting background (LA20) reveals two groups of ON-center RGC responses, one that is saturated and unresponsive and another that can still encode changes in contrast. This indicates that RGCs may be driven by two rod-mediated pathways of differing sensitivity.

ORAL-03-03

SYNAPTIC INPUTS ONTO WIDE-FIELD GANGLION CELLS IN MARMOSET RETINA

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Purpose: To measure the density of synaptic inputs onto the dendrites of two types of retinal ganglion cells. Specifically, we studied the small bistratified (SBS) and large sparse type of wide-field ganglion cells. **Methods:** Ganglion cells were photofilled following retrograde labelling after iontophoretic injection of 5% dextran tetramethylrhodamine biotin conjugate into the lateral geniculate nucleus of sufentanil-anaesthesised marmosets (Callithrix jacchus). Labelled cells were classified according to their morphology, dendritic field size, and the level of dendritic stratification. Antibodies against the C-terminal binding protein 2 (CtBP2), the glutamate receptor subtype 4 (GluR4), and the receptor-anchoring protein gephryin were used to identify excitatory (bipolar) and inhibitory (amacrine) synapses. **Results:** Bipolar inputs were found on dendrites of both the inner and outer tiers of SBS cells (10 cells). The average density of bipolar synapses was 4.0 ± 2.6 immunoreactive (IR) puncta / 100 µm^2. The average density of amacrine cell inputs was 4.2 ± 4.5 IR puncta / 100 µm^2 (3 cells). One SBS cell was processed simultaneously with markers for both amacrine and bipolar cell input. The ratio between the two input types was 6:5. For one large sparse cell the amacrine input was 1.6 ± 0.9 IR puncta / 100 µm^2. The bipolar input to sparse cells may arise from axons of diffuse bipolar 6 (DB6) cells. On average DB6 axon terminals showed 55.7 ± 22.3 areas of overlap (potential synaptic input) with large sparse cells (3 cells). **Conclusion:** Both excitatory and inhibitory inputs are present on dendrites of SBS and large sparse ganglion cells.

ORAL-03-04

RETINAL GANGLION CELLS IN THE PRIMATE RETINA RECEIVE DIRECT INHIBITORY SYNAPTIC INPUT

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Purpose: Vision is little changed over a wide range of ambient light intensity, an adaptive process that begins in the photoreceptors. Lateral inhibition at subsequent stages of retinal processing also contributes, but the relative contribution of synaptic interactions in the outer and inner plexiform layers is poorly understood, especially in primate. **Methods:** We measured the spatial organisation of retinal ganglion cells in an *in vitro* preparation of the marmoset (*Callithrix jacchus*) retina. Three adult males were dark-adapted then euthanased with an overdose of barbiturate; the eyes were removed, the retina dissected under dim red light and all subsequent procedures were under infrared illumination. Whole-cell patch-clamp recordings were made from cells (n = 18) in the ganglion cell layer in whole-mount retinas, in current- and voltage-clamp mode. Light-evoked synaptic currents were isolated by blocking voltage-gated sodium and potassium currents intracellularly; the excitatory and inhibitory conductances were calculated. **Results:** receptive fields of ganglion cells were of the classical type, with an excitatory centre and inhibitory surround. Excitatory synaptic input was greatest for small spots and less for large spots, evidence of presynaptic inhibition. Both small spots and uniform annuli could evoke direct inhibitory input to ganglion cells. In one ganglion cell tested the $GABA_A$ receptor antagonist SR95531 removed both presynaptic inhibition and direct inhibitory input. **Conclusion:** Spatial summation in primate retinal ganglion cells depends on direct excitatory and inhibitory inputs.

ORAL-03-05

DIRECTIONAL TUNING OF SLOWLY-ADAPTING MECHANORECEPTORS IN THE HUMAN NAIL BED

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Purpose: Of the slowly-adapting type II (SAII) receptors in the human hand, those associated with the medial and lateral borders of the nail bed (SAIInail) are unique in possessing small receptive fields and the capacity to respond to forces applied remotely to the finger pad. We assessed their capacity to discriminate between the angular directions of forces applied to the centre of the finger pad. Methods: Unitary recordings were made from 17 SAIInail units via tungsten microelectrodes inserted percutaneously into the median nerve of awake human subjects. The dorsal aspect of the receptor-bearing digit was fixed to a rigid support and a servo-controlled stimulator applied ramp-and-hold forces (amplitude 4 N, rate 8 N/s) via a flat disc over the centre of the finger pad. Stimuli were delivered normal to the skin and at 10°, 20° or 30° to the vertical in 8 radial directions. Results: With one exception all afferents demonstrated directional sensitivity at each vertical angle, and were broadly tuned to their individual preferred direction of force. The sharpness of tuning varied across units and the preferred directions were distributed in all angular directions with reference to the stimulation site. The median discriminatory capacity between radial directions was inversely related to the vertical angle, being 7° at 30°. Conclusions: Given their capacity to respond to forces transmitted through the finger pad, and their fine directional tuning over all radial and vertical directions, we conclude that SAIInail mechanoreceptors are ideally placed to unambiguously encode the total 3D profile of contact forces, and suggest that they play an important role in sensorimotor control of the hand.

ORAL-03-06

AUDITORY EXPERIENCE FINE-TUNES THE MATURATION OF SENSORY CELLS IN THE AVIAN INNER EAR

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Hair cells are exquisitely sensitive mechanoreceptors. The morphological polarity of the hair bundle protruding from the apex of the cell defines the axis of optimal mechanical sensitivity. Different hair-cell organs show specific, but always highly-organised patterns of hair-cell polarity. The establishment of those patterns in development is only partly understood. In the avian cochlea, hair-cell polarities deviate in a complex but highly precise pattern from a strictly radial orientation. Development of this pattern passes through a stage of uniform, radial orientation, before the mature polarities gradually emerge, suggesting a second phase of orienting signals. While the first phase occurs early and before the onset of hearing, the second phase clearly overlaps with auditory development. **Purpose**: To test whether hair-cell polarity is entirely governed by intrinsic signals or whether normal stimulation and function of hair cells is necessary for final adjustment. We investigated this by ablating the middle ear around the onset of hearing and thus drastically reducing the auditory input to the cochlea during the relevant phase of development. **Methods**: Six barn owls, altricial birds with a posthatching onset of hearing, underwent unilateral columellar removal within a few days after hatching. At P41-51, hearing thresholds of both ears were determined to assess the physiological effect of columellar removal. Both ears of each individual were then processed for SEM analysis of hair-cell polarities. **Results**: Threshold measurements confirmed that the animals had been monaurally deprived. Hair-cell polarities were subtly, but significantly altered in the deprived ears compared to their normal-hearing counterparts. **Conclusion**: To our knowledge, this is the first demonstration of a role for sensory experience in the maturation of sensory cells.

ORAL-03-07

INHIBITION IN SOUND LOCALISATION PATHWAYS

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Purpose: Sound localisation depends on minute differences in the arrival time and intensity level of sound at each ear. Distinct neuronal circuits, initiated in the cochlear nucleus by spherical bushy cells (SBCs) and globular bushy cells (GBCs), respectively process interaural time and interaural level differences. Previously we have described excitatory transmission in these circuits. Here we investigate the role of inhibition in sound localisation. **Methods:** Whole-cell recordings were performed from SBCs and GBCs in brainstem slices of CBA/CaH mice (n=5; P16-20). Evoked and spontaneous glycinergic inhibitory postsynaptic potentials (IPSCs) were recorded in the presence of bicuculline (10 μM), D-AP5 (30 μM), CNQX (10 μM) and TTX (1 μM, for spontaneous IPSCs) to block GABA, NMDA, AMPA, and sodium channels. The addition of strychnine (1 μM) abolished the IPSCs. Cells were filled with Neurobiotin (0.2%) for intracellular labelling and cell identification. **Results:** Preliminary data show the mean amplitude was 380 pA for evoked IPSCs and 151 pA for spontaneous IPSCs with mean rise times less than 1 ms and half-widths less than 50 ms. Electron microscope studies reveal that SBCs receive relatively greater numbers of inhibitory inputs than GBCs. Analyses are underway to correlate inhibitory inputs to cell responses. **Conclusion:** This combined anatomical and physiological approach will attempt to provide a comprehensive description of the role of inhibition in sound localisation.

ORAL-03-08

FREQUENCY SPECIFIC ACTIVATION OF INFERIOR COLLICULUS NEURONS THROUGH PENETRATING BRAINSTEM MICROSTIMULATION

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Introduction: Auditory Brainstem Implants (ABI) are being implanted clinically with minimal success as little is understood about how and where to stimulate the brainstem. By stimulating the Ventral Cochlear Nucleus (VCN) and recording in the Central Nucleus of the Inferior Colliculus (CIC) we have begun to characterize the brains response to electrical stimulation. **Methods:** Male Hooded Wistar Rats (n=6) weighing between 270-420g were anesthetized, fitted with hollow ear bars, and implanted with a 32-channel microelectrode in the VCN. Multiunit activity was recorded in the VCN in response to acoustic stimulation and the characteristic frequency of each electrode site was found. A quartz micropipette was stepped through the CIC to record single neurons in response to acoustic stimulation and electrical stimulation of the VCN using single biphasic charge-balanced pulses and pulse trains (100–2400pps). **Results:** Extracellular CIC neurons (n=17) exhibited either excitatory (n=9), suppressed (n=2) or nil (n=6) responses to single pulse VCN stimulation. Mean threshold of excitatory CIC neurons was 9μA (minimum = 4μA). Excitatory CIC neurons showed a high correlation between their CF and the CF of the VCN sites that excited them with lowest threshold ($r=0.86$, $p<0.005$) and lowest average first spike latency (FSL, $r=0.80$, $p<0.005$). CIC neurons exhibited both an increased spike rate and a decrease in FSL to increased stimulation rate. Intracellular recordings (n=3) showed the presence of excitation and strong inhibition following stimulation of the VCN. **Conclusion:** Microstimulation of the VCN can result in; low thresholds, high frequency-specific CIC activation, increased spike rate and decreased FSL. These findings will facilitate the development of new ABI stimulation strategies.

ORAL-04-01

EFFECTS OF GLUCOCORTICOIDS ON NEURONAL ACTIVITY, HORMONE SECRETION AND GLUTAMATE CONTENT AFTER BLOOD VOLUME EXPANSION (BVE)

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There are strong evidences suggesting the participation of the hypothalamic-pituitary-adrenal axis in the control of hidromineral balance. So, this study aimed to evaluate (1) the involvement of glucocorticoids in the secretion of oxytocin (OT) and corticosterone (CORT), (2) the activation of oxytocinergic neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus and (3) the total content of glutamate in the PVN, SON and median eminence (ME) of rats submitted to isotonic (I-) or hypertonic (H-) blood volume expansion (BVE). Male adult Wistar rats were subjected to I- (0.15M NaCl, 2ml/100g b.w., i.v.) or H-BVE (0.30M NaCl, 2ml/100g b.w., i.v.) two hours after the administration of dexamethasone (1mg/kg, i.p.) or vehicle. Brain and blood were collected for Fos-OT immunohistochemistry, determination of total glutamate content and hormone measurements. Rats (n=6-10) subjected to either I- or H-BVE showed increased plasma levels of OT and CORT. Double labelling for Fos and OT showed that the number of magnocellular neurons in PVN and SON were elevated in both I- and H-BVE rats (n=5-7). Pre-treatment with dexamethasone reduced OT (n=8-11) and CORT (n=8-9) secretion, as well as the number of Fos+OT neurons in the PVN and SON (n=5-7). The total content of glutamate in the PVN, SON and ME was increased after I- and H-BVE, and this response was not altered by previous administration of dexamethasone (n=6-11). These data suggest that glucocorticoids are involved in the control of rapid OT release and activation of oxytocinergic neurons, but are not likely to modulate the total amount of glutamate in hypothalamic structures in response to BVE.

ORAL-04-02

INTRA-AMYGDALA INJECTION OF $GABA_A$ AGONIST, MUSCIMOL, REDUCES TACHYCARDIA AND MODIFIES CARDIAC SYMPATHO-VAGAL BALANCE DURING RESTRAINT STRESS IN RATS

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Purpose: To determine the effect of bilateral microinjections of the $GABA_A$ receptor agonist, muscimol, into the amygdaloid complex on both the heart rate (HR) and cardiac autonomic activity during psychological stress. **Methods:** Experiments were performed in male Sprague-Dawley rats (n=9), with pre-implanted ECG electrodes. Animals were subjected to 30-min restraint after bilateral injection of either vehicle or muscimol into the amygdala. **Results:** HR increased sharply after the onset of the restraint and reached a peak 1-2 min later (from 344±6 to 440±20 BPM). Subsequently, HR began to fall, and during the next 10-15 min approached the steady-state level of 384±11. After vehicle, mean HR during each of three 10-min restraint epochs was significantly higher compared to the pre-restraint level. After muscimol, mean HR was significantly elevated only during the first 10 min of restraint. There was no difference in the early peak tachycardia between both conditions. Muscimol substantially accelerated the fall of the HR from the peak to the steady-state level, and thus the area under the curve value for muscimol (503±162 BPMmin) was significantly smaller then that for vehicle (1221±231 BPMmin); $P<0.05$. After vehicle, the high-frequency spectral power of the HR decreased and the low-frequency power increased during the restraint, resulting in a significant rise of the low frequency/high frequency ratio from 1.2±0.2 to 2.8±0.6 (n=9, $p<0.05$). Muscimol suppressed these stress-induced effects. **Conclusions:** inhibition of the amygdala neurons abolishes the sustained component of tachycardia during the restraint, but has no effect on the early tachycardic component. Restraint stress increases sympathetic and reduces parasympathetic outflow to the heart, and amygdala blockade prevents these changes.

ORAL-04-03

CHARACTERISATION OF THE CARDIOVASCULAR RESPONSE TO AIR-PUFF STARTLE STIMULI

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Purpose: Air-puff startle is a commonly used model of acute psychological stress, which is known to be associated with increased secretion of adrenocorticotropic hormone, and also with increased c-Fos expression in brain regions known to be involved in mediating physiological responses to stress, such as the dorsomedial hypothalamus and hypothalamic paraventricular nucleus. The aim of this study was to define more clearly the cardiovascular response to air-puff startle, as a first step to elucidating the brain mechanisms that subserve this response. **Methods:** Rats were anaesthetised with isoflurane (3%) for implantation of radio-telemetry probes, to allow subsequent measurement of arterial pressure (AP) and heart rate (HR) (control and startled rats, each n=5). A series of air-puffs were delivered to conscious rats from a cylinder of compressed air. **Results:** The air-puffs generated initial rapid, transient cardiovascular responses, involving both increases and decreases in both AP and HR. These initial responses were followed by a period of increased AP and HR. In between blocks of air-puffs, AP remained significantly elevated, whereas HR returned to levels not significantly different from baseline. **Conclusion:** The results suggest that there are multiple components to the cardiovascular response to air-puff. There is a rapid transient phase, which may be a result of mechanical and reflex changes. There is also a longer latency period of increased AP and HR, which may be mediated by a central command component involving activation of neurons in the DMH. The differential control of AP and HR between blocks of air-puffs suggests that these responses may, however, be mediated by separate populations of neurons. Future studies will examine the central pathways that mediate these responses.

ORAL-04-04

ACTIVATION OF 5-HT1A RECEPTORS SUPPRESSES THE CARDIOVASCULAR RESPONSE EVOKED FROM THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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Purpose: It has been shown previously that systemic or central administration of the 5-HT1a receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) suppresses the cardiovascular response to psychological stressors in conscious rats. Similarly, in anaesthetized rats, systemic administration of 8-OH-DPAT suppresses the cardiovascular response to activation of neurons in the dorsomedial hypothalamus (DMH), which is an essential brain region integrating the physiological response to acute psychological stressors. The hypothalamic paraventricular nucleus (PVN) also integrates cardiovascular responses to various stressors such as hypovolaemia, but not acute psychological stressors. The aim of this study was to determine whether the cardiovascular response evoked by activation of the PVN, like that evoked from the DMH, is modulated by central 5-HT1a receptors. **Methods**: Arterial pressure, heart rate (HR), and renal sympathetic nerve activity (RSNA) were recorded in 7 urethane anaesthetized rats. **Results**: Microinjection of bicuculline (20 pmol in 20 nl) into the PVN evoked increases in mean arterial pressure (MAP), HR, and RSNA. After systemic injection of 8-OH-DPAT (0.1 mg/kg iv), these increases in MAP, HR and RSNA were greatly reduced, by approximately 60-90% ($P<0.05$). After subsequent systemic injection of the 5-HT1a receptor antagonist WAY-100635, the evoked cardiovascular responses were partially restored. **Conclusion**: The results indicate that the inhibitory effect of activation of central 5-HT1a receptors is not limited to cardiovascular responses mediated via the DMH, but also includes responses evoked from the PVN. Thus, 5-HT1a receptors may exert a generalized modulatory effect on a wide range of cardiovascular responses integrated by forebrain nuclei.

ORAL-04-05

ACTIVATION OF SOMATOSTATIN 2A RECEPTORS ON SYMPATHOEXCITATORY NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA INHIBITS SYMPATHETIC TONE AND BLUNTS SYMPATHOEXCITATORY INPUTS

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Purpose: Sympathetic tone is generated by neurons in the rostral ventrolateral medulla (RVLM). We have recently demonstrated that the somatostatin (SST) receptor, SSTR2A is expressed in 34% of RVLM presympathetic neurons. We examined the effects of SSTR2A activation in the RVLM on resting and reflex sympathetic nerve activity (SNA), arterial pressure (AP) and heart rate (HR). **Methods:** SST, Lanreotide and BIM-23627 (SSTR2A agonist and antagonist, respectively) were microinjected into the RVLM of urethane-anaesthetised (1.3g/kg i.p.), paralysed, vagotomised and artificially ventilated Sprague Dawley rats (n = 32). AP, HR, phrenic nerve, splanchnic and lumbar SNA were recorded. Furthermore the effects of SST on subsequent RVLM glutamate evoked sympathoactivation (100 mM, 50 nL) or peripheral chemoreceptor activation (100% N2, 12sec), sciatic and aortic nerve stimulation were determined. **Results:** Bilateral RVLM injections of SST (50 nL 1.5 mM) caused SNA and AP to fall to levels seen following spinal transection. SSTR2A agonist or antagonist mimicked or blocked the effects of SST, respectively. Agonist effects were dose-dependent, reversible and not subject to tachyphylaxis. Baroreceptor evoked sympathoinhibition prevailed despite the reduced sympathetic tone. However, subsequent sympathoactivation by microinjected glutamate, somatosympathetic or chemoreceptor stimulation were significantly attenuated. **Conclusion:** Bilateral activation of SSTR2A expressed on a subpopulation of presympathetic RVLM neurons abolishes sympathetic vasomotor tone reducing AP. Secondly, SST2A receptor activation dampens RVLM neuronal excitability and blunts sympathoexcitatory afferent input. SST plays a profound role in the RVLM however natural stimuli that evoke its release are yet to be identified.

ORAL-04-06

PREPROGALANIN MRNA IN THE VENTROLATERAL MEDULLA OBLONGATA COLOCALISES WITH SUBPOPULATIONS OF RESPIRATORY, BUT NOT CARDIOVASCULAR NEURONS

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INTRODUCTION: The ventrolateral medulla (VLM) contains nuclei vital in the regulation of the cardiovascular and respiratory systems. The cardiovascular nuclei are characterised rostrally by TH expressing neurons that are glutamatergic and caudally by GABA-ergic neurons. The ventral respiratory group (VRG) is found immediately dorsal to the cardiovascular nuclei. The VRG neuronal network contains multiple chemically heterogenous subpopulations that do not contain tyrosine hydroxylase (TH) but can express neurokinin-1 receptors (NK1R). Previous studies have reported galanin immunofluorescence in various cardiovascular regions in higher brain regions, but its characterisation in the VLM has not been comprehensively investigated. PURPOSE: Examine the rostro-caudal distribution of preprogalanin mRNA expressing neurons (PPG) and its colocalisation with NK1R, TH and cholera toxin B (CTB), a retrograde tracer marking bulbospinal neurons in the ventrolateral medulla (VLM). METHODS: Combed in situ hybridization and immunofluorescence techniques were used on adult male Sprague Dawley rats brain tissue to label the targeted neurons (n=9). The CTB was injected in the intramediolateral cell column in the spinal cord. RESULTS: PPG expressing neurons were found in respiratory regions corresponding to the retrotrapezoid nucleus (RTN), pre-Bötzinger complex (pre-BötC) and the rostral ventral respiratory group (rVRG). The only cardiovascular region found to contain PPG was the A1 cell group. Subpopulations of PPG expressing neurons colocalise with NK1R in the pre-BötC/rVRG region of the VLM (15.81±2.035%) and with TH in the A1 cell group (40.36±7.49%). A subpopulation of non-TH PPG expressing neurons in the rVRG also project to the spinal cord (22.33±4.28%). CONCLUSION: These results provide supportive evidence for the involvement of galanin in respiratory regulation and possibly chemoreception.

ORAL-04-07

A1 AND C1 NEURONS IN THE RAT BRAINSTEM SHOW DIFFERENT PROFILES OF G-α MRNA EXPRESSION

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PURPOSE: To determine whether the mRNA for G-α isoforms G-! ai1, G-αi2, G-αi3, G-αl, G-αq, G-αo, G-αs, G-α11, G-α12, G-α13 are differentially expressed in tyrosine hydroxylase immunoreactiv(IR) neurons of A1 and C1 cell groups in rat brainstem.**METHOD**: Adult male Sprague Dawley rats received an overdose of sodium pentabarbitone (80mg/Kg IP) and were perfused with 4% paraformaldehyde. The brainstem was removed and 40µm sections were processed using in-situ hybridization and digoxeginin labeled probes for G-α protein mRNA combined with immunohistochemistry for tyrosine hydroxylase (TH).**RESULTS**: Gene expression of each G-α probes had a widespread unique distribution in the brainstem. All TH neurons examined in C1 (73±2) and A1 (29±3) region expressed G-αs (n=3) whereas, more than 80% of TH neurons in A1 (25±4, 25±2, 29±1, 28±1) and C1 (62±2, 70±3, 63±1, 59±5) region expressed G-αo, G-αi2, G-α12, G-αl (n=3). 50% of TH neurons expressed G-αi1 and G-α13 in A1 (24±1, 31±2) cell group and more than 75% in the C1(69±3,66±5) cellgroup (n=3).Less than 30% of the TH neurons expressed G-αi3 in A1(29±1) cell group and this proportion was even less than 20% in the C1(67±4) cell group (n=3).No TH neuron in either A1(25±2) or C1(81±1) region expressed G-α11 (n=3) protein mRNA.**CONCLUSION**: All G-α protein mRNA are present in both A1 and C1 cell groups with the exception of G-α11.This differential distribution G-α proteins reflects different functions of the two cell groups. Differential distribution of G-α proteins within each cell group may be associated with functionally different subpopulations. This suggests multiple signaling pathways are activated by G-α proteins from numerous upstream receptors contributing to cells output.

ORAL-4-08

ANGIOTENSIN RECEPTOR BINDING AND EFFECTS IN THE BRAIN OF ANGIOTENSINOGEN KNOCKOUT MICE

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Angiotensin II (Ang II), which is the main circulating effector hormone of the renin angiotensin system, is produced by enzymatic cleavage of angiotensinogen (AGT). Within the brain Ang II regulates several functions, including cardiovascular function and fluid homeostasis. The rostral ventrolateral medulla (RVLM) expresses angiotensin receptors and plays a critical role in blood pressure (BP) regulation. Purpose: The present study aimed to examine whether targeted deletion of the AGT gene altered angiotensin receptor responsiveness in RVLM. Methods: Angiotensinogen knockout ($AGT^{-/-}$, n=3) and wild-type ($AGT^{+/+}$, n=8) mice were anesthetized with urethane (0.75mg/g, IP) and chloralose (0.05mg/g, IP), tracheotomized and artificially ventilated with room air. The carotid artery was cannulated to measure BP. Glutamate (100pmol, 10nl) and Ang II (50pmol, 50nl) were microinjected unilaterally into the RVLM and cardiovascular responses recorded. Results: As has been shown in conscious mice, resting mean arterial pressure in $AGT^{-/-}$ mice (51±5mmHg) was lower than in $AGT^{+/+}$ mice (70±4mmHg). Microinjection of glutamate defined the position of the RVLM. Microinjection of Ang II into the RVLM caused a similar pressor response in both strains of mice ($AGT^{-/-}$ 14±6mmHg vs $AGT^{+/+}$ 10±3mmHg). Conclusion: Mice with targeted deletion of AGT have lower than normal resting BP but similar responses to activation of the RVLM by direct microinjection of Ang II. These results suggest that AGT deficiency is not associated with an upregulation of angiotensin receptor expression or responsiveness in the RVLM.

ORAL-05-01

VESICLE TRAFFICKING ABNORMALITIES IN ITSN1 NULL MICE

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Purpose: Since all individuals with Down syndrome (DS) (trisomy 21) develop Alzheimer's disease-like neuropathology, there must be a common disease mechanism. One of the earliest hallmarks of Alzheimer's disease pathogenesis is the presence of enlarged early endosomes, suggesting a disturbance in endocytosis. We identified a chromosome 21 gene, Intersectin-1 (ITSN1) that is up-regulated in DS brains and has a putative function in endocytosis. **Methods:** We generated *Itsn1* null mice, either by knocking out the entire gene or by knocking out only the long, brain predominant isoform. We investigated endosome size in brain sections using immunofluorescence for early endosome markers and measured NGF levels in the brain by ELISA. We also measured vesicle trafficking in chromaffin cells using carbon fibre amperometry and in neurones using synaptopHlourin uptake and release. **Results:** For n≥3 mice in every case, we found an increase in endosome area, reduced NGF levels in the septal region, a reduced number of exocytosis events in chromaffin cells and a slowing of endocytosis in neuronal cultures in knockout mice compared to normal controls. **Conclusions:** Our data is the first indication that *Itsn1* has a role in endocytosis in the mammalian brain and that a disruption in *Itsn1* expression causes a disturbance in endocytic function which may lead to impaired NGF retrograde transport.

ORAL-05-02

OPIOID-INDUCED MU-RECEPTOR INTERNALISATION AND DESENSITISATION IN MOUSE LATERAL PARABRACHIAL NEURONS

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Chronic use of strong analgesics such as morphine leads to tolerance and dependence. While the proposed mechanisms of opioid tolerance remain controversial, it appears that mu-opioid receptor (MOR) internalisation and physiological desensitisation are involved. Determining the relationship between internalisation and desensitisation has been hampered by an inability to perform both measurements in the same preparation and relating these to opioid tolerance in vivo. **Purpose:** the aim of this study was to simultaneously investigate MOR internalisation and desensitisation in lateral parabrachial neurons (LPB) using in vitro brain slice recordings together with MOR immunohistochemistry. All protocols were approved by Joint RNSH/UTS Animal Ethics Committee. Our preliminary experiments showed that sufentanil citrate (0.75 mg/kg sc) in vivo internalised MOR in LPB. **Methods:** adult male C57BL/J6 mice were deeply anaesthetised with isoflurane, decapitated and brain slices prepared. Several LPB neurons in a slice were labelled with biocytin (0.2%) using patch-clamp. Brain slices were then superfused with a desensitising concentration of met-enkephalin (30 μM) for 10 min to cause internalisation. They were then post-fixed with paraformaldehyde and immunostained for MOR and biocytin. For measurements of desensitisation, opioid-activated potassium currents were measured using whole-cell patch-clamp recordings during met-enkephalin application. **Results:** we found that met-enkephalin produced substantial MOR internalisation (n=3), while MOR remained on the membrane surface in control slices (n=3). Met-enkephalin also produced profound desensitisation, with MOR currents attenuated by 48% and 44% of maximum potassium current measured (n=2). **Conclusion:** ability to study MOR internalisation and desensitisation in the same native tissue will help to significantly understand underlying mechanisms of opioid tolerance.

ORAL-05-03

MECHANISMS UNDERLYING RCAN1 REGULATION OF EXOCYTOSIS AND VESICLE FUSION PORE KINETICS: IMPLICATIONS FOR DOWN SYNDROME AND ALZHEIMER'S DISEASE

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Purpose: Neuronal exocytosis is vital for synaptic activity, neuronal communication and to prevent neurodegeneration. Proteins aberrantly expressed in neurodegenerative disorders which regulate neuronal exocytosis may underlie neurodegenerative disease pathology. Regulator of Calcineurin 1 (Rcan1), located on chromosome 21, is overexpressed in brains of Down syndrome (DS) and Alzheimer's disease (AD) patients. We have illustrated using mouse lines which knock-out (KO) and transgenically overexpress (Tg) Rcan1 that Rcan1 regulates both the rate of exocytosis and vesicle fusion pore kinetics. Methods: Exocytosis from adrenal chromaffin cells cultured from 6-10 week old mice was monitored using carbon-fibre amperometry. Ca2+ imaging was also carried out on these cultures. Results: The number of secretory events observed during stimulation was decreased by 36% in Rcan1 Tg ($p<0.05$, n=23) and 50% in KO ($p<0.01$, n=21) cells compared to controls. Greater expression of Rcan1 also increased the speed of vesicle pore opening and closing at the point of fusion, resulting in lower levels of neurotransmitter release from individual vesicles and more "kiss-and-run" type fusion. These effects are not due to changes in Ca2+ entry (n=18-50) or the size of the readily releasable vesicle pool (n=8-16). We also investigated potential mechanisms underlying these changes relating to the known effects of Rcan1 on calcineurin activity, mitochondrial function and oxidative stress. Conclusion: Rcan1 regulates both the number of vesicles undergoing exocytosis and the amount of vesicle content released. This has direct implications for neuronal exocytosis and may underlie some of the reduced cognition and neurodegeneration observed in DS and AD.

ORAL-05-04

MECHANISMS OF PURINE P2X RECEPTOR ACTIVATION INDUCED EXCITATION IN THE TRIGEMINAL SENSORY SYSTEM

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Purpose: The subnucleus caudalis (Vc; medullary dorsal horn) is the site of termination of the majority of trigeminal small-diameter nociceptive primary afferent fibres. We have previously shown, *in vitro*, that P2X agonists may presynaptically facilitate excitatory neurotransmission in Vc. In order to elucidate the mechanisms of this interaction we have examined the roles of P2X receptors in Vc by using whole cell patch clamp in brainstem slices and on sensory neuron cell bodies. **Methods:** Sprague-Dawley rat pups (10-20 days) were anaesthetised with halothane, decapitated and horizontal slices (250μm) were cut from the caudal brainstem. Acutely dissociated trigeminal ganglion neurons were also used in this study. Whole-cell patch-clamp recordings (voltage or current clamp) were made. **Results:** The previously reported increase in excitatory neurotransmission in deep Vc laminae induced by the ATP analogue α,β-methylene-ATP (α,β-meATP) was blocked (no change in mEPSC rate or amplitude; n=7) following superfusion of the NMDA antagonist AP5 (40μM). To test if α,β-meATP activated an intracellular kinase cascade that altered NMDA receptor function, α,β-meATP (30μM) was applied in the presence of the kinase inhibitor, staurosporine (2μM). In this experiment α,β-meATP failed to alter mEPSC rate (P>0.9; n=7). In addition, recordings from trigeminal primary sensory neurons showed that α,β-meATP caused a depolarisation of 17.0 ± 2.3mV (n=5). **Conclusion:** These results suggest that P2X receptor-mediated excitation in deep laminae of Vc are dependent on NMDA receptors and involve a staurosporine-sensitive kinase.

ORAL-05-05

FLAVAN-3-OL DERIVATIVES ARE ALLOSTERIC MODULATORS OF $GABA_A$ RECEPTORS WITH ANXIOLYTIC PROPERTIES IN MICE

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Background: enhancement of the inhibitory action of $GABA_A$ receptors underlies the therapeutic actions of important agents including benzodiazepines, barbiturates and many anaesthetics. Previously, we have shown that the catechin derivatives 3-acetoxy-4'-methoxyflavan can positively modulate $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_1\beta_2$ $GABA_A$ receptors. These actions were independent of the high-affinity benzodiazepine site, and the exact mechanism remained unknown. **Aims and Methods**: to evaluate receptor subtype selectivity by testing these flavan derivatives on α_2 subtype $GABA_A$ receptors expressed in *Xenopus laevis* oocytes. Also, the binding site for these drugs was studied by developing analogues with antagonist properties. Furthermore, the anxiolytic action of the agent FL-131 was evaluated in Swiss mice using the elevated plus maze. **Results**: all four flavan steroisomers potentiated GABA-induced currents at both $\alpha_2\beta_2\gamma_{2L}$ and $\alpha_1\beta_2\gamma_{2L}$ receptors with similar potency (n=6-8). However, the efficacy at α_2 subtype receptors was significantly higher ($p<0.05$), reaching a greater maximal effect. The analogue FL-173 showed no actions on these receptors, but it proved to antagonize the modulatory action of FL-131 on $\alpha_1\beta_2$ receptors (IC_{50}=9 μM, n=6). Interestingly, it also antagonized the action of diazepam with a similar potency (n=8). The intraperitoneal administration of the agent FL-131 caused an increase in open arm exploration in mice at doses of 3, 5 and 10 mg/kg, compared to controls (n=12-20), with no changes in locomotor activity. **Conclusions**: flavan-3-ol derivatives are modulators of $GABA_A$ receptors with higher efficacy on α_2 subtype, which could explain the anxiolytic action of the agent FL-131 on mice. Their allosteric actions take place through the binding to the low-affinity benzodiazepine site on these receptors.

ORAL-05-06

DEPOLARISATION SENSITIVE PHOSPHORYLATION SITES IN AMPHIPHYSIN I REGULATE ENDOPHILIN BINDING

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Synaptic vesicle endocytosis (SVE) in nerve terminals is responsible for the retrieval of empty synaptic vesicles after exocytosis. It is mediated by group of proteins called the dephosphins which includes amphiphysin I. Amphiphysin is rapidly dephosphorylated by calcineurin after nerve terminal depolarisation, suggesting a triggering role in SVE. We analysed amphiphysin I isolated from nerve terminals to identify all the phosphorylation sites to determine which phospho-sites might regulate protein-protein interactions required for SVE. We used ^{32}P-labelling of nerve terminals, tryptic digestion, titanium dioxide phosphopeptide enrichment, two dimensional phosphopeptide mapping, and nano-HPLC tandem mass spectrometry. Site-directed mutagenesis of a subset of the amphiphysin I phosphorylation sites was then used to characterise protein-protein interactions *in vitro* using GST pull-downs experiments. Thirteen phospho-sites were detected using this procedure. Evidence was obtained indicating at least two protein kinases phosphorylate amphiphysin *in vivo*. Five phospho-sites were shown to contain the majority of the ^{32}P incorporated into nerve terminals, and the others were minor. Each of the five main phospho-sites was rapidly dephosphorylated on KCl-induced depolarisation of the nerve terminals, whereas the other sites exhibited only minor changes. Site-directed mutagenesis and *in vitro* pull-down experiments revealed that at least two sites regulated the amphiphysin I interaction with one of its main protein binding partners, endophilin I. Although the results reveal complex multi-site phosphorylation of amphiphysin I in nerve terminals we highlight the potential importance of five of thirteen phospho-sites for SVE. The results form the basis for future understanding the role of individual sites or clusters of phospho-sites in SVE.

ORAL-05-07

RETINOL STIMULATES TYROSINE HYDROXYLASE ACTIVITY BY INCREASING SER40 PHOSPHORYLATION IN BOVINE ADRENAL CHROMAFFIN CELLS

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Tyrosine Hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis. Long-term regulation of TH is achieved by modulation of TH expression. Chronic exposure (7-8 days) to vitamin A (retinol) and its metabolites, especially retinoic acid (RA), regulates neuronal differentiation by increasing the expression of neural specific markers, including TH. Short-term regulation of TH is achieved by increasing the phosphorylation of Ser40, which increases TH activity by relieving the feedback inhibition caused by catecholamine binding. The short term effects of retinol and its metabolites on TH are unknown. We therefore investigated the effect(s) of retinol and RA on TH phosphorylation and TH activity using bovine adrenal chromaffin cells. Retinol (10 μM) induced a 2-fold increase in TH activity at 10 minutes of incubation that lasted up to 2 hours. Ser40 phosphorylation was acutely increased, reaching a peak (4 fold) at 15 minutes of incubation and then decreasing to 2-fold control levels for the next 1.75 hours. Ser40 phosphorylation and TH activity were inhibited by the PKC inhibitor GO6893 at 15 minutes. PKC activation was dependent on the retinol induced influx of extracellular calcium. These results show for the first time that retinol induces short-term effects on TH regulation. These effects were non-genomic (i.e. independent of nuclear retinoic receptor activation) and were dependent on retinol induced calcium influx and PKC activation. The concentrations of retinol used in these studies are similar to the levels obtained in vivo in response to pharmacological treatments and the effects on TH activation are therefore likely to be meaningful in vivo

ORAL-05-08

RHO-KINASE REGULATES ASTROCYTIC MORPHOLOGY AND GLUTAMATE TRANSPORTERS

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Homeostasis of extracellular L-glutamate (Glu) is maintained by excitatory amino acid transporters (EAATs) and altered function of EAATs occurs in various neurological conditions. Astrocytic EAATs perform the majority of Glu uptake, however little is known about the factors regulating EAAT activity and targeting. The activity of astrocytic EAATs may be modulated by interactions with proteins including GTRAPs, which link to the actin cytoskeleton and regulate EAAT localization. Thus, EAAT regulation and astrocytic morphology are both linked to the actin cytoskeleton. **Purpose:** These studies aimed to investigate changes in astrocytic morphology and EAAT activity induced by the Rho-kinase inhibitor, HA1077 (Fasudil), and interactions with signaling mechanisms used by lysophosphatidic acid (LPA). **Methods:** Primary cultures of astrocytes were established from forebrains of C57BL6 mice (at least n=3 independent cultures). Astrocytes were treated with LPA (0 - 100 μM) for 24 h followed by treatment with or without HA1077 (100 μM) for a further 24 h. EAAT function was determined by studies of transport, localization and cellular distribution. Cytochemistry for GFAP and phalloidin (to label F-actin) gave insights into astrocytic morphology. **Results:** HA1077 altered astrocytic morphology, with GFAP labeling revealing stellation of astrocytes, with re-organization of F-actin. Treatment with LPA had no effects on astrocytic morphology and failed to prevent stellation in response to HA1077. HA1077 significantly increased uptake of [^{3}H]D-aspartate within 24 hours by doubling V_{max} ($p<0.001$; K_d unchanged). Cell surface expression of EAAT1 and EAAT2 was significantly increased as determined by immunoblotting and immunocytochemistry. Treatment with LPA alone did not alter EAAT activity, but pre-treatment for 24 hours prevented the increase in uptake caused by HA1077. **Conclusion:** These findings demonstrate multiple mechanisms, including Rho/ROCK pathways, regulating astrocytic shape and EAAT function.

ORAL-06-01

MODELLING NEGATIVE SYMPTOMS OF SCHIZOPHRENIA IN RODENTS

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Purpose: The negative symptoms of schizophrenia are often subtle from a clinical perspective, but nevertheless, well described animal models of social behaviour are available and have been used in various animal models of schizophrenia. For example, social interactions are typically studied between pairs of male rats. The aim of this experiment was to validate an automated scoring system for the social interaction test in male and female rats. Methods: Social behaviour was measured between pairs (n>5 pairs per group) of unfamiliar 10-week old Sprague-Dawley rats (matched according to sex, age and body weight) in an open field under low light (30 lx). The rats were housed individually or in pairs for 10 days prior to a 10 minute social interaction test. The social interaction test was scored using both a manual event recorder (the Observer) and an automated video tracking system (Ethovision). Results: The distance between pairs of rats (or proximity) measured using Ethovision was correlated with mobility ($r=0.81$, $P<0.01$), and only weakly correlated with social investigation time as scored manually using the Observer ($r=0.52$, $p=0.1$). Factor analysis indicated that the automated and manually scored measures loaded onto separate factors, which essentially indicates that they measured different aspects of the test. Conclusions: Automated methods are needed for high throughput screening of behavioural phenotyping of rodents. By contrast, manual scoring, while time consuming, provides detailed information about underlying behaviour. The data suggest that automated and manually scored measures assess qualitatively different aspects of social behaviour and both approaches should be used for a thorough investigation of the social interaction test.

ORAL-06-02

THE WILLIAMS-BEUREN SYNDROME GENE GTF2IRD1 IS EXPRESSED IN GABAERGIC NEURONS AND UNDERPINS THEIR RESISTANCE TO THE GABAERGIC ANTAGONIST PENTYLENETETRAZOL AND ALTERED ANXIETY RELATED BEHAVIOUR

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Purpose: *GTF2IRD1* is one of 20 genes hemizygously deleted in Williams Beuren syndrome (WBS) and encodes a unique family of transcription factors the first of which was discovered in our laboratory. Methods: We have generated *Gtf2ird1* knock-out and *lacZ* knock-in reporter mice. Results: Knock-out mice display decreased levels of inhibition/anxiety, and altered sociability (n=10 for each genotype), phenotypes reminiscent of several behavioural features of WBS. Knock-out mice also display motor coordination and motor learning deficiencies and produce an abnormal vocalisation in certain stressful situations (n=10). Expression analysis shows high levels of *Gtf2ird1* expressed in the GABAergic neuronal population, suggesting that the behavioural phenotype observed in these mice and individuals with WBS could be mediated through abnormal GABAergic signalling. This was confirmed by the abnormally high resistance of knock-out mice to the GABAergic antagonist pentylenetetrazol (n=10). Conclusions: Taken together, these data indicate that *Gtf2ird1* is involved in the regulation of GABAergic neuron function and underpins the complex neurological pathways that are disrupted in WBS. We aim to elucidate the molecular function of *Gtf2ird1* by analysing gene and protein expression using gene-chip and 2-D protein gel technologies. These experiments will be carried out on GABAergic neurons enriched from *Gtf2ird1* knock-out X GAD-67-GFP mice (that express GFP exclusively in GABAergic neurons) by cell sorting.

ORAL-06-03

THE INFLUENCE OF IL-1BETA, IL-6 AND TNF-ALPHA POLYMORPHISMS ON CNS FUNCTION OF COGNITIVE PERFORMANCE, DEPRESSION AND ANTIDEPRESSANT PHARMACORESPONSE

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Purpose: Recent research suggests complex effects of cytokines in the central nervous system with relevance to cognitive function, depression and antidepressant pharmacoresponse. Methods: Results of three analyses by this group on cytokine effects of IL-1beta, IL-6 and TNF-alpha polymorphisms in the CNS in humans will be presented: (1) a genetic association study of variants of IL-1beta, IL-6, TNF-alpha and cognitive function in healthy elderly, (2) an association study of IL-1beta, IL-6, TNF-alpha polymorphisms and depression and (3) an association study of IL-1beta, IL-6, TNF-alpha polymorphisms and antidepressant pharmacoresponse in depression. Results: In a sample of N=369 healthy elderly people, the study provides first results on detrimental effects -1418 C-- $\beta$of the IL-1>T polymorphism on memory performance and -308 G-- αneuroprotective effects of the TNF->A polymorphism on processing speed in elderly individuals. In another sample of N=340 depressed patients and age and gender matched healthy controls we fond genotypes of the cytokines IL-1beta, IL-6 and TNF-alpha to be associated with a diagnosis of depression. The same genetic variants showed in a pharmacogenetic analyses poorer treatment outcome with antidepressants in depression. Conclusions: The studies demonstrate the relevance of the genetic variants of the cytokines IL-1beta, IL-6 and TNF-alpha in CNS processes with relevance to cognitive function, depression and pharmacogenetics in antidepressant treatment. Further studies are needed to explore the exact molecular mechanism for these effects.

ORAL-06-04

CHRONIC EXPOSURE TO MDMA ("ECSTASY"): MECHANISMS UNDERLYING TOLERANCE TO THE ANXIOGENIC EFFECTS

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Purpose The illicit drug 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") is an amphetamine derivative that continues to increase in popularity. Acute exposure to MDMA causes a rapid release of serotonin and other monoamines and repeated exposure results in persistent deficits in serotonin neurotransmission. MDMA is anxiogenic as measured in the elevated plus maze and in tests of open field exploration. Repeated exposure to MDMA results in tolerance to this effect. **Methods** In the present study we examined the contribution of serotonin (5HT) 2a and 2c receptors in tolerance to the anxiogenic effect of MDMA in 15 gps of rats (n=8-12/gp). **Results** MDMA (0.0 – 3.3 mg/kg, IP) increased the latency to emerge from a hide box to an open field. Tolerance to this effect was demonstrated for rats that received exposure to MDMA (4 X 10.0 mg/kg at 2 hourly intervals) two weeks prior to the test. The 5HT2a/2c agonist, mCPP, also increased the latency to emerge from the hide box but tolerance to this effect was not observed in MDMA pretreated rats. The acute effect of both MDMA and mCPP was attenuated in a dose-dependent manner by pretreatment with the 5HT2c antagonist, RS202221 but only the effect of MDMA was attenuated by pretreatment with the 5HT2a/2c antagonist, ritanserin. **Conclusion** These data suggest that 5HT2c mechanisms contribute to the acute effects of both MDMA and mCPP but that tolerance to the anxiogenic effects of MDMA likely involves neuroadaptations in the 5HT2a receptor.

ORAL-06-05

HUNGER AND SATIATION IN HUMANS ACTIVATE THE HYPOTHALAMUS AND NUCLEUS ACCUMBENS: A FUNCTIONAL IMAGING STUDY

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Purpose: Functional brain imaging studies (1,2,3) of hunger and satiation in humans have directly compared hunger with satiation, but have not be able to separately examine these states. Arterial spin labeling (ASL) functional MRI measures of regional cerebral blood flow (rCBF) enables state comparisons over periods of hours. Our objective was to compare hunger and satiation states with a baseline condition to examine whether hypothalamic and striatal nuclei were activated in either state separately. **Methods:** ASL scans acquired with 3 Tesla MRI from 15 subjects (42.4±13.1 years, 3 males) were collected during three states: after 18 hours of fasting (hunger), immediately after eating a standard meal (satiation), and 90 minutes after eating (baseline). **Results:** Hunger activations (hunger – baseline) occurred in the prefrontal, posterior parietal and orbitofrontal cortices, superior temporal gyrus, hypothalamus, peri-aquaductal grey and cerebellum. Satiety (satiation – baseline) activated the midbrain and pons, as well as the posterior cingulate, prefrontal cortex, precuneus, orbitofrontal cortex, hippocampus, nucleus accumbens, putamen, thalamus, hypothalamus and cerebellum. **Conclusion:** We have extended results from previous functional imaging studies of hunger and satiation using ASL. Consistent with animal studies hypothalamic activation was observed during hunger, whilst satiation activations in the striatum and midbrain implicate reward circuits in the processes of satiation. **References:** 1. Tataranni, P. A., et al., (1999) *Proc Natl Acad Sci U S A* **96**, 4569-74. 2. Gautier, J. F., et al., (2000) *Diabetes* **49**, 838-46. 3. Tataranni, P. A. & DelParigi, A. (2003) *Obes Rev* **4**, 229-38.

ORAL-06-06

COGNITION AND IMMUNE FUNCTION IN THE RAT MODEL OF DEVELOPMENTAL VITAMIN D (DVD) DEFICIENCY

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Introduction: Developmental vitamin D deficiency has a profound effect on brain development and behaviour. The aim of this study was to explore the effect of DVD-deficiency on cognition and, given vitamin D is also a potent immunomodulator, investigate possible changes in immune function in the adult offspring. **Methods:** Briefly, female rats were fed a vitamin D-deficient diet from six weeks prior to conception until birth, and then transferred to a diet containing vitamin D. Control rats were fed a normal diet throughout the experiment. A two-way shuttle box was used to examine active avoidance in ten-week old control and DVD-deficient rats. A comprehensive study of *in vitro* and *in vivo* measures of immune structure and function was also conducted. **Results:** DVD-deficient animals learnt the active-avoidance task at a slower rate than controls ($p \leq 0.005$, n=20). Interestingly, this difference was ameliorated when DVD-deficient animals were extensively handled for five days prior to testing (n=8), suggesting that novelty and attention to the task may be altered in DVD-deficient animals. The immune study (n≥8 per group) revealed that DVD-deficient rats had enlarged spleen ($p \leq 0.005$) and peripheral lymph tissue ($p \leq 0.05$). Moreover, *in vitro* stimulation of peripheral blood mononuclear cells from DVD-deficient animals elicited an increase in cytokine production (IFNγ, TNFα, IL-2 and IL-10) ($p \leq 0.05$). **Conclusion:** The fact that transient vitamin D deficiency during development induces both cognitive impairment and an activated immune system has immediate implications for the auto-immune and neuropsychiatric disorders currently linked to vitamin D status.

ORAL-06-07

THE IMPACT OF TNF ON COGNITIVE FUNCTION DURING AGING PROCESSES

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Purpose: Recent evidence suggests a role for tumor necrosis factor alpha (TNF) in the functioning of the central nervous system (CNS). The aim of this work was to examine the effect of a deficiency of TNF (TNF-/-) and its main receptors (TNF-R1-/- and TNF-R2-/-) on cognitive function throughout aging. **Methods:** A standardized survey on cognition-like behavior assessing learning and retention, spatial learning/memory, cognitive flexibility and learning effectiveness was used in B6.WT and B6.TNF gene targeted mice strains (B6.wild-type, B6.TNF-/-, B6.TNF-R1-/-, B6.TNF-R2-/- mice), across three age periods. Gene expression in the brain and peripheral cytokine assessment was conducted, along with histological measurements. **Results:** All studied mice strains demonstrated successful exploration and learning processes during the training phases of the tests, which made the specific cognition like tests valid in these mice strains. In the specific cognition-like tests, the B6.TNF-/- mice demonstrated significantly poorer learning and retention in the novel object test as compared to B6.WT, B6.TNF-R1-/- and B6.TNF-R2-/- mice. In addition, spatial learning and learning effectiveness were significantly poorer in B6.TNF-/- mice as compared to B6.WT mice. Moreover, B6.TNF-R1-/- or B6.TNF-R2-/- mice performed generally better than TNF-/- mice but poorer than B6.WT mice. While the absence of TNF was correlated with poor cognitive functioning, the deletion of both TNF-receptors was involved in partially reduced cognitive functioning. **Conclusion:** Low-levels of TNF under non-inflammatory immune conditions appear essential for normal cognitive function. TNF displays an interesting candidate gene for cognitive function. Translational research is required to investigate associations between genetic variants of TNF and cognitive function in healthy subjects and neuropsychiatric samples.

ORAL-06-08

FG7142, A BENZODIAZEPINE RECEPTOR PARTIAL INVERSE AGONIST, PREVENTS EXPRESSION OF OVER-EXPECTATION IN PAVLOVIAN FEAR CONDITIONING

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Purpose: Learning not to fear depends on the actions of predictive error. When the expected outcome of a conditioning trial exceeds the actual outcome, fear is lost. The phenomenon of over-expectation is useful for studying the role of predictive error in fear loss because fear is reduced despite continual pairings of the conditioned stimulus (CS) with shock. We examined whether the expression of over-expectation, like extinction, was mediated by the activation of GABAA receptors. **Methods:** Six experiments were carried out with rats to examine; overexpectation, the reversal of its expression by the benzodiazepine partial inverse agonist FG7142, the dose-dependency of this effect, and to examine any possible effect of FG7142 on the acquisition of conditioned fear, its expression, and the expression of the blocking effect. **Results:** We demonstrate that fear to a tone CS that has been paired with shock is attenuated if, after the initial tone-shock conditioning, rats are conditioned with further pairings of a compound of the tone and a flashing light (which had also received previous pairings with shock) with shock. This reduction in freezing is prevented by systemic injection of the benzodiazepine inverse agonist FG7142 prior to test. This effect of FG7142 was dose-dependent and not due to any general tendency of FG7142 to increase freezing. FG7142 does not effect expression of blocking or acquisition of conditioned fear. **Conclusion:** As in other examples of learning not to fear (e.g., extinction), overexpectation does not simply erase original learning. Fear over-expectation, like fear extinction, imposes a GABAergic mask on expression of conditioned fear. This mask is specific to reductions in fear that result from negative error-correction.

ORAL-07-01

ROLE OF MATRIX METALLOPROTEINASE-9 IN HUMAN AND MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease resulting from motor neuron degeneration. Study of the mutations in the Cu/Zn superoxide dismutase (SOD1) underlying the familial ALS may be utilised as a model to identify pathological mechanism and potential therapeutic target for the sporadic ALS. Transgenic mouse TgSOD1G93A is a widely used model, which closely replicates both clinical and pathological hallmarks of human ALS. Matrix metalloproteinase-9 (MMP-9) belongs to a family of proteolytic enzymes that are capable of degrading and remodeling the extracellular matrix. **Purpos:** MMP-9 is proposed to play a role in ALS, however, its role remains unclear due to conflicting reports in human and mouse studies. Further definition may lead to an understanding of its potential as a biomarker in ALS. **Methods:** To determine if MMP-9 levels are altered in the TgSOD1G93A mice with slow disease progression, we measured MMP-9 using zymography and western blot. Behavioral tests were employed to observe motor function. **Results:** We found that MMP-9 activity and expression levels were significantly decreased in end stage compared to pre-symptomatic TgSOD1G93A mice (n=10), whereas MMP-9 was not different between age-matched wild type mice. Weight loss was the first clinical changes in TgSOD1G93A followed by fine hind limb tremors. MMP-9 expression levels were also significantly decreased in ALS patients compared to the age-matched controls (n≥14). **Conclusion:** Our study indicates that MMP-9 could be a potential ALS biomarker. However, it remains to be determined if MMP-9 inhibition is beneficial in ALS.

ORAL-07-02

MULTIPLE ROLES OF SOD1 PROTEINS IN A CELLULAR MODEL OF MOTOR NEURON DISEASE (MND): NEUROPROTECTIVE AND CYTOTOXIC OUTCOMES

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Purpose: We previously showed that cells expressing inclusions of the disease-associated SOD1A4V were highly susceptible to apoptosis. However, dispersed SOD1, either wildtype (WT) or A4V, protected cells against death induced by staurosporine (STS). 1) Is the enzymic activity of SOD1 needed for neuroprotection in MND? 2) Is neuroprotection by dispersed SOD1 manifested against death inducers other than STS? **Methods:** NSC-34 cells were transfected with WT and mutant (A4V and G85R) SOD1-EGFP constructs, then challenged with STS, etoposide and H_2O_2. Apoptosis was monitored through caspase-3 activation and nuclear morphology, mitochondrial recruitment and activation of Bax, and redistribution of cytochrome *c*. **Results:** 1) SOD1G85R, an MND-linked mutant SOD1 without enzymatic activity, generates inclusions, and makes NSC-34 cells more susceptible to apoptotic signalling (n=12, p≤0.001). Cells bearing SOD1G85R inclusions have increased Bax recruitment and its activation, cytochrome *c* release and downstream caspase-3 activation (n=3). Dispersed form of SOD1G85R protects cells against apoptosis induced by STS compared to untransfected subpopulation (n=12, p≤0.001). 2) Both etoposide and H_2O_2 treatment caused to increased cell death in cells bearing mutant SOD1 inclusions with the involvement of mitochondrial apoptotic signalling (n=3, p≤0.001). Significantly, dispersed SOD1 (including WT and both mutant forms) clearly protects cells against apoptosis induced by both etoposide and H_2O_2 (n=3, p≤0.01). No protection was seen in cells expressing GFP control vector only. **Conclusions:** 1) Neither toxic nor protective effects of SOD1 in NSC-34 cells require its enzymic activity. 2) The neuroprotection afforded by dispersed SOD1 is effective against a range of apoptotic inducers and an oxidative stressor. Understanding complex aspects of SOD1 biology should provide clues for the molecular pathology of MND and its possible treatment.

ORAL-07-03

AXONAL TRANSPORT DEFECTS ARE ASSOCIATED WITH PARKINSONISM AND MEMORY IMPAIRMENT IN TAU TRANSGENIC MICE

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In Alzheimer's disease (AD) and frontotemporal dementia (FTD) the microtubule associated protein tau becomes hyperphosphorylated and eventually aggregates and deposits in neurons. However, clinical symptoms such as memory impairment and motor dysfunction precede protein deposition and neurodegeneration. Here, we present a novel transgenic mouse model that expresses mutant tau which shows an early-onset memory deficit preceding the histopathological characteristics of the FTD Pick's disease. In addition, these transgenic mice present Parkinsonism which is responsive to L-Dopa, and amyotrophy. All symptoms occur in the absence of overt neurodegeneration. Pathomechanistically, we found an impairment of axonal transport that is due to an aberrant interaction of phosphorylated tau with the axonal transport machinery. This also results in decreased transport of vesicles containing the dopamine synthesizing enzyme tyrosine hydroxylase (TH) in nigrostriatal dopaminergic neurons. Our results suggest that defective axonal transport is an early disease mechanism in AD and FTD. In particular impaired delivery of TH in the nigrostriatal system may underlie Parkinsonism in FTD.

ORAL-07-04

THE β-AMYLOID PROTEIN OF ALZHEIMER'S DISEASE INCREASES NEURONAL CRMP-2 PHOSPHORYLATION BY A RHO-GTP MECHANISM

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Purpose: Neuritic abnormalities are a major hallmark of Alzheimer's disease (AD) pathology. Aβ deposits cause changes in neuritic processes, contributing to neurodegenerative and cognitive deficits observed in individuals with AD. The aim of the present study was to examine the effects of Aβ on neurite outgrowth and to determine the activation of downstream signalling mechanisms which mediate these effects. **Methods:** We administered Aβ at 1 μM to retinoic acid-differentiated SH-SY5Y cells and investigated neurite outgrowth (n=3 experiments per administration). We also determined the state of aggregation of Aβ40 and Aβ42 administered "fresh" (non-aggregated) or "aged" (aggregated) by atomic force microscopy (AFM). We investigated whole brain lysates and coronal brain sections from APP(Swe) Tg2576 as well as wild type mice (n=3 at 6, 12 and 18 months-of-age for each genotype and age). The levels of Rho-GTPase activity were examined by Rho-GTP precipitation, immunoprecipitation, Western blot analysis and immunohistochemistry assays. **Results:** We show that Aβ decreases neurite outgrowth from SH-SY5Y human neuroblastoma cells. To explore molecular pathways by which Aβ alters neurite outgrowth, we examined the activation and localisation of RhoA and Rac1 which regulate the level and phosphorylation of the collapsin response mediator protein-2 (CRMP-2). Aβ increased the levels of the GTP-bound (active) form of RhoA in SH-SY5Y cells. This increase in GTP-RhoA correlated with an increase in an alternatively spliced form of CRMP-2 (CRMP-2A) and its threonine phosphorylated form. Both a constitutively active form of Rac1 (CA-Rac1) and the Rho kinase inhibitor, Y27632, decreased levels of the CRMP-2A variant and decreased threonine phosphorylation caused by Aβ stimulation. The amount of tubulin bound to CRMP-2 was decreased in the presence of Aβ but Y27632 increased the levels of tubulin bound to CRMP-2. Increased levels of both RhoA and CRMP-2 were found in neurons surrounding amyloid plaques in the cerebral cortex of the APP(Swe) Tg2576 mice. We found that there was an increase in threonine phosphorylation of CRMP-2 in Tg2576 mice and the increase correlated with a decrease in the ability of CRMP-2 to bind tubulin. **Conclusions:** The results suggest that Aβ-induced neurite outgrowth inhibition may be initiated through a mechanism in which Aβ causes an increase in Rho GTPase activity which, in turn, phosphorylates CRMP-2 to interfere with tubulin assembly in neurites.

ORAL-07-05

CRMP2 IS A NOVEL GSK3 AND CDK5 SUBSTRATE THAT IS HYPERPHOSPHORYLATED IN ALZHEIMER'S DISEASE

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Purpose: GSK3 and Cdk5 are brain-enriched kinases that are important for the health and normal functioning of neurons. Identification of their substrates is crucial for understanding their mode of action. However, only a few physiological substrates have so far been identified. **Methods:** We have used a new screening technique called KESTREL to identify novel substrates of GSK3 from rat brain. Physiological substrates were confirmed using *in vitro* kinase assays, cell culture and transgenic animal studies. **Results:** Collapsin Response Mediator Protein 2 (CRMP2) is a brain-enriched microtubule-binding protein that was found to be phosphorylated by GSK3 at Ser518/Thr514/Thr509, but only after prior phosphorylation by Cdk5 at Ser522. Phosphorylation at these sites regulated axon elongation in rat hippocampal neurons. Importantly, CRMP2 was hyperphosphorylated by GSK3 and Cdk5 in cortex tissue from human Alzheimer's disease (AD) patients (n=36), as well as two mouse models of AD that develop plaques. However, it was not hyperphosphorylated in other 'tauopathy' diseases (n=20) or in two mouse models that develop neurofibrillary tangles only. Hyperphosphorylation of CRMP2 in AD mouse models was not caused by significant changes in Cdk5 and/or GSK3 activity. However, the Ser522 site was found to be remarkably resistant to dephosphorylation by phosphatases. Therefore it is possible that a small increase in Cdk5 activity during the pathogenesis of AD might be sufficient to cause a large increase in CRMP2 phosphorylation. **Conclusion:** CRMP2 is a novel, physiological substrate of GSK3 and Cdk5 that regulates neurite outgrowth, and hyperphosphorylation of CRMP2 might be a specific marker for human Alzheimer's disease.

ORAL-07-06

27-HYDROXYCHOLESTEROL REGULATES AMYLOID-BETA PEPTIDE PRODUCTION VIA ABC TRANSPORTERS

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Cholesterol is an integral part of the neuronal plasma membrane and recent evidence has shown that it regulates amyloid precursor protein (APP) to form amyloid-beta peptides, which are a major constituent of cerebral amyloid plaques associated with Alzheimer's disease. Excess cholesterol is converted to 27-hydroxycholesterol by the brain and by other peripheral tissues and 27-hydroxycholesterol is the predominant hydroxycholesterol in human circulation. Unlike cholesterol, 27-hydroxycholesterol can readily cross the blood brain barrier and flux into the brain, however the role of 27-hydroxycholesterol in the brain and in particular in APP processing is not well known. Here we examined the impact of 27-hydroxycholesterol on amyloid-beta peptide generation. We show that 27-hydroxycholesterol at 10 μM concentration reduces amyloid-beta peptide generation by 55% (n=3; $p<0.05$) in CHO cells that stably express human APP695 and by 29% (n=3; $p<0.05$) in primary human neurons. The cellular APP levels were also measured and were not altered by 27-hydroxycholesterol. Since 27-hydroxycholesterol is known to be a ligand for the nuclear receptor LXR we tested the impact of 27-hydroxycholesterol on the expression of ABCA1 and ABCG1 in primary human neurons. We show that it significantly upregulates ABCA1 and ABCG1, at both RNA and protein levels (n=3). In conclusion, we demonstrate that 27-hydroxycholesterol regulates APP processing most likely via upregulation of ABCA1 and ABCG1 expression.

ORAL-07-07

IDENTIFICATION OF PHARMACOLOGICALLY DISTINCT γ-SECRETASE ACTIVITIES

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Gamma-secretase carries out the final step in the release of Alzheimer's disease Aβ amyloid peptides. It also cleaves the transmembrane domains of Notch, p75 and other families of type I receptors, limiting its potential as a therapeutic target. The activity is contained within molecular complexes comprised of presenilin (PS), which constitutes the catalytic subunit, and nicastrin, Aph1 and Pen-2. There exist two PS homologues and three Aph1 isoforms, therefore alternative combinations may provide six distinct complexes. **Purpose:** To investigate and compare γ-secretase activities in brain and peripheral tissues. **Methods:** Brain, liver, kidney, lung, spleen, muscle and thymus of adult mice (n=4) were homogenised and membrane fractions, solubilized with 1% CHAPSO, were analysed by western blotting for PS and nicastrin, and for γ-secretase activity on a recombinant APP substrate in the presence or absence of various inhibitors. **Results:** Enzymatic activity was correlated with PS expression and was highest in the brain, lung, and liver. Lung and liver activities were inhibited by pepstatin, MG132 and NSAID, but resistant to inhibitors of brain γ-secretase such as DAPT and L-685,458. Lung γ-secretase produced higher levels of Aβ42 than brain γ-secretase. Aph1 species may determine the difference between these γ-secretase activities since Aph1b is predominant in the lung whereas Aph1a is the major species expressed in the brain. **Conclusion:** Our results demonstrate the existence of pharmacologically distinct γ-secretase activities and show that complexes producing different ratios of Aβ42/Aβ40 can be targeted selectively.

ORAL-07-08

KUNITZ PROTEASE INHIBITOR-CONTAINING AMYLOID PRECURSOR PROTEIN ISOFORMS IMPAIRED MITOCHONDRIAL FUNCTION

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Purpose: Reduced glucose metabolism is an early feature in Alzheimer's disease (AD), preceding the onset of the clinical symptoms of dementia and the appearance of the neuropathological amyloid-beta (Aβ) deposits. Studies have shown that this metabolic deficit is associated with altered expression and activity of enzymes involved in mitochondrial energy production. However, the cause of this aberration is unclear. Aβ is proteolytically derived from amyloid precursor protein (APP), and there are three major splice forms of APP in the human brain; APP_{695}, APP_{751} and APP_{770}. In the brain, the predominant isoform transcript appears to be APP_{695}, coupled with moderately high levels of APP_{751} and APP_{770}. The APP_{751} and APP_{770} isoforms contain a Kunitz protease inhibitory (KPI) motif that APP_{695} lacks, and APP_{770} possesses an additional OX2 domain that is absent in both APP_{695} and APP_{751}. In AD-afflicted human brain tissues, APP_{751} and APP_{770} expression were elevated. **Methods:** In this study, we used a novel immortalized neuronal cell line derived from the cortex of postnatal day 1 APP knockout (APP-KO) mice. This APP-KO cell line was subsequently transfected with human APP_{695}, APP_{751} or APP_{770} cDNA. **Results:** We detected increased production of Aβ42 and impairment of mitochondrial function in cell lines expressing the KPI-containing APP isoforms. In addition, these KPI-APP isoforms have greater propensity to co-localize with various mitochondrial-specific protein markers. **Conclusion:** This increased subcellular localization by the KPI-containing APP isoforms may contribute to the observed aberrant mitochondrial function.

ORAL-08-01

ROLE OF FAS LIGAND AND TNF IN CELL APOPTOSIS FOLLOWING FOCAL TRAUMATIC BRAIN INJURY

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BACKGROUND: Neuronal apoptosis contributes to ongoing damage following traumatic brain injury (TBI). This study aimed to determine whether inhibition of the extrinsic pathway of apoptosis via genetic mutation of the Fas receptor or therapeutic neutralisation of FasL and TNF, decreases cell death, lesion volume, and improves neurological outcome in a mouse model of focal TBI. **METHODS:** Mice expressing non-functional Fas receptor (*lpr*) were killed at 1, 4 and 7d post-TBI and compared to wild type controls (WT). Lesion volume was determined on HE-stained cryosections. Apoptotic cells were counted following TUNEL and caspase-3 immunohistochemistry throughout the cortical contusion. Neurological outcome was assessed at 1h and every 24h post-TBI. Similar analysis was performed on mice injected with antibodies against TNF and FasL (Ab: 2 mg/kg I.V., 30min post-TBI). Controls received pre-immune IgG or saline. **RESULTS:** Lesion volume and number of TUNEL+ cells of *lpr* mice were significantly reduced compared to WT mice (p=0.014 and p=0.005, respectively, n=5/group). Numbers of TUNEL+ cells correlated with caspase-3+ cells at 24h (Spearman's rho=0.410, p=0.005), suggesting that TUNEL+ cells are apoptotic. *Lpr* mice showed improved neurological outcome from 2d to 7d post-TBI (p<0.05, n=10/group). No differences in neurological outcome, lesion volume or number of TUNEL+ cells were observed in mice treated with anti-FasL/TNF antibodies (n=5/group). **CONCLUSION:** Fas mutant mice show improved neurological outcome following TBI, possibly due to reduced lesion volume and cell apoptosis. Conversely, neutralisation of FasL/TNF did not result in significant improvements, suggesting that therapeutic inhibition of Fas receptor may be the optimal neuroprotective strategy following TBI.

ORAL-08-02

MORPHOMETRIC MEASURES OF STRUCTURAL BRAIN CHANGES IN A RAT MODEL OF CLOSED-HEAD TRAUMATIC BRAIN INJURY

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Purpose: Traumatic brain injury (TBI) is a major community health problem with high incidence of long-term morbidity. The fluid percussion injury (FPI) model in rats is an accepted model of closed-head trauma to study the neurobiology of brain injury. The study aimed were to examine subacute (one month post-trauma) effects on the volume of key brain regions following FPI. **Methods**: 8 to 12 week old male Wistar rats were anaesthetized and a 5mm left-sided craniotomy drilled to access the dura. A 3.5 atmosphere pulse was delivered via a FPI device and animals (n=7) were allowed to recover. Sham rats (n=7) underwent the same procedures without delivery of the pulse. One week prior to and 6-7 weeks after FPI (37±4 days), animals had T2-weighted MRI scans (4.7 Tesla magnet, Bruker Avance). Volumes of selected brain regions were measured blindly using Analyze™, including the ventricles, cortex, the hippocampi, amygdala, and thalami. **Results**: Injured (vs. sham injured) animals showed a significantly greater decrease in volume of ipsilateral cortex (-32.6 ± 3.7 vs. -16.1 ± 5.5 mm3; p=0.03) and an increase in the ipsilateral lateral ventricle (+5.1± 1.9 mm3 vs. +0.3 ± 0.4 mm3, p=0.04) and 3rd ventricle (FPI +3.0 ±0.9 vs Sham +0.7 ± 0.5 mm3; p=0.05) volumes. No differences were identified in other measured brain regions. **Conclusion**: FPI results in morphological changes in brain structures particularly affecting the ipsilateral cortex and periventricular brain regions. These structural changes may play a role in long-term adverse psychoneurological outcomes of head trauma.

ORAL-08-03

ANIMAL MODELS OF STROKE

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PURPOSE: Cerebral ischemia has been induced in animals for hundreds of years in order to gain an understanding of normal and pathological vascular physiology. More recently, the principle purpose of such models has shifted away from investigation of physiology to the evaluation of drug efficacy. We sought to review factors influencing stroke size and responses to interventions, with a view to better understanding the data. **METHOD:** Stroke studies were identified using PubMed, cross-referencing and hand-searching. Controlled experiments using volumetric endpoints were retained for further analysis. Infarct data was normalised across each species, and meta-analysis was undertaken to examine relationships between stroke size, level of neuroprotection, and experimental characteristics. **RESULTS:** Findings from almost 3000 experimental studies were included in the analysis. Model parameters were found to have a major impact on stroke size. For instance, infarct volume was seen to increase with duration of ischemia up to 2 hours, but was smaller where the occlusion was permanent, and where more than one vessel was occluded. Temporal factors also affected the putative size of infarct, with measurements made out to two weeks having smaller strokes. Somewhat surprisingly, levels of neuroprotection appeared independent of time measured, challenging the notion that long term studies are essential for valid results. Brain damage was also exacerbated by nitrous oxide and ketamine, feeding the animal prior to surgery and when ethanol and PEG were used as control infusions. Age was unrelated to the degree of brain damage, but was associated with a poorer outcome. **CONCLUSION:** In spite of the extensive number of stroke studies, we are only just coming to grips with how the methodology itself influences the outcome in these models. More detailed analyses of experimental stroke models will facilitate our understanding of the validity and reliability of stroke models for preclinical evaluation.

ORAL-08-04

THE EFFECT OF POST-TRAUMATIC HYPOXIA ON NEUROINFLAMMATION, TRYPTOPHAN OXIDATION AND MELATONIN PRODUCTION AFTER TRAUMATIC BRAIN INJURY

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A post-traumatic hypoxic event is observed in up to 40% of severe traumatic brain injury (TBI) patients and is known to exacerbate brain inflammation, oxidative stress and tissue damage. The relationship of neuroinflammation, and the oxidative stress via the activation of the tryptophan pathway and melatonin production was investigated in various neurological diseases. However, this interaction was never explored after TBI combined with post-traumatic hypoxia. **METHODS:** Ventricular CSF was collected daily for 5 days from severe TBI patients, with or without systemic hypoxia (n=5/group), admitted to the Alfred Hospital. Samples were analysed for cytokines (IFN-γ, IL-4, IL-6, IL-10 and GM-CSF) and melatonin by ELISA, while tryptophan, kynurenine, kynurenic acid, and 3-hydroxyanthranilic acid (3-OHAA) were detected by HPLC. **RESULTS:** On the day of trauma, normoxic patients showed higher IFN-γ levels (52.7±29.5 vs. 15.0±5.5pg/ml). In contrast, IL-4 and GM-CSF were higher in the hypoxic group (0.77±0.13 vs. 0.31±0.02; 28.9±13.1 vs. 5.6±2.3, respectively). Post-traumatic hypoxia increased 3-OHAA, the precursor of glutamate receptor agonist quinolinic acid, in CSF at day1 (195.9±163.9nM) and decreased in all other days (1-50nM). Melatonin was higher in non-hypoxic patients with a peak at day3 (1.4±0.3 vs. 16.4±8.7nM). **CONCLUSIONS:** This study suggests that post-traumatic hypoxia exacerbates neuroinflammation and alters tryptophan metabolism and melatonin production after TBI. Higher levels of melatonin may reduce cytokine and 3-OHAA concentrations, due to its anti-oxidative and antiinflammatory properties. Further studies will investigate the beneficial effects of melatonin administration in a rodent model of TBI subjected to post-traumatic hypoxia.

ORAL-08-05

INCREASED ANXIETY-LIKE BEHAVIOUR IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

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Purpose: Mood disturbances, including elevated anxiety and depression, are common and disabling long-term sequelae of traumatic brain injury in humans. Previously, these behaviours have been considered as psychological and social consequences of the trauma, but neurobiological alterations induced by the trauma have also been implicated. This longitudinal study, using a rat model of TBI (fluid percussion injury), was designed to assess the anxiety- and depression-like behaviours in TBI. **Methods:** Male non-epileptic Wistar rats (n=21) received a ~3.5 atm fluid pressure pulse directed to the right sensorimotor cortex, or sham injury (n=17). At 1, 3 and 6 months following injury, rats underwent three assessments of anxiety and depression-like behaviours: the elevated plus maze, the open field test, and the sucrose-preference test. **Results:** Injured animals displayed increased anxiety-like behaviour as evidenced by reduced % time spent in open arms in the plus maze (p=0.001, two-way ANOVA), and reduced time spent in the centre area of the Open Field (p=0.013, two-way ANOVA) at all time points studied. No differences were observed in the sucrose-preference test of depression. **Conclusion:** This report provides the first evidence of persistent anxiety-like disturbances in a model of traumatic brain injury in rats. This finding indicates that the common occurrence of these symptoms in humans who have suffered a TBI is likely to have a neurobiological basis. Studies in this model could provide insights into the mechanisms underlying affective disorders in brain injured patients.

ORAL-08-06

MONOCYTE CHEMOATTRACTANT PROTEIN (MCP-1) IN INFLAMMATION FOLLOWING TRAUMATIC BRAIN INJURY (TBI)

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The chemokine MCP-1 is implicated in macrophage/microglia recruitment following TBI, a process thought to exacerbate secondary tissue damage. This study aims to elucidate MCP-1's role after focal TBI using MCP-1 knockout (KO) mice. METHODS: Using a weight-drop device, TBI was induced in MCP-1 KO and wildtype (wt) C57Bl/6 mice. Neurological dysfunction was assessed at 1h then daily, by the Neurological Severity Score (NSS) and a ledged beam test. Protein levels of 18 inflammatory cytokines were measured by ELISA in injured cortex homogenates at 2,4,12 and 24h post-TBI. Brain sections of wt and KO mice collected at 2,4,12 and 24h, 4,7 and 14d, were examined for lesion volume (H&E) and macrophages/ microglia (F4/80). RESULTS: MCP-1 KO mice (n=11) showed improved neurological recovery compared to wt (n=12), with lower NSS scores at 1-2 weeks post-injury, and fewer beam test errors. Seven cytokines were elevated following TBI, reaching maximal expression at 4h in wt mice, however a delayed, amplified peak was observed at 12h in MCP-1 KO mice. Lesion volumes were similar at all timepoints regardless of strain. Macrophage infiltration peaked at 4d, with fewer cells counted at 4d (15%) and 7d (30%) in KO mice (n=3-4). By 2 weeks post-TBI the spread of macrophages was significantly reduced in KO mice (50%,n=6). CONCLUSION: MCP-1 KO mice exhibited improved functional outcome at 1 week post-injury, possibly consequential to reduced macrophage accumulation at this time. Interestingly, this was associated with the altered acute release of inflammatory mediators in MCP-1 KO mice. Continuing analysis up to 4 weeks post-TBI may further define potential neuroprotective effects of MCP-1 deficiency.

ORAL-08-07

SYMPATHETIC NEUROVASCULAR TRANSMISSION IS NOT POTENTIATED ABOVE A T4 SPINAL CORD TRANSECTION IN RATS

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Purpose: Spinal cord injury increases neurally evoked contractions of arteries supplied by preganglionic sympathetic neurones located below the level of the injury [1]. While it is likely that this change is produced by a decrease in ongoing sympathetic nerve activity following transection of bulbospinal pathways, it remains possible that humoral factors contribute to alterations in neurovascular function. To address this question, the effects of T4 spinal cord transection on transmission to the rat median artery have been investigated. This artery is supplied by preganglionic neurones located above the lesion. **Methods**: The lesion was performed under anaesthesia with ketamine/xylazine (60/10 mg/kg, i.p.). Two weeks postoperatively the rats were anaesthetised (100 mg/kg pentobarbitone, i.p.) and killed by exsanguination. Arteries were dissected and mounted isometrically in myographs. Comparisons were made with arteries from unoperated rats. **Results**: In median arteries (n=8), no change in responses to perivascular nerve stimulation was observed. There was also no change in reactivity to the α-adrenoceptor agonists phenylephrine or clonidine. However, blockade of neurally evoked contractions by application of the α-adrenoceptor antagonists, prazosin (10nM) and idazoxan (0.1μM), was reduced ($P<0.05$). **Conclusion**: Unlike in tail arteries, where neurovascular transmission was markedly potentiated 2 weeks postoperatively [1], spinal cord transection did not increase neurovascular transmission in median arteries. It did increase the component of contraction that was resistant to α-adrenoceptor blockade, as observed in tail artery [1], which may reflect the effects of some circulating factor. The findings support our conclusion that a reduction in sympathetic nerve activity is the cause of augmented neurovascular responses below a spinal lesion. 1. Yeoh et al. (2004). J. Physiol. 556, 545-555.

ORAL-08-08

NOVEL ACTION OF THE BIOLIPID SPHINGOSINE 1-PHOSPHATE. MODULATION OF NOCICEPTION VIA S1P1 RECEPTORS

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Purpose: With progression of many diseases the accompanying pain gets increasingly difficult to treat and novel analgesic principles for an improved therapy are urgently needed. The project aimed at identifying the role of the biolipid sphingosine 1-phosphate (S1P) and its receptors in the transduction of pain. Methods: We used quantitative RT-PCR, In-Situ-Hybridization and immunohistochemistry to detect sources and targets of S1P in nociceptive neurons in murine dorsal root ganglia and spinal cord. The involvement of S1P in pain transduction was investigated by measurements of the sensitivity of mice to heat and capsaicin using the Hargreaves test, a nerve-skin preparation and cultured DRG neurons. Results: The S1P synthesizing enzymes, sphingosine kinase 1 and 2 (SK1, SK2) could be detected in DRG (n = 4) and spinal cord (n = 5) at the transcriptional and translational level (both n = 5) with highest mRNA expression levels for S1P1 and S1P3 receptors in DRG and S1P1 and S1P5 receptors in spinal cord. S1P1 and S1P3 receptor immunoreactivity was present in peripherin- and TRPV1-immunoreactive DRG neurons and processes, whereas S1P2 protein was restricted to Nf200 positive myelinated nerves. S1P induced in vivo hyperalgesia (n = 10) that was massively reduced in TRPV1-KO mice (n = 10). Furthermore S1P dose dependently sensitized heat-activated and capsaicin-dependent inward currents in cultured DRG neuron preparations (n = 6). This sensitization could be mimicked by application of the S1P1 receptor agonist SEW2871 (n = 3). Conclusion: The results demonstrate that the sphingolipid S1P sensitizes nociceptors via S1P1 receptor stimulation and activation of TRPV1.

ORAL-09-01

SPECTRAL SELECTIVITY OF LUMINANCE VISION IN REEF FISH

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Purpose. Luminance vision has high spatial resolution and is used for form vision and texture discrimination. In humans, birds and bees luminance channel is spectrally selective – it depends on the signals of the long-wavelength sensitive photoreceptors (bees) or on the sum of long- and middle- wavelength sensitive cones (humans), but not on the signal of the short-wavelength sensitive (blue) photoreceptors. The reasons of such selectivity are not fully understood. The aim of this study is to reveal the inputs of cone signals to high resolution luminance vision in reef fish. **Methods.** 16 freshly caught damselfish, *Pomacentrus amboinensis,* were trained to discriminate stimuli differing either in their colour or in their fine patterns (stripes vs. cheques). Three colours ('bright green', 'dark green' and 'blue') were used to create two sets of colour and two sets of pattern stimuli. The 'bright green' and 'dark green' were similar in their chromatic properties for fish, but differed in their lightness; the 'dark green' differed from 'blue' in the signal for the blue cone, but yielded similar signals in the long-wavelength (green) cones. **Results.** Fish easily learned to discriminate 'bright green' from 'dark green' and 'dark green' from 'blue' stimuli. Fish also could discriminate the fine patterns created from 'dark green' and 'bright green'. However, fish failed to discriminate fine patterns created from 'blue' and 'dark green' colours, i.e. the colours that provided contrast for the blue-sensitive photoreceptor, but not for the long-wavelength sensitive one. **Conclusion.** High resolution luminance vision in damselfish, *Pomacentrus amboinensis*, does not have input from the blue-sensitive cone, which may indicate that the spectral selectivity of luminance channel is a general feature of visual processing in both aquatic and terrestrial animals.

ORAL-09-02

SUPPRESSIVE SURROUNDS AND CONTRAST ADAPTATION IN MARMOSET LATERAL GENICULATE NUCLEUS NEURONS

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Purpose. The sensitivity of neurons in the dorsal Lateral Geniculate Nucleus (LGN) depends on the spatial and temporal context within which stimuli are presented. Here we show that the responsiveness of both the classical (CRF) and extra-classical (ECRF) receptive fields depends on their prior history of stimulation. **Methods.** We recorded the extracellular response of single-units in the LGN of two anaesthetised and paralysed adult marmosets. The contrast responses of the CRF and the suppressive ECRF were measured with concentric patches of drifting grating, the contrasts of which varied independently. These measurements were made before and during prolonged exposure (adaptation) to high contrast drifting gratings confined, in separate experimental blocks, to either the CRF or the ECRF. **Results.** Magnocellular (M)-cells: adaptors confined to the CRF reduced the contrast sensitivity (spikes/s/contrast) of the CRF (on average by 74%, two-tailed t-test $p < 0.05$; $n = 12$), but had no effect on that of the ECRF ($p = 0.1$). As expected, CRF adaptors drifting at relatively high temporal frequencies (16 Hz) were more effective than those drifting at low temporal frequencies (1 Hz). Adaptors confined to the ECRF reduced the contrast sensitivity of the ECRF (by 58%, $p < 0.05$; $n = 7$), without substantial effect on that of the CRF ($p = 0.9$). Parvocellular (P)-cells: the contrast sensitivities of the CRF and ECRF of P-cells where unaffected by any type of adaptor (in all cases $p > 0.4$). **Conclusion.** The sensitivity of M-cells, but not that of P-cells, depends on the prior history of stimulation over both the CRF and the ECRF.

ORAL-09-03

ORIENTATION TUNED VISUAL NEURONS IN THE HOVERFLY

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Purpose: Despite having tiny brains and relatively low resolution compound eyes many fly species frequently engage in precisely controlled aerobatic pursuits of conspecifics. Recent investigations into high-order processing in the fly visual system have revealed a class of neurons, coined Small Target Motion Detectors (STMDs), capable of responding robustly to target motion against the motion of background clutter. Surprisingly, STMDs show several physiological characteristics that are similar to motion vision neurons of the vertebrate cortex. Orientation tuned 'columns' are one such characteristic of the cat cortex. We wanted to investigate to what extent target neurons of the hoverfly brain are orientation tuned. **Methods:** We recorded intracellularly from morphologically identified STMD neurons (n=100) in the lobula complex of the hoverfly *Eristalis tenax* (males, n=70). We identified neurons as STMDs based on a selective response to small moving targets, but a complete rejection of widefield stimuli on a visual monitor (100 x 75 degrees). To test for orientation selectivity, we drifted small oriented bars across the monitor in different directions while measuring the intracellular response, and filled the neurons with Lucifer Yellow after completion of the physiological recording. **Results:** Retinotopically organized STMDs display exquisite sensitivity to small targets, and include both direction selective and non-direction selective classes covering a large area of visual space. We identified many STMDs that were direction selective, but only a few classes that showed strong orientation tuning. These neurons, however, show a strong preference to optimally oriented targets moving in either direction. **Conclusions:** It has previously been found that higher order visual processing in the insect shows stunning similarities to processing in the cat cortex. Here we show that these similarities extend to orientation tuning of identified neurons.

ORAL-09-04

ATTENTION MECHANISMS IN HIGHER ORDER VISUAL NEURONS IN THE DRAGONFLY

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Purpose: *Hemicordulia tau* is a predatory dragonfly that hunts in flight. Dragonflies posses high acuity compound eyes permitting accurate target detection with a 97% prey capture rate. We recently classified a higher order visual neuron, Centrifugal Small Target Motion Detector 1 (CSTMD1) that has an exquisite selectivity for small targets.We hypothesized that the extreme selectivity requires powerful spatial inhibitory mechanisms. To investigate whether such inhibition derive form inhibitory input from target selective elements, we investigated the response of CSTMD1 as a function of location of a second (distractor) target. In addition, we hypothesized that transfer of inhibition between visual hemispheres provides a neural basis for attention in situations when multiple targets are in view, commonly observed in nature. **Methods:** We recorded intracellularly, from neurons (n=5) in the 3rd optic ganglion (lobula) whilst displaying two small moving targets on a high-speed CRT display. We have also used intracellular labelling to investigate the likelihood that long-range inhibitory effects are mediated by the heterolateral anatomical interactions. In addition, we hypothesized that transfer of inhibition between visual hemispheres provides a neural basis for attention in situations when multiple targets are in view, commonly observed in nature. **Results and Conclusions:** The results show that responses to a target drifted through the receptive field are inhibited by a second target, the strongest inhibition is seen when the targets are close whilst a weaker suppression of response is seen when the targets are at a large separation, suggesting binocular interactions.

ORAL-09-05

MULTIPLE STAGES OF ORIENTATION-SPECIFIC CONTEXTUAL MODULATION IN HUMAN VISUAL CORTEX

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Purpose: Contextual modulation is a fundamental characteristic of human vision. Here, we used fMRI to investigate the basis of orientation-specific centre-surround effects in visual cortex. **Methods:** Subjects' (n = 4) brains were scanned at 3T while performing a dimming task at fixation. The circular stimulus aperture was divided into a "test" annulus and the remaining "inducing" region. Five types of stimulus blocks were presented in a balanced design: test only; inducer only; parallel test and inducer; orthogonal test and inducer; blank (fixation only). Each 16-second block contained 16 different orientations of stimuli, presented in a pseudo random order, such that parallel and orthogonal blocks differed only in the relative orientation of test and inducer and not in the distribution of absolute orientations. **Results:** GLM and %-signal change analysis indicated significantly lower BOLD activation in response to gratings with parallel versus orthogonal surrounds across the early retinotopic areas of visual cortex. **Conclusions:** Parallel surrounds generated the greater suppression of the BOLD response, consistent with existing psychophysical data on contrast-contrast and the tilt illusion. This effect increased in magnitude from V1 through V2 and V3 to V3A and V4, indicating that orientation-specific interactions occur at multiple stages along the cortical processing hierarchy.

ORAL-09-06

HIERARCHY OF DIRECTIONAL INTERACTIONS IN VISUAL MOTION PROCESSING

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Purpose: Perceived direction of motion is subject to simultaneous (direction repulsion) and successive (direction adaptation) contextual effects. In both cases, perceived direction is typically repelled from the direction of the inducing stimulus. Here, we used psychophysical methods to investigate the relationship between these effects. **Methods:** Experiment 1 - subjects (n = 6) adapted to a transparent motion stimulus in which two sets of dots of different speeds (2º/s and 7º/s) moved in different directions (± 25º from vertical). The magnitude of the direction aftereffect (DAE) to this transparent adapting stimulus was measured in four conditions: slow test dots only; slow test dots in presence of fast dots; fast test dots only; fast test dots in presence of slow dots. Experiment 2 – using unidirectional test stimuli, DAEs were measured to transparent adapting stimuli tailored to individual subjects (n = 4) to distinguish whether adaptation was influenced by direction repulsion within the adapting stimulus. **Results:** Experiment 1 – the measured DAE was greater for test stimuli containing dots of both speeds. Quantitatively, DAE magnitude for transparent test stimuli was consistent with direction repulsion occurring subsequent to direction adaptation within the test stimulus. Experiment 2 – the magnitude of DAEs for transparent adapting stimuli were predicted from the physical rather than the perceived adapting directions, consistent with direction adaptation occurring prior to direction repulsion within the adapting stimulus. **Conclusions:** Together, the results of these experiments demonstrate that direction adaptation is mediated earlier than direction repulsion in the visual motion processing hierarchy.

ORAL-09-07

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

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Purpose: It is hypothesised that lesions of the visual cortex suffered early in life are far less severe than those suffered in adulthood. We are particularly interested in understanding the pathways underpinning the residual visual capability observed in certain paradigms following a lesion of the primary visual cortex (V1) during the early postnatal period. **Methods:** In the marmoset monkey (*Callithrix jacchus*) we unilaterally ablated ~10-15º of the primary visual cortex (V1; operculum and part of the calcarine) at postnatal day 14 (*n*=2). Two years later, connections between the middle temporal area (area MT) of the visual cortex, lateral geniculate nucleus (LGN) and medial portion of the inferior pulvinar (PIm) were visualized through intraocular injections of fluorescent-labelled cholera toxin subunit B and injections of Fast Blue into area MT. In addition, the architecture of these areas were examined with SMI-32 and calbindin immunolabelling, and Nissl substance and Gallyas staining. **Results:** Compared with control data, the cortical surface area of MT ipsilateral to the ablation had increased, as well as the number of retrograde-labelled cell bodies in PIm - the primary nucleus of pulvinar that receives direct retinal input and projects to area MT. In addition, morphological changes were seen in a population of spared calbindin immunoreactive cells present in the LGN projection zone corresponding to the ablated portion of V1. **Conclusion:** These data suggest the residual visual capability observed following V1 lesion during infancy could be, in part, attributed to the increase in the direct retino-thalamic inputs to area MT.

ORAL-09-08

PUPIL DILATION PRECEDES AND PREDICTS PERCEPTUAL RIVALRY SWITCHES: IMPLICATING A ROLE FOR NORADRENERGIC MECHANISMS IN PERCEPTUAL SELECTION

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Purpose: During sustained presentation of an ambiguous stimulus, an individual's perceptual experience will generally switch between the different possible alternatives rather than stay fixed on one interpretation. In behavior, evidence suggests that the locus coeruleus (LC) – the brainstem nucleus responsible for synthesis and release of noradrenaline (NA) throughout the cortex – may play a crucial role in both the selection of a behavioral response and ensuring continual reassessment of the available alternatives. We hypothesized that the LC-NA system may play a similar role in selecting between different perceptual alternatives. Because pupil dilation is known to reflect LC activation levels (pupil dilator muscles are controlled exclusively by NA), this study used pupil diameter as a surrogate measure of LC-NA activity. **Methods:** We recorded fluctuations in pupil diameter using the Eyelink 1000, while participants (n=6) observed 4 different rivalry stimuli (Necker cube, plaid motion segregation, structure from motion and auditory stream segregation). **Results:** In accordance with predictions, pupil dilation was seen prior to perceptual transition for all four ambiguous visual and auditory stimuli tested. Not only did pupil diameter increase reliably across subjects and stimulus types, but the relative amount of dilation 600ms prior to the perceptual switch was a significant predictor of the subsequent duration of perceptual stability. This pupil dilation could not be explained by blink/saccade artifacts, motor response or stimulus driven changes in retinal input. **Conclusion:** These results demonstrate strong links between pupil dilation and perceptual switches induced by ambiguous stimuli. We interpret these findings as evidence that NA may be involved consolidating the transition to a new perceptual state. Specifically, we are claiming that perceptual rivalry may reflect a form of perceptual decision making and that the LC-NA complex may play exactly the same role in perception as it is understood to be playing in behavioural selection.

ORAL-10-01

CHOLINE TRANSPORTER LABELS CHOLINERGIC NERVES IN THE HUMAN ENTERIC NERVOUS SYSTEM

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Acetylcholine is a major neurotransmitter in the intestine. Cholinergic neurons are labelled using antibodies against choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT). Choline uptake is essential in the synthesis of acetylcholine and occurs via the high affinity choline transporter (CHT). CHT-immunoreactivity is present in neurons containing VAChT and ChAT in the human central nervous system and rat enteric nervous system (Harrington 2007, Cell Tiss Res 327:421). This study examined if CHT labels cholinergic nerve fibres in human intestine. Human ileum and colon biopsies (n=4) were fixed, frozen, sectioned and processed for fluorescence immunohistochemistry using antibodies against CHT, synaptophysin, cChAT, VAChT, nitric oxide synthase (NOS), substance P (SP), vasoactive intestinal peptide (VIP) and c-Kit. CHT-immunoreactivity was present in many nerve fibres in circular and longitudinal muscle, myenteric and submucosal ganglia, submucosa and mucosa. CHT completely colocalised with VAChT and cChAT. There was some colocalisation with SP, but little with VIP or NOS in nerve fibres in myenteric ganglia or circular muscle. CHT was present in nerve fibres in the mucosa in nearly all VIP and some SP nerves. Interstitial cells of Cajal were closely intertwined with nerves containing CHT-immunoreactivity in the circular muscle. This study shows CHT labelled cholinergic enteric nerves in human ileum and colon and suggests it is a useful additional cholinergic marker. Importantly, CHT labels nerve fibers in the mucosa that are not labeled by VAChT providing the first visualization of these known cholinergic nerves.

ORAL-10-02

HOW CAN ACETYLCHOLINE HAVE INHIBITORY ROLES? LOCATION OF MUSCARINIC RECEPTORS IN HUMAN INTESTINE

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Purpose: Acetylcholine (ACh) is well recognised for causing muscle to contract, but physiological studies suggest ACh has roles in inhibitory pathways and muscle relaxation in the intestine. The aim of this study was to visualise the cellular location of the 3 major muscarinic receptors (M1r-M3r) in human bowel to identify likely roles in inhibitory pathways. **Methods:** Human paediatric colon biopsies (n=3) were fixed, sections and wholemounts were incubated with antisera against M1r, M2r or M3r, followed by fluorescent secondary antibodies and viewed using confocal microscopy. Tissue was double labelled with synaptophysin to identify nerve fibres, c-kit for interstitial cells of Cajal (ICC) and VAChT for cholinergic, Substance P for tachykinergic and nitric oxide synthase (NOS) for inhibitory neurons. **Results:** M2r & M3r were abundant on muscle cells. M1r/M3r activate PLC while M2 inhibits adenylate cyclase. In this location, M3r can induce contraction while M2r induce relaxation. M1r and M3r were on neurons in myenteric ganglia and nerve fibres in CM, where they can modify release of transmitters. M2r were located presynaptically on nerve fibres. M1r were on cholinergic nerve fibers where they could control release of ACh and on nitrergic nerve fibers where they could control release of NO, the major inhibitory transmitter. M2r and M3r were on nerve fibers close to intramuscular ICC. M1r were also submucosal neurons. M1r were on endothelial cells in submucosal arterioles. M3r-IR was present on mucosal epithelial cells. **Conclusion:** This study shows that muscarinic receptors are in key sites to directly control muscle relaxation and contraction, to mediate both excitatory and inhibitory nerve transmission, and affect vasodilation and secretion.

ORAL-10-03

ENTERIC 5-HT CIRCUITS DIFFER BETWEEN INBRED MOUSE STRAINS

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Purpose: *tph2* encodes tryptophan hydroxylase 2, the serotonin (5-HT) synthesizing enzyme expressed by central and enteric neurons. Some inbred mouse strains are homozygous for a single-nucleotide polymorphism (C1473G) in *tph2* that affects 5-HT synthesis. C/C individuals express a higher activity enzyme in brain than G/G individuals, correlated with behavioural differences [1]. Hence, enteric 5-HT circuits may differ between strains. ***Methods:*** Segments of jejunum (3-4 cm) were removed from 2 Balb/c (G/G) and 5 C57Bl/6 (C/C) mice, incubated in 5-HT (500 nM, 1-2 h, 37°C) and fixed in Zamboni's fixative (2-24 h, 4°C). Myenteric plexus was processed for immunohistochemical labelling of 5-HT plus nitric oxide synthase (NOS) or calretinin. Z-series of stained ganglia were collected using a confocal microscope. Appositions between 5-HT$^+$ terminals and calretinin$^+$ or NOS$^+$ somata were quantified as previously described [2]. ***Results:*** Very few 5-HT$^+$ terminals apposed NOS$^+$ somata in either strain (mean ± sem; C57Bl/6: 3 ± 0.4, n=63 cells; Balb/c: 2 ± 0.4, n=49; $p<0.005$). Calretinin$^+$ somata with either Dogiel type I (C57Bl/6: 7 ± 0.8, n=29; Balb/c: 4 ± 0.5, n=59) or Dogiel type II (C57Bl/6: 6 ± 3.2, n=6; Balb/c: 1 ± 0.6, n=11) morphology were apposed by more 5-HT$^+$ terminals in C57Bl/6 than Balb/c mice. This was only significant for Dogiel type I cells ($p<0.01$). ***Conclusion:*** NOS$^+$ enteric neurons may not be involved in 5-HT-mediated pathways in either strain. However, significantly more 5-HT$^+$ terminals innervate calretinin$^+$ neurons in C57Bl/6 (high activity) than Balb/c (low activity) jejunum. Hence, *tph2* genotype is not a simple predictor of 5-HT involvement in enteric motor patterns. ***References:*** [1] Kulikov et al. (2005) Genes Brain Behav 4:482-5. [2] Neal & Bornstein (2007) Neuroscience 145:556-67.

ORAL-10-04

EFFECTS OF AGE, PRESENCE OF NEURONS AND ENDOTHELIN-3 ON THE ABILITY OF ENTERIC NEURAL CREST CELLS TO COLONIZE RECIPIENT GUT

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There is enormous interest in the potential of neural cell therapy for treating Hirschsprung's disease, which is characterized by an absence of neurons from the distal intestine. However, it is unclear whether enteric neural crest cells (ENCCs) can colonize segments of gut in which the mesenchyme has differentiated or contains enteric neurons. Furthermore, Hirschsprung's disease can be caused by mutations in genes expressed by ENCCs, such as Ret, or by the mesenchyme, such as endothelin-3 (Et-3), and it is unknown whether neural cell therapy will be viable in all cases of Hirschsprung's disease. To investigate effects of age and presence of neurons, we used co-cultures and compared the ability of GFP+ ENCCs from caeca or midgut of E11.5 mice to colonize explants of hindgut from E11.5 and E14.5 *Ret*$^{+/+}$ and *Ret*$^{-/-}$ mice, which lack enteric neurons (n>10 for each). We also examined the ability of ENCCs to colonize recipient gut from E11.5 *Et-3*$^{+/+}$, *Et-3*$^{+/-}$ and *Et-3*$^{-/-}$mice. ENCCs colonized explants of hindgut from E11.5 *Ret*$^{+/+}$ and *Ret*$^{-/-}$ mice equally well. In contrast, ENCC migration along NC-colonised hindgut explants from E14.5 *Ret*$^{+/+}$ mice was dramatically reduced, while migration along uncolonised hindgut from E14.5 *Ret*$^{-/-}$ mice was significantly reduced. There was no significant difference in the ability of ENCCs to colonize gut from E11.5 *Et-3*$^{+/+}$ and *Et-3*$^{+/-}$ mice. However, ENCCs migrated significantly shorter distances along hindgut explants from E11.5 *Et-3*$^{-/-}$ mice. These results show that age, presence of neurons and lack of endothelin-3 within the recipient gut mesenchyme all reduce the ability of ENCCs to colonize segments of gut.

ORAL-10-05

EFFECTS OF MUTATIONS IN ENDOTHELIN-3 ON COLONIC MOTILITY AND NEURON DENSITY

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Purpose: Mutations in genes encoding members of the endothelin-3 (Et-3) signaling pathway cause Hirschsprung's disease, a congenital condition associated with an absence of enteric neurons in the distal gut. Spontaneous propagating motility patterns were examined in *Et-3*$^{-/-}$, *Et-3*$^{+/-}$ and *Et-3*$^{+/+}$ mice aged postnatal day P8-P12, the maximum viable age for the *Et-3*$^{-/-}$ mice. **Methods:** Video recordings of mouse colon *in vit*ro were used to construct spatiotemporal maps of spontaneous contractile patterns. Myenteric ganglia in post-caecal, mid and distal colon of *Et-3*$^{-/-}$, *Et-3*$^{+/-}$ and *Et-3*$^{+/+}$ mice aged P10 were examined after immunohistochemical processing using antibodies to Hu and NOS. Reactivity for NADPH-diaphorase was examined to determine the length of the aganglionic region in *Et-3*$^{-/-}$ mice and if there is an aganglionic zone in *Et-3*$^{+/-}$ mice. **Results:** There were no significant differences in the intervals between successive colonic migrating motor complexes (CMMCs) or in other properties of CMMCs between *Et-3*$^{+/-}$ and *Et-3*$^{+/+}$ mice. The effect of blockade of nitric oxide synthesis on CMMCs was similar in *Et-3*$^{+/-}$ and *Et-3*$^{+/+}$ mice. There was no difference in density of Hu^{+} cells or proportion of Hu^{+} cells that were NOS^{+} between *Et-3*$^{+/-}$ and *Et-3*$^{+/+}$ mice in any region. In *Et-3*$^{+/-}$ mice, an aganglionic zone was not present. In *Et-3*$^{-/-}$ mice the aganglionic region extended over ~70% of the colonic segment. No discernable CMMCs were observed in these mice. In the ganglionated zone, the density of Hu^{+} cells was ~50% lower but the proportion of NOS^{+} cells was significantly greater than in *Et-3*$^{+/-}$ or *Et-3*$^{+/+}$ mice ($p<0.05$). **Conclusion:** These data suggest that impairments in colonic motility correlate with density of enteric neurons.

ORAL-10-06

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ORAL-10-07

IN VIVO PATCH CLAMP RECORDING OF SYNAPTIC EVENTS EVOKED IN SUPERFICIAL DORSAL HORN NEURONS AFTER STIMULATION OF THE FEMALE REPRODUCTIVE TRACT IN THE MOUSE

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Pelvic pain is a common and debilitating condition in females. One obstacle to effective treatment is the lack of knowledge about how the nervous system processes sensations originating in pelvic organs. Previous studies using extracellular recording techniques (Berkley et al 1993 J Neurophysiol. 69:583) or C-Fos labelling (Tong et al 2003 Anesthesiology 99:205) have shown that dorsal horn neurons in thoracolumbar (T13-L3) and lumbosacral (L6-S1) spinal cord segments respond to stimulation of the female reproductive tract (FRT). These techniques, however, do not resolve subthreshold synaptic events or provide information on the characteristics of the recorded cell. **Purpose:** We have modified our in vivo preparation of the mouse spinal cord (Graham et al 2004 J Physiol. 561: 749) to record synaptic potentials from dorsal horn neurons after stimulation of the FRT. **Methods:** Mice (> P21, n=13) were anaesthetized with urethane (2.2 g/kg i.p, then 25% of initial dose after 20 mins). A laminectomy exposed the thoracolumbar or lumbosacral spinal cord segments. Mechanical stimulation of the cervix was delivered via a blunt probe inserted into the vagina. **Results:** Cervical stimulation evoked synaptic potentials in dorsal horn neurons in both the L2 and S1 spinal cord segments. In 15/18 neurons only subthreshold synaptic inputs were recorded following cervical stimulation. **Conclusions:** Together, these results confirm that spinal projections of cervical afferents are widely dispersed. Furthermore, our results indicate that much of the synaptic activity evoked in dorsal horn neurons following FRT stimulation is subthreshold. This suggests that previous studies may have significantly underestimated the extent of FRT projections into the spinal cord dorsal horn.

ORAL-10-08

ACTIVATION OF MICTURITION-RELATED SACRAL SPINAL NEURONS DIFFERS BETWEEN RATS WITH NORMAL AND OVERACTIVE BLADDER

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Non-voiding contractions are a hallmark of overactive bladder (OAB), which affects 10-15% of the population in developed countries. Spontaneously hypertensive rats (SHR) have non-voiding contractions and reduced bladder capacity; and pharmacological studies suggest that spinal mechanisms are involved. Here, we investigated whether micturition-related sacral spinal neurons differ between female rats with normal and overactive bladders. We induced Fos-immunoreactivity by evoking micturition reflexes through bladder infusions of saline under urethane anaesthesia. Bladder pressure was continuously recorded. Control rats were not infused. Segments L5-S2 from pairs of infused and control rats were cut transversely; Fos plus choline acetyltransferase (ChAT) were localized immunocytochemically. Neurons showing immunoreactivity for Fos, ChAT or Fos+ChAT were counted in 10 sections from each rat. Infused SD (n=4) had few non-voiding contractions whereas 3/6 infused SHR and 4/6 infused WKY had many non-voiding contractions. Large numbers of Fos-immunoreactive neurons were present in all infused SD. In infused SHR and WKY, Fos-positive neurons were very variable in number and less numerous than in infused SD. Control rats had fewer Fos-immunoreactive neurons than infused rats. In all infused rats, Fos-positive neurons occurred mainly in and dorsal to the parasympathetic nucleus, where double-labelled neurons were concentrated, and around the central canal. We are currently testing whether the number of Fos-immunoreactive neurons correlates with the number of non-voiding contractions or other physiological parameters. Our findings indicate that neural control of micturition differs between rats with OAB and rats with normal bladder function. Furthermore, many WKY appear to have OAB and may be inappropriate controls for studies on bladder function in SHR.

ORAL-11-01

ONTOGENIC VARIATION IN BLOOD-BRAIN BARRIER AND WHITE MATTER DAMAGE AFTER SYSTEMIC INFLAMMATION

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Purpose: To investigate the relationship between inflammation-induced blood-brain barrier and white matter damage during an extended period of brain development. **Methods:** Five age groups of South American opossums were used: P14-23, P21-30, P35-44, P42-51 and P54-63. Prolonged inflammation was induced by intraperitoneal injection of 0.2mg/kg lipopolysaccharide (LPS, *E.coli* 055:B5, Sigma) at P14, P21, P35, P42 and P54 over a 9 day period by administration of 5 serial injections at 48h intervals. All experiments were conducted 24h after the 5th injection, ie at P23, P35, P44, P51 and P63. Treated animals were compared with age-matched controls. 4-6 animals were used for each age and treatment. Animals were terminally anaesthetised with inhaled halothane before tissue collection. Assessment of blood-brain barrier permeability, white matter volume, microglia number and astrocyte number were conducted in coronal 5 micron sections of Bouin's fixed, paraffin embedded brain tissue. mRNA and protein of inflammatory mediators were measured in brain tissue and plasma. **Results:** Myelin was reduced within the external capsule in animals treated with LPS from P35-44 compared to age-matched controls. Increased BBB permeability was observed at this age and was still present in older animals. However white matter damage was only present in the P35-44 age group. Microglia response to inflammation within the external capsule was highest at P35-44. The up regulation of inflammatory mediator COX-2 follows a different pattern from P35 onwards compared to the early stages of development. **Conclusion:** White matter damage occurs in the developing brain as a result of the coincident developmental timetable of the inflammatory response, microglia and vasculature.

ORAL-11-02

IMPACT OF NEONATAL INFECTION ON ADULT HIPPOCAMPAL GLUCOCORTICOID RECEPTOR, MINERALOCORTICOID RECEPTOR AND CORTICOTROPHIN RELEASING HORMONE mRNA ABUNDANCE

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Purpose Perinatal stress has been shown to alter receptor function in the hippocampus and modify the adult stress response in a number of animal models. As yet no studies have investigated the effect of infection on the developing HPA axis. The current study examines the impact of neonatal infection on adult hippocampal glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) abundance, corticotrophin releasing hormone (CRH) and circulating corticosterone (CORT). **Methods** Balb/c mice (in each group, $n \leq 14$) were intranasally infected at birth with *Chlamydia muridarum* (400 ifu) or vehicle. At nine weeks of age animals were euthanized, brains removed and frozen. The hippocampus was removed and RNA was extracted using a parallel RNA/DNA/protein extraction method. GR, MR and CRH abundance was measured with βactin as the reference gene using qRT- PCR. CORT was measured from serum using a commercial RIA kit. **Results** Compared to the same-sex vehicle group, neonatally infected adult females showed a significant increase in GR and MR mRNA, while the males showed a significant decrease in receptor abundance. CRH mRNA abundance was similar across all groups. The neonatally infected group shows a decrease in CORT levels in females and infected males having significantly higher circulating CORT relative to the vehicle males.**Conclusion** The present study demonstrates for the first time in the mouse that neonatal infection has a sexually dimorphic effect on adult GR and MR mRNA abundance and circulating CORT. Both sexes demonstrated a reciprocal relationship between receptor abundance and circulating CORT. Microarray analysis has been undertaken to determine the global effects of neonatal infection on hippocampal structure and function.

ORAL-11-03

LONG-TERM ALTERATIONS IN NEUROIMMUNE RESPONSES AFTER NEONATAL EXPOSURE TO LIPOPOLYSACCHARIDE

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Purpose: Some aspects of the systemic inflammatory response in the adult animal have been suggested to be susceptible to modification by early-life infection, and this may be mediated by alterations to hypothalamic-pituitary-adrenal axis activity. We therefore investigated whether early life immune exposure will alter immune and endocrine responses to immune stress in adulthood. **Methods:** Wistar rats (n = 9) were administered *Salmonella enteritidis* lipopolysaccharide (LPS, 0.05mg/kg, ip) or saline on days 3 and 5 of life. In adulthood, subjects were administered LPS (0.10mg/kg, ip) or saline (equivolume), and the febrile response and activity of animals were continuously monitored for 12 hours. Food and water consumption during this period was recorded. Levels of IL-1β in the brain were measured at the end of the experiment using an enzyme-linked immunosorbent assay. From a different set of rats receiving identical neonatal and adult treatments, blood was collected at 0, 90, 180, and 360 minutes following adult LPS or saline treatment, and serum corticosterone as well as lymphocytes and immunoglobulins were measured. **Results:** Adult animals that had been neonatally exposed to LPS displayed attenuated fevers in response to LPS in adulthood. Activity was reduced and corticosterone production was increased after exposure to LPS in adulthood irrespective of neonatal treatment. Alterations to IL-1β levels in the brain were observed. No difference was found in the consumptions of food and water as a function of neonatal treatments. **Conclusion:** The results suggest that neonatal LPS can produce lifelong alterations to CNS-mediated inflammatory responses in adult rats.

ORAL-11-04

DEVELOPMENTAL VITAMIN D DEFICIENCY (DVD) AND BRAIN DOPAMINE ONTOGENY

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Purpose: Our group has pioneered research indicating that Developmental vitamin D (DVD) deficiency during gestation alters both brain development and function. **Results**: We have convergent evidence indicating a disturbance in dopamine signalling in this model. 1stly the superior colliculus (the proto-basal ganglia) is the initial site where the vitamin D receptor is expressed in foetal brain; 2ndly we show a reduction in Catechol-O-methyl transferase (a major metabolic enzyme for dopamine) in these foetal brains; 3rdly dopamine metabolites in the DVD deplete neonatal brain reflect this enzymatic change. When we allow these animals to mature under vitamin D normal conditions we repeatedly observe alterations in both spontaneous and psychomimetic enhanced locomotion. Consistent with the theme of persistent changes in dopamine signalling in this model we now present new data showing that dopamine transporter density and/or affinity are altered in DVD deplete female offspring whilst DA 1 receptor density and dopamine cell number is reduced in DVD deplete male offspring (all $P<0.05$ $n>8$). Finally, parathyroid hormone (PTH) levels are 2-3 fold greater in vitamin D deficient Dams across gestation. **Conclusions:** Taken together these findings confirm that the absence of vitamin D during foetal brain development induces long-lasting changes in multiple aspects of dopamine signalling. Studies in bone development may be informative for an understanding of potential developmental mechanisms. For instance Nurr-1 is a nuclear transcription regulator important in both bone and dopamine neuron development. Like vitamin D it heterodimerises with the retinoic acid receptor, RXR, to initiate transcription. Most importantly Nurr-1 is dramatically upregulated by PTH. A study of dopamine ontogeny in foetal DVD rat brain is therefore now warranted.

ORAL-11-05

ADDITIVE EFFECTS OF INTRAUTERINE AND POSTNATAL OVERNUTRITION ON ADIPOSITY AND CENTRAL APPETITE REGULATORS IN OFFSPRING

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Differentiation of central neural pathways involved in appetite regulation and energy metabolism occurs from the last week of gestation until weaning in the rat. Thus, exposure to excess nutrients due to prolonged maternal obesity and litter size reduction during this period could unfavorably "program" appetite and metabolic control. It is unclear to what extent maternal and postnatal nutritional states interact to exacerbate unfavorable metabolic consequences in offspring. We induced dietary-obesity in female Sprague Dawley rats by high-fat-diet (30%) feeding before mating. The same diet continued throughout gestation and lactation. At day 1 after birth, the sizes of some litters were reduced to 3 (normal size 12). Pups were born at similar weight regardless of maternal diet. At day 20, pups born of obese mothers, or raised in small size litters, were heavier than those from lean mothers, or those raised in normal size litters (lean mother & normal litter 33.2 ± 0.3g; lean mother & small litter 47.4 ±1.9g; obese mother & normal litter 47.4 ± 1.4g; obese mother & small litter 61.3 ± 2.5g). Both intrauterine and postnatal overnutrition caused increased adiposity and plasma leptin concentrations, and glucose intolerance, while maternal obesity increased offspring plasma triglyceride levels. Hypothalamic neuropeptide Y (NPY, feeding stimulator) was downregulated by maternal obesity, and this was exaggerated by postnatal overnutrition; reciprocal effects were observed in proopiomelanocortin (POMC, feeding inhibitor). Thus both intrauterine and postnatal overnutrition predisposed offspring to early-onset obesity. The altered hypothalamic NPY and POMC may be an adaptive response to overnutrition.

ORAL-11-06

THE EFFECTS OF NICOTINE ON NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN THE DEVELOPING PIGLET BRAINSTEM

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Purpose: Postnatal cigarette smoke exposure is a major risk factor for sudden infant death syndrome (SIDS). Utilizing a piglet model of early postnatal nicotine exposure, we tested the hypothesis that nicotine exposure increases the expression of the nicotinic acetylcholine receptor (nAChR) subunits α7 and β2 in the brainstem medulla. We also tested for gender-specific effects. **Methods:** Nicotine exposed piglets (n=14) were compared to non-exposed controls (n=14), with equal gender proportions in each group. Immunohistochemistry was performed to identify α7 and β2 nAChR subunits, and was subsequently quantified in 7 nuclei of the medulla, at both the rostral and caudal levels. **Results:** Comparing nicotine exposure to controls, α7 was significantly decreased in the dorsal motor nucleus of the vagus at the rostral level (rDMNV) ($p=0.01$), while β2 was significantly increased in the caudal DMNV (cDMNV) ($p=0.05$) and caudal nucleus of the spinal trigeminal tract (cNSTT) ($p=0.03$). β2 was greater in the male cNSTT ($p=0.01$). Conversely, females had greater β2 compared to males in the caudal hypoglossal nucleus (cXII) ($p<0.01$) and caudal inferior olivary nucleus ($p=0.03$). Within the nicotine group, males had significantly lower β2 in the cDMNV compared to females ($p=0.02$). Comparing nicotine exposed males to control males, there was a significant increase in β2 in the cXII of nicotine exposed males ($p<0.01$). **Conclusion:** Overall, the α7 changes were exposure specific with no gender differentiation, whereas β2 showed some gender selectiveness. Together, these findings provide evidence that postnatal nicotine exposure significantly changes the expression of nAChRs in the developing brainstem, with both regional and gender differences.

ORAL-11-07

RATS PRENATALLY EXPOSED TO HEROIN – THEIR RESPONSE TO METHAMPHETAMINE IN ADULTHOOD

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Previous studies in our lab examining the effects of prenatal heroin exposure on postnatal (PN) behavioral development in rats showed an increase in locomotor activity and rearing and a decreases habituation rate in PN 3weeks. Although there is a difference in gender response, behavioral changes recovered to the normal level in adulthood. **Purpose:** To examine whether prenatal heroin exposure would alter the vulnerability to aversive stimuli in adulthood. **Method:** Sprague-Dawley rats (n=9 each comparison group) that were exposed to heroin (10mg/kg/day,s.c.) prenatally from gestational day 8 to 20 were studied at PN 3months. Offspring borne to dams from pair-fed and free food and water groups were compared to the heroin-exposed group. The response to methamphetamine (METH) challenge (1mg/kg) in the offspring was investigated to ascertain change in METH-induced behavioural sensitization. Locomotor activity and rearing were parameters measured in both male and female offspring. **Results:** After METH (1mg/kg) dosing for 5 days, heroin-exposed male rats showed a significant sustained decrease in spontaneous locomotor activity but not in METH-induced sensitization. After a 10-day drug-free period, female, control and pair-fed rats showed a recovery from a decrease in spontaneous activity and rearing with male heroin-exposed rats persisted to be hypoactive. **Conclusion:** Although rats in adulthood showed compensatory recovery from early changes after prenatal heroin expousure, male rats may have an increase susceptibility to future drug exposure in adulthood.

ORAL-11-08

ALTERED PAIN RESPONSES TO LIPOPOLYSACCARIDE, BUT NOT INTERLEUKIN-1BETA, FOLLOWING PRENATAL ENDOTOXIN EXPOSURE

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Previous work has demonstrated that both the systemic and central administration of interleukin-1 beta (IL-1b), and its induction by the administration of peripheral lipopolysaccharide (LPS), results in elevations in pain sensitivity, or "hyperalgesia". Purpose: Given that the production of IL-1b following prenatal exposure to endotoxin is altered, this study aimed to identify whether LPS-induced hyperalgesia profiles would also change following such exposure. Methods: Pregnant F344 rats received endotoxin (PE; 200ug/kg, s.c, n=10) or saline (n=10) on gestational days 16, 18 and 20. On postnatal 90, offspring's pain thresholds were assessed prior to and 4 hours following peripheral administration of LPS (100ug/kg, s.c.) and recombinant rat IL-1b (rrIL-1b; 10ug/kg, i.p.). Three assays of pain were employed – the hot plate, tail immersion and von Frey tests. Results: Control offspring exhibited typical LPS-induced hyperalgesia at four hours post stimulation in tests of both thermal (hot plate and tail immersion) and mechanical nociception (von Frey). Evidence of hyperalgesia was observed in the PE offspring on the hot plate test. However, male PE offspring displayed decreased pain sensitivity following LPS stimulation in the von Frey tests, while both male and female PE offspring displayed decreased pain sensitivity on the tail immersion test. No change in pain perception was evident on any pain test following administration of rrIL-1b. Conclusions: Prenatal exposure to endotoxin resulted in sexually- and pathway-dimorphic alterations to pain-behaviours associated with basal states and the sickness (LPS-induced) response. These results were not observed following rrIL-1b administration, suggesting that the spinal hyperalgesia observed is a result of direct actions of LPS rather than via an IL-1b pathway. These findings implicate the prenatal period as a critical determinant in programming later nociceptive functioning.

ORAL-12-01

INVESTIGATION OF COPPER-REGULATED PROTEIN EXPRESSION IN NEURONS USING ANTIBODY MICROARRAYS AND IN SILICO PROTEIN NETWORK ANALYSIS

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Purpose: Neurodegenerative illnesses such as Alzheimer's disease (AD) are characterized by aberrant biometal metabolism. Recent advances in proteomic tools have helped to investigate the effects of aberrant metal homeostasis on protein turnover and signaling. **Methods:** In this study, we used antibody microarray analysis of cells with altered cellular Cu levels to identify how protein metabolism is modulated by Cu. Neuronal and non-neuronal cells (n = 4) were treated with cell permeable Cu-complexes including Cu-clioquinol and glyoxal-bis(*N*(4)-methylthiosemicarbazonato)-Cu(II) (Cu-GTSM). Cell cultures were analysed using the Clontech Antibody Microarray 500 or specific phospho-protein arrays (RayBiotech). The data were then analysed using the software program Pathway Studio (Ariadne Genomics) to identify protein interaction networks. **Results:** Using these methods, we have found that altered intracellular Cu levels induced substantial changes in DNA repair and maintenance proteins such as Ku80, cell cycle proteins including D-type cyclins and membrane receptor tyrosine kinase activity (EGFR). These protein changes have been validated by Western blot analysis and we are currently investigating how the altered metabolism of proteins by Cu modulates cell signalling and survival. **Conclusions:** Our results demonstrate the power of antibody microarrays for the identification of novel proteins that may be involved in neurodegenerative disorders.

ORAL-12-02

N-CADHERIN AND β-CATENIN LEVELS IN ALZHEIMER DISEASE AND ALCOHOLICS

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Neurodegenerative diseases such as Alzheimer disease (AD) and chronic alcoholic brain damage show regionally-selective loss of neurones and synapses. N-cadherin is a synaptic junction protein that bridges pre- and postsynaptic excitatory terminals *via* β-catenin attachment to the cytoskeleton. Brain tissue from pathologically confirmed AD, alcoholic and control cases was obtained at autopsy with informed written consent and frozen at -80°C in 0.32 M sucrose. Areas investigated included hippocampus and inferior temporal cortex, which are susceptible to AD pathology, and occipital cortex, which is relatively spared. The damage-susceptible frontal cortex of alcoholic cases was compared with controls. Levels of N-cadherin and β-catenin were measured by "in-gel" immunodetection on crude membrane preparations of frozen human brain tissue. A tendency for higher levels of N-cadherin was observed in AD cases (n = 9) than in controls (n = 9), although not significantly. β-catenin levels were markedly higher ($P < 0.01$) in AD cases (n = 15) than in controls (n = 15). β-catenin levels were also markedly higher ($P < 0.01$) in the frontal cortex of alcoholic cases (n = 9) than in controls (n = 9) but no change was noted in the levels of N-cadherin. AD cases were scored according to pathological severity of AD accounting for neuronal loss, tangle and plaque load, and gliosis. A score of 0 indicated no pathology; 1, mild or modest pathology; 2, moderate pathology; and 3, severe pathology, for each area studied. The levels of β-catenin showed a significant positive correlation with increasing pathological score ($P < 0.01$). These results could indicate a dysfunction of excitatory synapses in AD and chronic alcoholism which shows differential synaptic pathology.

ORAL-12-03

QUANTIFICATION OF GEPHYRIN LEVELS IN ALZHEIMER'S DISEASE AND CONTROL POST MORTEM BRAINS USING A RECOMBINANT GEPHYRIN TRUNCATE AS STANDARD

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'Gephyrin,' derived from the Greek word meaning 'bridge', binds inhibitory glycine and $GABA_A$ receptors to the subsynaptic cytoskeletal elements, serving as an important receptor-microtubule linker for the assembly and stabilisation of inhibitory post-synaptic terminals in the central nervous system. Excitotoxicity, or neuronal loss due to excessive excitatory transmission, depends on the balance of excitatory and inhibitory factors, and has been postulated by several research groups as a crucial factor contributing to synapse loss in Alzheimer's disease. Thus aberrant gephyrin levels in AD might contribute to disease pathology by altering the normal inhibitory modulation of excitation impulses. This is the first study of gephyrin in relation to AD. Gephyrin protein levels were investigated in two AD susceptible areas and a spared area in *post mortem* brain tissues from normal (n = 15) and AD (n = 15) patients. Quantification of the protein levels was achieved by interpolation from known concentrations of a recombinant truncated gephyrin protein containing the immunogenic epitope. A significant reduction ($P < 0.01$) in gephyrin was seen in the disease condition. Analysis, using indices of pathological severity, suggested that gephyrin levels decline until a moderate pathological condition is reached, but rise thereafter. This may indicate a compensatory mechanism for the excessive excitatory damage in the final stages of the disease. A second immunoreactive band was detected, presumed to be a splice variant of gephyrin, in all the cases in all three brain areas which requires further characterisation. This study contributes to the understanding of excitotoxicity in AD.

ORAL-12-04

ROLES OF OXIDATIVE-STRESS AND ENERGY DEPRIVATION IN MODULATING ALZHEIMER'S DISEASE-ASSOCIATED PROTEINS

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Purpose: Oxidative stress and reduced metabolic rate occur in the Alzheimer's disease (AD) brain and are associated with neurodegeneration. Evidence of oxidative stress has been found to precede the major development of pathological AD hallmarks - senile plaques (comprised of beta-amyloid peptide deposits) and neurofibrillary tangles (comprised of hyperphosphorylated tau protein). Our aim is to determine whether physiological abnormalities associated with aging can cause AD-like pathological changes in neurons. Effects of oxidative stress and energy deprivation on the generation of beta-amyloid peptides and tau phosphorylation will be determined. **Methods:** We use an embryonic chick primary neuronal cell culture model to determine effects of glucose deprivation (incubation in glucose-free medium) and oxidative stress (exposure to hydrogen peroxide). Secreted endogenous beta-amyloid peptides are immunoprecipitated from cell medium after incubating the cultures in the presence (n=6) or absence (n=6) of hydrogen peroxide or glucose-free medium for 2 to 24 hours. Lysates are made and full-length amyloid precursor protein (APP), total tau, phosphorylated tau and stress-associated markers detected by immunoblotting. Cell viability is determined in parallel by trypan blue staining. **Results:** We observe a two to three-fold increase in the levels of beta-amyloid peptides in cell medium from hydrogen peroxide treated cultures compared to controls. Additionally, we observe an increase in the ratio of phosphorylated tau to total tau in cell lysates from glucose-deprived cultures. **Conclusion:** (1) Events associated with aging such as reduced glucose metabolism and oxidative stress could contribute to pathological changes to neurons in AD - increased generation of beta-amyloid peptides and hyperphosphorylation of tau. (2) The embryonic chick primary neuronal cell culture model is suitable for investigating mechanisms of AD-like changes to neurons.

ORAL-12-05

GLAST1B: A SENSITIVE MARKER OF DYSFUNCTIONAL NEURONS

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Purpose: We have identified GLAST1b (exon 9-skipping GLAST) as a novel marker of dysfunctional neurons. We have now examined the functional expression of GLAST1b in neonatal animals subject to hypoxic insults. **Methods:** Immunohistochemistry and Western blotting for GLAST1b was performed on brains of pigs (n=6) that had been subject to hypoxic insults and compared with control animals (n=6). Additional brains (n=4) were used to prepare brain slices to detect transport of D-aspartate and other ligands by glutamate transporters. CSF from animals was also analysed for the presence of GLAST1b. **Results:** In brain regions where astroglial glutamate transporters were lost after an hypoxic insult, GLAST1b expression was induced in neurons. Western blots indicated that GLAST1b expressed by neurons lacked the normal GLAST amino terminal region, clarifying why conventional (N-terminal) GLAST antibodies did not normally detect this protein. Preliminary results indicated that GLAST1b or fragments thereof could be detected in CSF. Uptake experiments showed that D-aspartate was readily accumulated into astrocytes in control pigs, but not accumulated into astrocytes which had lost immunocytochemically detectable GLT1a. The examination of neuronal uptake of D-aspartate and related ligands such as D-glutamate is ongoing. **Conclusions:** GLAST1b represents a novel, physiologically relevant and sensitive marker for the detection of neurons at risk of dying in response to hypoxic insults and stroke. More widely, in disease states such as Alzheimer's disease, "at risk" neurons may express a form of GLAST that we now believe to be GLAST1b. The detection of GLAST1b, or antibodies to such in CSF or blood may have direct clinical value.

ORAL-12-06

THE KYNURENINE PATHWAY IN AMYOTROPHIC LATERAL SCLEROSIS

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AIMS: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. The kynurenine pathway (KP) catabolizes tryptophan and generates neuroactive compounds, such as picolinic acid (PIC) and quinolinic acid (QUIN), is emerging as a possible pathogenic component of ALS. The first enzyme in the KP, indoleamine-2,3 dioxygenase, can be stimulated by cytokines. This study aims to characterize the KP in ALS patients, in NSC-34 cell line and in primary human fetal motor neurons (hMN); and assess the effect of QUIN toxicity on NSC-34 cells. **METHODS**: We used GC/MS to quantify QUIN and PIC levels in CSF and serum from ALS patients (n=150) and controls (n=35). With cell cultures, RT-PCR and immunocytochemistry were used to characterize KP enzymes and catabolites; LDH test to assess the effect of QUIN, with and without the NMDA antagonists MK801, APV and memantine. **RESULTS**: Serum and CSF QUIN levels were significantly higher for ALS patients and serum PIC levels were significantly lower compared to controls. NSC34 cells and hMN cells stained positive for KP enzymes and catabolites; RT-PCR on NSC34 cells showed the presence of most of the KP enzymes. LDH test showed a dose dependant increase of LDH with QUIN, partially inhibited by MK801 and completely inhibited by MK801, APV and memantine combined. **CONCLUSION**: Our results provide *in vivo* and *in vitro* evidence to support the involvement of the KP in ALS.

ORAL-12-07

CLONING AND CHARACTERIZATION OF A NOVEL CHOLESTEROL P-TYPE ATPASE TRANSPORTER IN NEURODEGENERATIVE DISEASE

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Neurodegenerative disorders such as Parkinson and Niemann Pick diseases share a common abnormality in cholesterol transport. Although the molecular mechanisms underlying neurodegeneration remain to be fully determined, major advances have been made by elucidating the biochemistry of the several key proteins, including Niemann Pick C2 (NPC2) in Niemann Pick diseases. Here we report the cloning and characterization of a human P-type ATPase and its interaction with NPC2 and cholesterol. This work suggests for the first time an involvement of the novel cholesterol ATPase transporter in neurodegenerative diseases.

ORAL-12-08

AGING-RELATED CHANGES IN ASTROCYTES IN THE RAT RETINA: IMBALANCE BETWEEN CELL PROLIFERATION AND CELL DEATH REDUCES AVAILABILITY OF ASTROCYTES TO NEURONS

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Purpose: Astrocytes are intimate partners with neurons in virtually every function of the central nervous system and are therefore likely to play a significant role in aging-related loss of neuronal function. The aim of this study was to identify changes that occur in astrocytes in the central nervous system during physiological aging. **Methods:** Astrocytes in retinal wholemount preparations from Wistar rats (n=52) aged from 3 (young adult) to 25 months (aged) were investigated qualitatively and quantitatively following immunofluorohistochemistry. Glial fibrillary acidic protein, S100 and Pax2 were used to identify astrocytes and blood vessels were localized using *Griffonia simplicifolia* isolectin B4. Cell proliferation was assessed by bromodeoxyuridine incorporation and cell death by TUNEL-labelling and immunolocalization of the apoptosis markers active caspase-3 and endonuclease G. **Results:** The density and total number of parenchymal astrocytes increased between 3 and 9 months of age but decrease markedly between 9 and 12 months. Proliferation of astrocytes was detected at 3 months but not beyond that age. However, with aging the proportion of astrocytes that were $TUNEL^+$ and relative expression of active caspase3 and endonuclease G increased. In addition, in aged retinas astrocytes exhibited gliosis-like morphology and loss of Pax2 reactivity. A small population of $Pax2^+/GFAP^-$ cells was detected in both young adult and aged retinas. **Conclusion:** This study points to a reduction in the availability of astrocytes to neurons with aging. This and other aging-related changes reported here may have a significant impact on the ability of astrocytes to maintain homeostasis and support neuronal function in old age.

ORAL-13-01

AGE-RELATED DIFFERENCES IN CORTICAL EXCITABILITY AND INHIBITORY PROCESSES DURING INTERLIMB COORDINATION

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Purpose: To investigate the neurophysiological correlates of age-related changes in the coordination of hand and foot movements. **Methods:** Older adults (n = 15; age, M = 66.7 yrs) and young adults (n = 15; age, M = 21.9 yrs) performed cyclical isodirectional and non-isodirectional hand-foot movements with contralateral and ipsilateral limb combinations. Silent period (SP) duration following transcranial magnetic stimulation (TMS) was measured from the right extensor carpi redialis (ECR) during the interlimb coordination tasks. Cyclical foot movements were also performed while the right hand was maintaining tonic contraction. **Results:** Older adults demonstrated lower coordination stability than younger adults, particularly when performing non-isodirectional movements with ipsilateral limbs. Higher MEP amplitudes and shorter silent periods were evident under phasic compared to tonic activation conditions. Young adults showed significantly longer silent period durations during phasic activation of ipsilateral limbs compared to contralateral limb coordination. In contrast, silent period durations did not differ between contralateral and ipsilateral limb coordination in older adults. **Conclusion:** These results suggest that deterioration in motor performance with advancing age may be associated with a decreased ability to modulate inhibitory function.

ORAL-13-02

PLASTICITY AT HUMAN CORTICOSPINAL-MOTONEURONAL SYNAPSES

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Repeated delivery of pairs of timed pre- and post-synaptic action potentials induces lasting potentiation or inhibition at many synapses (1). In humans, spike timing-dependent plasticity in the motor cortex may underlie altered responses to transcranial magnetic stimulation (TMS) after repeated paired stimulation of peripheral nerve and motor cortex (2). **Purpose:** We hypothesized that conditioning with paired stimuli would induce plastic changes at a spinal level in the human motor pathway. **Methods:** Subjects (n=8) took part in 4 experiments. On each day, electromyographic responses (cervicomedullary motor evoked potentials, CMEPs) were evoked in right biceps brachii by stimulation of corticospinal axons with electrical pulses between the mastoids (180-320 mA). CMEPs were recorded before and after conditioning with 50 pairs of stimuli (0.1 Hz). In each pair, brachial plexus stimulation evoked a maximal M-wave (Mmax; 30-100 mA) and TMS (round coil, 47-80% stimulator output) elicited a motor evoked potential of ~5% Mmax in biceps. On each day, different interstimulus intervals (ISI) were used, with the cortical stimulus before the peripheral stimulus (+12 and +22 ms) or vice versa (-3 and -13 ms). **Results:** Prior to conditioning, CMEPs were similar sizes on each day (area, 6.1±1.9% Mmax; P=0.133). After conditioning with ISIs of +22 and -13 ms, CMEPs became depressed (by 48±28% and 24±29%, respectively; P<0.001), whereas no significant change occurred with the other ISIs. Depression of the CMEP developed over 20-30 minutes and the response remained depressed at 60 mins post-conditioning when testing ceased. **Conclusion:** The results suggest that paired peripheral and descending volleys can induce plastic changes at human corticomotoneuronal synapses. (1) Dan & Poo (2004) Neuron 44:23-30. (2) Wolters et al (2003) JNeurophysiol 89:2339-2345.

ORAL-13-03

THE EFFECT OF TEST INTENSITY ON SHORT-INTERVAL INTRACORTICAL INHIBITION IN DIFFERENT EXCITABILITY STATES

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Purpose: As short-interval intracortical inhibition (SICI) varies with test MEP size (1) test TMS intensity is often adjusted to control for different excitability states. However, the effect of test intensity on SICI in different excitability states has not been systematically examined. The aim of this study was to investigate the effect of test intensity in different excitability states on SICI. Methods: Eleven volunteers participated in the study. Single and paired-pulse (3 ms ISI) TMS were delivered to the hand area of left M1 using a focal figure-of-eight coil and MEPs recorded in the right first dorsal interosseous (FDI). Test intensities ranged from 90%-150% resting motor threshold (RMT) and the conditioning intensity was fixed at 70% RMT. SICI was examined in three excitability states (a) at Rest, (b) during isometric abduction of the left FDI (Contra) and (c) during isometric abduction of the right FDI (Active). Results: MEPs were largest for Active followed by Contra, then Rest ($p < .02$). For all conditions maximal SICI occurred at 110-120% RMT and SICI was reduced at lower and higher intensities ($p < .05$). The major effects of increased excitability were decreased SICI and reduced variation with test intensity, resulting in a Condition x Intensity interaction ($p < .001$). Conclusion: SICI showed a similar dependence on intensity for all conditions despite large differences in MEP size. Results suggest that SICI is affected by test intensity and excitability state, and not MEP size, consistent with the conclusion of Zoghi et al. (2). This has implications for the practice of adjusting test intensity to accommodate changes in MEP size across conditions. (1) Roshan et al. (2003) Exp Brain Res, 151:330-337 (2) Zoghi et al. (2003) J Physiol, 550:933-946.

ORAL-13-04

POST-ACTIVATION DEPRESSION OF THE HUMAN H REFLEX MEASURED USING THRESHOLD TRACKING

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Purpose. The H reflex is a simple, largely monosynaptic, spinal reflex. As stimulation rate increases reflex amplitude is limited by post-activation depression, also termed homosynaptic depression (HD). HD is conventionally studied measuring changes in the output (reflex amplitude) to a constant input (stimulus intensity). Threshold tracking reverses this, keeping the output constant (reflex amplitude) while altering the input (current required to generate a specific reflex amplitude), thus permitting the study of a relatively constant population of α-motoneurones. **Methods**. In 17 subjects soleus EMG was recorded in response to tibial nerve stimulation at 0.05, 0.1, 0.3, 1 and 2 Hz. This was repeated with voluntary contractions at 2.5, 5 and 10% MVC. We recorded the amount of current required to generate an H reflex that was 10±-0.5% of Mmax. **Results**. HD of the H reflex was evident at 0.1-2 Hz using threshold tracking, but did not continue to increase after 1 Hz, and was significantly reduced during voluntary contractions, although rate effects were still evident. The H:M ratio decreased with increasing stimulus rate up to 1 Hz. In one subject the H reflex was tracked to 5, 10, 15 and 20% Mmax at 0.3, 1 and 2 Hz. In this subject HD varied systematically with rate and with the proportion of the activated motoneurone pool. **Conclusion**. Threshold tracking effectively clamps the output so that the reflex involves a constant population of α-motoneurones. The changes in stimulus intensity required to generate the test reflex therefore represents changes in the net synaptic drive onto the motoneurone pool. These data could allow insight into the compound EPSP produced in the motoneurones.

ORAL-13-05

THE ROLE OF MOTOR COMMAND SIGNALS IN HUMAN POSITION SENSE IS PRESERVED IN DIFFERENT LOADING CONDITIONS

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We asked 8 blindfolded subjects to indicate their perceived wrist angle with a pointer under two conditions. During the isometric condition the subject's wrist was locked into a test position and they performed an isometric contraction, into flexion or extension, equal to 30% of their maximum voluntary contraction (MVC). At the same time they indicated their wrist angle with the pointer. The second task was isotonic, in which the wrist was free to move into flexion and extension but subjects were required to maintain a test angle using visual feedback, while being loaded into flexion or extension with a weight of 30% MVC. Again they indicated perceived wrist position during the contraction. For both tasks the subject's wrist muscles were conditioned with brief contractions at short length, of either the wrist flexors or wrist extensors, to control the muscle contraction history and muscle spindle firing rate. The muscle conditioning had an effect, indicating subjects were utilizing muscle spindle inputs. The direction of the muscle contraction also had an effect showing that subjects perceived their wrist to move in the direction of the contraction. There was no difference between the isometric and isotonic tasks. By performing these two tasks at the same joint this result resolves a conflict between results that have shown the position illusion to move in the direction of the contraction (Gandevia et al., 2006 J Physiol) and results with have shown that the illusion moves in the direction opposite to the contraction (Walsh et al. 2004 J Physiol; Ansems et al. 2006 J Physiol).

ORAL-13-06

CONCURRENT RECORDING OF MUSCLE SYMPATHETIC NERVE ACTIVITY AND BRAINSTEM FMRI SIGNAL INTENSITY: 'REAL-TIME' IMAGING OF CARDIOVASCULAR CONTROL IN HUMAN SUBJECTS

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Purpose: Muscle sympathetic nerve activity (MSNA) varies in its pattern across subjects and cannot be predicted simply from measuring blood pressure or heart rate. In order to understand the central neural processes responsible for determining the pattern of MSNA we attempted to record MSNA while performing functional magnetic resonance imaging (fMRI) of the brainstem. **Methods:** Using an MRI compatible headstage we recorded bursts of MSNA via tungsten microelectrodes inserted into the common peroneal nerve in three awake subjects. Gradient echo, echo-planar fMRI was performed using a 3T scanner (Philips Achieva). 200 volumes (46 axial slices, TR=8 s, TE=40 ms, flip angle=90 deg, raw voxel size =1.5 mm3) were collected in a 4s-ON, 4s-OFF protocol. Total sympathetic burst amplitudes were measured during the 4 s period between scans. Blood Oxygen Level Dependent (BOLD) changes in brainstem signal intensity (SPM5, uncorrected $p<0.001$) were measured during the subsequent 4 s period to take into account the +5 s neurovascular coupling delay and the -1 s required for conduction of the sympathetic bursts from the brainstem to the peripheral recording site. **Results:** Using the total sympathetic burst amplitude recorded during the previous 4 s epoch as the input model, we found covariation in BOLD signal intensity within the regions of the rostral and caudal ventrolateral medulla and medullary raphe. **Conclusions**: We have demonstrated the feasibility of concurrent recording of MSNA and brainstem fMRI and conclude that the approach can provide "real-time" imaging of the neural processes responsible for the generation of sympathetic nerve activity in awake human subjects.

ORAL-13-07

EXPERIMENTAL BACK PAIN CAN ALTER RESPIRATORY MUSCLE ACTIVITY

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Control of trunk muscles is complicated by the integration of distributed inputs from respiratory, voluntary and balance centres of the central nervous system and peripheral mechanisms. Pain alters activity of trunk muscles during voluntary tasks. However, the effect of pain on respiratory activity is unknown. Although respiration is critical for homeostasis, respiratory behaviour could be modified by pain. **Purpose:** To determine if low back pain modifies activity of trunk muscles that have duel postural and respiratory demands. **Methods:** Subjects stood in tandem with right foot forward. Respiratory rate and phase was monitored via a pneumotach and mouthpiece. Fine-wire electromyography (EMG) electrodes were inserted bilaterally with ultrasound guidance into obliquus internus and transverse abdominis. Recordings were made for 15 respiratory cycles with and without experimental low back pain (5% hypertonic saline injection 5 cm left of the L3 spinous process). Each respiratory cycle was divided into inspiratory and expiratory phases, and each phase was divided into 5 epochs of equal length. EMG amplitude was quantified as the average rectified value (ARV) and motor-unit threshold crossings in each epoch. Values were normalised to the peak value across the 10 epochs during the trials without pain. Data were analysed for each epoch and with values averaged over the 5 epochs for each phase. **Results:** Data is reported from 5 subjects. Mean pain level during the pain trial was 4.5±2.9/10 (visual analogue scale). Respiration rate (breaths/minute) was not altered with pain (no-pain 14.4±2.8; pain 17.2±5.1). ARV right transverse abdominis decreased during inspiration with pain (paired t-test $P<0.02$). **Conclusion:** These data indicate that control of respiratory muscles can be influenced by experimental low back pain. This finding may underlie breathing changes in people with clinical pain.

ORAL-13-08

SINGLE PERIPHERAL NEURONES OUTPERFORM HUMANS ON A VIBROTACTILE AMPLITUDE DISCRIMINATION

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Purpose: With microneurography it is possible to analyze neural mechanisms in unanaesthetised humans performing a psychophysical task. We compared subjects' discrimination performance at different vibration amplitudes with the performance of simultaneously recorded single neurones. **Methods:** Impulses were recorded from single tactile afferents (n=20) with tungsten microelectrodes inserted percutaneously into the median nerve of awake human subjects (n=6). By placing a vibrotactile stimulator in the centre of the receptive field, the neurone's amplitude response function to a sinusoidal vibration (20Hz) was characterized. Using a 2-interval forced choice paradigm, we then measured each subject's Just-Noticeable-Difference (JND) for vibrations with pedestals of different amplitudes using an adaptive staircase. Subjects' performance was compared to that of a hypothetical observer making a decision on each trial based on the spike count of the simultaneously recorded neurone. **Results:** Neurones showed a characteristic response profile: a sub-threshold range for which neurones did not fire any spikes, a response range which increased linearly, and a saturated range for which response was constant. All neurones had a response range narrower than the range of amplitudes that subjects could detect. However, when pedestal amplitude was chosen to be within the neurone's response range, the single neurone nearly always outperformed the subject. **Conclusion:** Vibrotactile amplitude discrimination samples individual peripheral neurones each accurate only over a narrow range. The superiority in performance of individual neurones over observers indicates either that the range of human sensitivity comes at a cost to discrimination through pooling across neurones with different response functions or that significant noise is introduced between periphery and the cortical decision stage.

ORAL-14-01

LARGE SCALE DNA SEQUENCING OF GENES INVOLVED IN PARKINSON'S DISEASE USING THE PD GENECHIP - A PLATFORM FOR NEUROSCIENCE RESEARCH

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Background: Parkinson's disease (PD) is one of the most common neurodegenerative diseases, affecting 1% of Australians over the age of 55. Advances in our understanding of PD have been fuelled by the identification of genes (PD genes), and mutations therein, which cause disease. It is now recognised that common (SNP) variation in the PD genes is associated with disease susceptibility in sporadic cases. **Purpose**: A catalogue of all DNA sequence variation in the PD genes would help to increase our understanding of the pathogenesis of PD, however, it would require sequencing of literally hundreds of PD patients and healthy controls. Conventional approaches to DNA sequencing are not suited to such a study because they are both labour intensive and expensive. Methods: Using Affymetrix CustomSeq technology, 185 DNA sequences from 16 different PD genes, including *SNCA*, *Parkin*, *UCHL1*, *DJ-1*, *PINK1*, *LRRK2* and other plausible candidates, were tiled onto a silicon chip at Affymetrix – making a total of 44,320 bases. For 11 of the 16 genes, exons, exon/intron borders, 5' and 3' untranslated regions were included, the latter to enable the identification of regulatory SNPs. **Results**: We have developed a protocol for high throughput DNA sequencing of selected PD genes using the PD GeneChip and have re-sequenced DNA samples collected from 75 Victorian PD patients, including some with early age-at-onset of symptoms (≤55 y.o.), a family history of disease (≥2 affected relatives) and sporadic PD. A summary of these sequence data will be presented. **Conclusion**: The PD GeneChip is a platform technology that will enable PD research, and it has the potential to be developed as a low-cost diagnostic tool. This study will open the way for this technology to be employed for the genetic analysis of other human neurological and non-neurological diseases.

ORAL-14-02

A NOVEL CULTURE TECHNIQUE FOR LOW-DENSITY HIPPOCAMPAL NEURONS

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Neuronal cultures provide a powerful tool in neuroscience for dissecting molecular and cellular mechanisms. Hippocampal neurons, amongst others, have become the most widely used cell type. They were employed in analyzing dendritic spine morphology, synaptic plasticity as well as dissecting pathomechanisms in neurodegenerative diseases. These cultures are often limited by low and short survival rate of cells. Different procedures have been developed to overcome these problem, including hippocampal slice cultures, mixed cultures or co-cultures with both tissue explants and astroglial cells. However, culturing at low density is often not possible or highly-dependent on sophisticated methods. Therefore, we have established an easy and robust technique for culturing hippocampal neurons at low-cell density for long-term survival. This was achieved by culturing embryonic (E16.5) murine hippocampal neurons, surrounded by a spatially separated ring of cortical cells for neurotrophic support. Both cell types were prepared from the same embryo providing sufficient material for many experiments. This approach allows for the comparative study of single embryos from different genetic background. Hence, this ring support system enable the long-term culture of hippocampal neurons at low cell density, suitable for studies of cellular morphology.

ORAL-14-03

A FUNCTIONAL APPROACH FOR COUNTING PRESYNAPTIC VESICLES

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The fidelity of synaptic transmission depends on the number of synaptic vesicles that can be mobilised from different vesicle pools in the presynaptic terminal. We describe a new method for counting the number of functionally available vesicles that can be released from synapses in hippocampal cultures. Our general strategy is to first poison all the vesicles with Bafilomycin (Baf), a H^+-pump inhibitor, collapsing the energy gradient necessary for refilling vesicles with neurotransmitter. This renders vesicles 'invisible' following an initial round of exocytosis, ensuring that they will be counted only once. **Methods:** Whole-cell voltage clamp recordings were made from autaptic glutamatergic neurons (12-24 d in culture). Baf (5 μM) was applied using a picospritzer. **Results:** Brief application of Baf consistently caused an initial potentiation followed by a stimulus-dependent rundown of the autaptic EPSC (n = 7 cells). During rundown, miniature EPSCs declined in frequency without any change in amplitude, ruling out the presence of partially-filled vesicles. Baf-induced rundown was purely stimulus-dependent (and not time-dependent), indicating that there was no leakage of glutamate out of Baf-treated vesicles. No recovery of this Baf-induced rundown was observed after recording periods of >1 hour. **Conclusions:** Baf binds to vesicles irreversibly. Baf-treated vesicles retain their full neurotransmitter content until their first round of release. Thus, each Baf-treated vesicle contributes only one quantum of neurotransmitter to the postsynaptic response over the time course of the experiment. Use of this approach allows one to count the number of vesicles in the readily-releasable pool of synaptic vesicles and to measure vesicle recycling between pools. This information is critical for an understanding of synaptic dynamics.

ORAL-14-04

TIME COURSE AND SPATIAL EXTENT OF V1 SUPPRESSION VIA INTRACORTICAL BOTULINUM TOXIN

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Botulinum toxin is known to suppress synaptic activity by cleavage of SNARE complex proteins, thus impairing the docking of synaptic vesicles. **PURPOSE** Intracortical injection of Botulinum toxin is in development as a model of reversible, medium- to long- term inactivation of selected cortical targets. The model has potential advantages over existing methods of inactivation for studies if neural plasticity, but its technical and performance specifications remain to be determined. **METHODS** In this series of pilot experiments, we evaluated the onset time and spread of effect of pressure injection of Botulinum toxin type E into primary visual cortex (V1) of 4 anaesthetized (pentothal, 4 mg.kg.h-1, N2O/O2, 7:3) cats. **RESULTS** Serial extracellular recording of single and multiunits over intervals of 12-36 hours post-injection (0.5 ng/kg in 1 μl) indicate that initial toxin effects are detectable within approximately 30 minutes after application, with >95% suppression of evoked spike activity within 8-12 hours. Dye injections using toxin diluted with 2% evans blue, as well as multiunit electrode mapping of tissue surrounding the injection site indicate that toxin effects spread over a radius of at least 4 mm within 24 hours of injection, with minimal spread of effects to corresponding visual areas in the uninjected hemisphere. **CONCLUSION** This series of experiments confirms the feasibility of intracortical botulinum toxin as a method of selective inactivation of cortical areas. Studies are ongoing to assess the degree and time course of recovery from toxin-induced suppression of cortical activity.

ORAL-14-05

SEARCHING FOR DIRECTION: HOW DO INVASIVE NEURAL PRECURSOR CELLS MOVE?

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Purpose Cell invasion is the basis of several fundamental biological systems including developmental morphogenesis and disease progression. Cell invasion involves cell motility and proliferation. The details of how cells move within an invasive population cannot always be determined by measuring population-level properties of the invasion system. This is a major impediment limiting our ability to describe cell invasion. **Methods** Using a cellular automata simulation technique, we perform *in silico* experiments (n=40) and demonstrate how to distinguish between different motility mechanisms using individual-level cell trajectory data. **Results** We show that cell trajectory data can distinguish between directed and undirected cell motilities within invasive populations. This approach can be used to interpret time-lapse imaging data. **Conclusion** Distinguishing between directed and undirected motility is possible by measuring properties of individual cell trajectories and has profound implications regarding our ability to design strategies to manage development and disease associated with cell invasion.

ORAL-14-06

POLYMER-COATED ELECTRODES FOR THE DELIVERY OF CHARGE AND NEUROTROPHINS TO COCHLEAR NEURONS

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Purpose: Neurotrophins protect auditory neurons from degenerating after deafness but clinical translation requires safe treatment methods. We investigated a biocompatible conducting polymer coating (polypyrrole; Ppy) for cochlear implant electrodes that provides neurotrophic support for neurons without impeding the electrical function of the device. **Methods:** Platinum electrode arrays (cochlear implants) were coated with Ppy, Ppy/NT3 or Ppy/^{125}I-labelled NT3. Coated electrode arrays were implanted for a 2-week period into 2-week deafened guinea pig cochleae. Electrical stimulation (biphasic current pulses) was applied to the implanted electrodes of half the animals for 8 hours per day over the 2 week period. The spread of released ^{125}I-NT3 was assessed and the effects of released NT3 or ^{125}I-NT3 on nerve survival post-deafening and post-implantation were quantified. **Results:** NT3 was released from Ppy/NT3-coated electrode arrays through passive diffusion, but enhanced and controlled release was achieved by applying electrical stimulation. Deafened guinea pigs implanted with Ppy showed 1.23-fold reduced neural density in the implanted ear indicative of insertion damage that often occurs during cochlear implantation (n=4). With Ppy/NT3-coated electrodes, insertion damage was reduced (now 1.11-fold) but the neural density was still worse than the unimplanted ear (n=9). However, applying electrical stimulation to Ppy/NT3-coated electrodes resulted in 1.10-fold greater neural survival in the implanted ear compared to the unimplanted ear indicating that Ppy/NT3 protects neurons from deafness-induced damage as well as insertion damage (n=2). **Conclusions:** Neurons in a deafened cochlea will benefit from Ppy/NT3-coated electrodes in two ways; insertion damage is overcome and deafness-induced neural degeneration is minimised.

ORAL-14-07

REGIONAL SKIN TEMPERATURE AS AN INDICATOR OF UNDERLYING MUSCLE PERFUSION

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Purpose: A maximal voluntary breath hold (inspiratory-capacity apnoea, ICA) causes little change in ongoing skin sympathetic nerve activity (SSNA) but a sustained increase in muscle sympathetic nerve activity (MSNA), resulting in vasoconstriction within the muscle vascular bed but not within skin. We hypothesised that this reduction in muscle perfusion could be detected non-invasively by thermographic monitoring of the overlying skin. **Methods:** In the present study we measured the heat emitted over the tibialis anterior (TA) muscle in 6 awake subjects with a high-sensitivity infrared thermographic camera (ThermaCAM P45, FLIR Systems, Sweden). After a 3 min baseline was recorded, changes in skin temperature over TA were measured in response to a 60 s ICA. Changes in skin temperature were also measured in a 3 min recovery period following the ICA. **Results:** ICA caused skin temperature over TA to decrease significantly from 31.0 ± 0.1ºC (SE) to 30.7 ± 0.1ºC (95% CI: baseline = 30.9ºC-31.2ºC, ICA = 30.5ºC–31.0ºC; p= 0.03). Skin temperature in the recovery period remained significantly lowered compared with baseline 31.0 ± 0.1ºC vs 30.8 ± 0.1ºC (95% CI: baseline = 30.9ºC-31.2ºC. recovery = 30.6ºC–31.0ºC; p= 0.03). **Conclusions:** Previous studies have shown that sustained changes in blood flow are required to alter the thermal inertia of the skin. As SSNA shows only a transient increase during the inflation phase of the ICA, and since MSNA is not sustained during the static phase, we suggest that the thermographic emission recorded over TA reflects the underlying muscle vasoconstrictor activity and resultant decrease in muscle perfusion. This approach may be useful in assessing muscle perfusion non-invasively during wound repair.

ORAL-14-08

A COMPUTATIONAL MODEL RELATING NEURAL ACTIVITY TO CEREBRAL BLOOD FLOW

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Purpose: Functional magnetic resonance imaging (fMRI) is a major tool for non-invasive imaging of brain function. It detects increased neural activity indirectly by measuring changes in the magnetic properties of cerebral blood flow that result from changes in flow and oxygen content. Our purpose is to construct a mathematical model of the communication chain that links neural activity to blood flow in neighbouring arterioles. **Methods**: In the model, neural activity leads to glutamate release at synapses and the overflow acts on astrocytes causing them to release EETs (epoxyeicosatrienoic acids). The astrocytes abut onto arteriolar smooth muscle cells and the EETs act to hyperpolarize these cells, leading to the closure of L-type calcium channels resulting in vasodilation and hence increased blood flow. **Results**: The computational model incorporating the above steps successfully accounts for the main observed changes in blood flow in both visual cortex and somatosensory cortex following their stimulation by high-contrast drifting grating or by single whisker stimulation, respectively. The model also predicts a linear increase in blood flow with increasing numbers of activated astrocytes, but a non-linear increase with increasing glutamate release. **Conclusion**: The current model utilises one pathway, that mediated by EETs, and obtains satisfactory agreement with currently available experimental data. Other agents, such as adenosine and nitric oxide, have also been implicated in the vasodilation process and could be included in the model when more extensive experimental data becomes available.

ORAL-15-01

CAN TOO MUCH STARGAZIN CAUSE EPILEPSY?

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Transmembrane AMPA receptor regulatory proteins (TARPs) play a critical role in trafficking and anchoring AMPA receptors to the synapse and therefore in defining neuronal excitability. **Purpose:** Linkage analysis in GAERS, a genetic animal model of absence epilepsy, identified a quantitative trait locus on chromosome 7 containing the gene for the TARP, stargazin. A mutation in this gene, resulting in decreased stargazin expression, is the causative abnormality in the stargazer mouse which has absence epilepsy. We hypothesised that abnormalities in stargazin sequence or expression may contribute to the epileptic phenotype in GAERS. **Methods:** RNA was extracted from somatosensory cortex and thalamus, brain regions critical for the generation of absence seizures, of juvenile and adult GAERS and control animals. Quantitative PCR was performed for stargazin and ribosomal 18S RNA. Sequencing of the coding region and Northern Blots were done to examine for the presence of mutations and splice variants. **Results:** Stargazin mRNA expression was increased in the somatosensory cortex of juvenile (76%, n=10, $p<0.05$) and adult GAERS (74%, n=8, $p<0.001$). Additionally, in juvenile animals there was a smaller increase in stargazin mRNA expression in the thalamus (26%, n=10, $p<0.05$). No gene mutations or splice variants were detected. **Conclusions:** GAERS have increased expression of stargazin mRNA in the somatosensory cortex, which contains the focus generating the seizures in rats. This is present in the pre-epileptic juvenile GAERS and therefore is not merely a secondary consequence of the seizures. These data are the first to implicate an increase in stargazin expression in the pathogenesis of epilepsy, with the stargazer mouse having decreased expression.

ORAL-15-02

THE TOTAL NUMBER OF DENTATE GRANULE CELLS INCREASES IN EPILEPTIC RATS FOLLOWING STATUS EPILEPTICUS

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Purpose: A consistent histological finding in temporal lobe epilepsy (TLE) is "dispersion" of the granular cell layer of the hippocampus. Enhanced neurogenesis is also seen in the granular cell layer, however it is unknown whether these new cells simply replace cells lost during the epileptogenic process or represent an excessive proliferative response, increasing the total cell number in this region. This study used unbiased stereological methods to quantitate the total number of cells in the dentate granular cell layer and CA1 pyramidal cell layer in the post-kainic acid (KA) status epilepticus (SE) rat model of TLE. **Methods:** 13-week old male wistar rats were treated with KA (2.5-5 mg/kg i.p.) to induce SE. Control animals received saline. Five weeks following treatment animals post SE (n=9) and controls (n=15) were transcardially perfused and their brains prepared for cryo-sectioning. Stereological estimates of total neuronal numbers were performed on thionin stained sections using Stereoinvestigator™. **Results:** The total number of dentate granule cells was significantly increased in the epileptic animals compared to controls ($1.35x10^6$ Vs. $1.04x10^6$, $p<0.01$). In contrast the total number of CA1 pyramidal cells was decreased in the epileptic rats ($2.56x10^5$ Vs. $3.5x10^5$, $p<0.05$). **Conclusions:** In addition to the previously recognized granular cell "dispersion" in the post-KA SE rat model there is an increase in total cell number. This suggests that neurogenesis in this region is not just compensating for lost neurons, but rather reflects an aberrant proliferative response that potentially has pathogenic significance.

ORAL-15-03

A NOVEL MUTATION IN THE RAT $CA_v3.2$ T-TYPE CA^{2+} CHANNEL CACNA1H GENE INCREASES ABSENCE SEIZURE EXPRESSION

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Rationale: Recent evidence implicates the $Ca_v3.2$ T-type Ca^{2+} channel in the pathogenesis of genetic absence epilepsy, although whether functional abnormalities in this channel play a causative role is unknown. Linking an absence phenotype to a mutation in this channel would provide *a priori case* for a causative role. To this end, we have identified that GAERS rats (a genetic rat model of absence epilepsy) carry a homozygous single nucleotide missense mutation in a highly conserved region the III-IV linker region of the $Ca_v3.2$ T-type Ca^{2+} gene (R1584P). This study examined the *in vivo* electrophysiological effects of this mutation. **Methods:** Male F2 progeny of both NECxGAERS and GAERSxNEC double-cross matings underwent EEG recordings at 18 weeks, which were blindly analyzed to determine whether seizure activity correlated with the animals' genotype. **Results:** The R1584P genotype correlated strongly with the expression of absence seizures in F2 rats. Rats homozygous (n=12) for the mutation had more seizures per minute ($p=0.02$) and spent a greater percentage of recording time in seizure activity ($p=0.03$) compared to those with the wild-type genotype (n=8). On both measures heterozygote rats (n=24) had values in between the homozygotes, consistent with a co-dominant effect. **Conclusions:** The *in vivo* results indicate that the $Ca_v3.2$ R1584P mutation plays a causative role in the expression of absence seizures in GAERS. Future studies including effects on $Ca_v3.2$ membrane expression, splice variants and cellular electrophysiological properties *in vivo* may provide insight into the cellular mechanisms by which the R1584P mutation acts to promote seizures in the GAERS model.

ORAL-15-04

EFFECT OF FOCAL CORTICAL AND THALAMIC NPY INJECTIONS ON GENETIC ABSENCE SEIZURES AND APPETITE

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Purpose: Neuropeptide Y (NPY) has potent effects against genetically determined, thalamocortical seizures in Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Here we determined the sites within the thalamocortical circuit at which NPY exerts its anti-seizure effects. Methods: Adult male GAERS were implanted with recording electrodes and cannulae bilaterally into the reticular thalamus (nRT), ventrobasal complex (VB) or deep layers of the S2/S1ULp region of somatosensory cortex. In freely moving rats serial doses of NPY/saline were delivered in randomised order, followed by 90min EEG recording. nRT, VB injections were: 0.2 µl of 0, 0.15, 0.5, 1.5 and 3 mmol/L NPY, and cortex: 2 µl of 0, 0.15, 0.5 and 1.5 mmol/L NPY. Results: A dose-dependent suppression of seizures was seen following injection into the S2/S1ULp cortex (median % time in seizure = 7.4%, 3.6%, 2.9% and 0.5% respectively, $p=0.02$, n=5). nRT injections produced a smaller, but significant, dose dependent suppression of seizures (median % time in seizure = 8.8%, 7.0%, 5.8%, 5.6%, 3.4, $p=0.03$, n=6). However, VB injections did not result in seizure suppression ($p=0.13$, n=9). The seizure suppression was greater in the cortex than nRT (median suppression vs. vehicle: 95.9% vs 59.3%, $p=0.02$). nRT injections resulted in a increase in food intake at the highest dose ($p=0.03$), with no effect at the other sites. Conclusion: The extent of seizure suppression following the localized injections into S2/S1ULp region supports the "cortical focus" theory for these "generalized" absence-like seizures. For the first time a role for the thalamus in appetite regulation has been implicated, with an orexigenic effect of NPY in the nRT.

ORAL-15-05

CHANGED CHOLINERGIC MARKERS IN BRODMANN'S AREA 6 FROM SUBJECTS WITH SCHIZOPHRENIA

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There is an increasing body of evidence that suggests that changes in cortical muscarinic receptors (CHRM) occur in the CNS of subjects with schizophrenia1. To better understand the changes in muscarinic receptors in the dorsolateral prefrontal cortex we used in situ radioligand binding to measure [^{3}H]pirenzepine binding to CHRM1/4 and [^{3}H]4DAMP to CHRM3 in Brodmann's area 6 from 20 subjects with schizophrenia and 20 age/sex match controls. In addition, we used Western blot analyses to measure levels of SP1 (a transcriptional regulator of the CHRM1) and BACE (known to be regulated by CHRM1 activity) in the same cohorts of subjects. [^{3}H]pirenzepine binding was present in two discrete layers in the cortex and the density of radioligand binding was decreased in both layers in subjects with schizophrenia (Mean±SEM: layer 1; schizophrenia = 75±11 vs. control = 123±3.8 fmol/mg ETE, p<0.01; layer 2; schizophrenia = 103±14 vs. control = 159±5.8 fmol/mg ETE, p<0.001). By contrast levels of [^{3}H]4DAMP binding (Mean±SEM: schizophrenia = 83±4.2 vs. control = 86±2.3 fmol/mg ETE, p=0.56), SP1 (Mean±SEM: schizophrenia = 1.18±0.08 vs. control = 1.39±0.08 ratio IC, p=0.35) and BACE (Mean±SEM: schizophrenia = 1.44±1.7 vs. control = 1.72±2.1 ratio IC, p=0.22) were not altered in subjects with disorder. These data show a decrease in levels of CHRM1/4, but not CHRM3, SP1 and BACE, in Brodmann's area 6 from subjects with the disorder. 1. References Raedler, T.J. et al. (2007) Towards a muscarinic hypothesis of schizophrenia. Mol.Psychiatry 12, 232-246.

ORAL-15-06

CLOZAPINE SIGNALS THROUGH THE EGF-ERK PATHWAY IN CORTICAL NEURONS. A THERAPEUTIC TARGET FOR REFRACTORY SCHIZOPHRENIA?

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Purpose: Antipsychotic drugs are ineffective in up to half of patients with schizophrenia and can carry disabling side-effects. An exception is clozapine, which is superior to other agents in treatment-resistant schizophrenia. This singular efficacy may incorporate interactions with several GPCRs and subsequent targeting of signalling pathways such as the mitogen activated protein kinase-extracellular signal regulated kinase (MAPK-ERK) cascade. This pathway is critical to early brain development and adult synapse formation, processes altered in schizophrenia. We have previously reported that whilst antipsychotic drugs including clozapine acutely inhibited ERK activation, only clozapine stimulated ERK with continued treatment. This stimulation however, was not modulated by Gi/o/q-coupled receptors and PKA/C-signalling systems classically associated with these agents. Hence we examined clozapine's effect on alternative growth factor signalling systems. **Methods:** Phosphorylation of the MAPK isoforms, ERK1/2 by clozapine in the absence/presence of pathway-specific inhibitors was measured in murine cortical cultures (n=3 to 6) and *in-vivo* mouse studies (n=4) by immunoelectrophoresis. **Results:** The epidermal growth factor receptor (EGFR) inhibitor, AG1478 dose-dependently inhibited pERK1/2 (IC_{50} 0.083 and 0.106μM, respectively) in the presence of clozapine whereas the platelet-derived growth factor receptor inhibitor, tyrphostin A9 did not. Immunofluoresence data then indicated that clozapine treatment increased EGFR phosphorylation (Tyr1068) commensurate with ERK signalling. Parallel studies in mice confirmed that clozapine treatment caused rebound cortical ERK activation. **Conclusion**: This is the first evidence that the effects of clozapine may involve the EGF signalling system not previously linked to antipsychotic drug action. Our findings may therefore point to a plausible mechanism by which treatment-resistant patients are responsive to clozapine therapy and also highlight the EGF system as a potential new treatment target.

ORAL-15-07

INVESTIGATING THE ROLE OF CREB IN SCHIZOPHRENIA: BEHAVIOURAL STUDIES IN CREB-DEFICIENT MICE

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Purpose: Cyclic AMP-response element binding protein (CREB) is a transcription factor involved in receptor signal transduction, memory processes and drug abuse. CREB is associated with dopaminergic and serotonergic activity in the brain and novel variants of CREB have been found in schizophrenia. We therefore assessed CREB mutant mice for locomotor hyperactivity and prepulse inhibition (PPI), behavioural animal models of aspects of schizophrenia focusing on psychosis and sensory gating, respectively. **Methods:** $CREB^{Nestin\text{-}Cre/loxlox}$ mice (n=7), which have brain-specific loss of CREB production, were compared to wildtype control (n=29) and heterozygote littermates (n=7) using a pseudo-randomized within-animal protocol. Locomotor activity was measured with Tru-Scan photocell cages (Coulbourn, USA) and PPI was assessed with SRLab automated startle boxes **Results:** The locomotor hyperactivity induced by 5 mg/kg of amphetamine, but not that by 5 mg/kg of phencyclidine, was significantly smaller in CREB mutant mice than in wildtype controls and heterozygote mice (total distance moved during two hours: 30415±3642cm vs. 45602±4312cm and 56107±5514cm, respectively). The effect of amphetamine on PPI tended to be greatest in CREB mutants, however, these mice also showed a reduction in startle responses which may have influenced the results. There was no genotype difference in the effect of MK-801 on PPI. **Conclusion:** $CREB^{Nestin\text{-}Cre/loxlox}$ mice show markedly reduced acute amphetamine-induced locomotor hyperactivity, a behavioural animal model of psychosis. These preliminary results could reflect altered dopamine receptor-mediated signal transduction or dopamine receptor expression in the mutant mice; possibilities which will have to be addressed in future studies. These experiments support a role of CREB in psychosis.

ORAL-15-08

A MUSCARINIC RECEPTOR DEFICIT ENDOPHENOTYPE OF SCHIZOPHRENIA?

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Our data suggests that there are subjects with schizophrenia who differ from others with the syndrome in that they have low levels of cortical muscarinic M1 receptors (CHRM1) 1. We believe that these subjects might represent a discrete endophenotype. To explore this hypothesis by determining if other neurochemical changes segregate with this endophenotype, we are extending our studies on cohorts of 74 controls and 80 subjects with schizophrenia. In these cohorts, [^{3}H]pirenzepeine binding and CHRM1 mRNA are decreased in Brodmann's Area 9 from subjects with schizophrenia compared to controls ([^{3}H]Pirenzepine: Mean ± SEM: 133.9 ± 7.25 vs. 182.7 ± 4.50 fmole/mg ETE; p<0.0001. mRNA: Mean ± SEM: 15.27 ± 1.5 vs. 27.32 ± 2.5 x103 dpm/mg ETE, p<0.0001). The cohort was found to include a group of subjects with schizophrenia (n = 22) who had very low levels of pirenzepine binding compared to both controls and other subjects with schizophrenia (Mean ± SEM: 44.3 ± 6.88 vs. 182.7 ± 4.50 and 167.8 ± 4.52 fmole/mg ETE respectively; p<0.0001). Our extended studies suggest that [^{3}H] kainate binding is decreased in subjects with the muscarinic receptor deficit endophenotype compared to controls and other subjects with schizophrenia (n = 10 in each group, p<0.0001). Significantly, this is not the case for the other ionotropic glutamate receptors (NMDA; p = 0.354, AMPA; p = 0.932). These preliminary findings add weight to the hypotheses that there is a muscarinic receptor deficit endophenotype of schizophrenia and these subjects have a different pathological profile to other individuals encapsulated in the syndrome of schizophrenia. In addition, our data raise the possibility of a specific cholinergic/glutamatergic interaction within the cortex in this subgroup. We are continuing our investigations to further understand the ramifications of the muscarinic receptor deficit seen in this group of subjects with schizophrenia. Reference List 1. Dean,B., McLeod,M., Keriakous,D., McKenzie,J. & Scarr,E. Decreased muscarinic(1) receptors in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol. Psychiatry 7, 1083-1091 (2002).

ORAL-16-01

CALCIUM CHANNEL BLOCKERS LIMIT SECONDARY DEGENERATION IN A PARTIAL INJURY MODEL

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Purpose: Following partial injury to the central nervous system (CNS), intact healthy tissue undergoes secondary degeneration characterised by, for example, neuronal death and inflammatory cell infiltration. Although the topographic arrangement of the visual system offers a means of injuring discrete retinal ganglion cell (RGC) axon populations, most studies to date have relied upon an optic nerve crush model which induces a variable amount of initial axotomy across the face of the nerve and, consequently, a variable degree of secondary damage. Here we have characterised a partial optic nerve injury model and examined the effects of Lomerizine, a newly described calcium channel blocker which is currently in phase II trials as an anti-glaucoma agent and which is localised specifically to the CNS following oral administration. Methods: The dorsal side of the optic nerve in PVG rats was exposed and cut to a depth of 200µm (~1/5th) using a diamond radial keratotomy knife. Lomerizine dihydrochloride (30mg/Kg) was given orally in butter twice daily. Survival of RGCs in the spared ventral retina was assessed in wholemounts using retrograde fluorogold labelling; inflammatory cell infiltration was assessed using immunohistochemistry (ED-1: macrophages; ferritin: microglia). Results: Preliminary data indicate that our partial optic nerve lesion model is characterised by a predictable and increasing degree of secondary cell death in intact retina between 1 and 4 weeks. However, at 28 days, Lomerizine limited the secondary loss of RGCs (Lomerizine: 1958.3±258.3 RGC/mm2, n=2; vehicle: 1345.8±67.5 RGC/mm2, n=8; sham operated: 2244.4±104.8 RGC/mm2, n=3) and may reduce inflammatory cell infiltration. Conclusion: Lomerizine dihydrochloride may limit secondary degeneration following partial CNS injury.

ORAL-16-02

CHARACTERISATION OF NEUROGENESIS FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY (TBI) IN THE ADULT RAT

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Purpose: Neurogenesis in the adult mammalian brain is induced in response to various types of brain injury, including focal TBI. However, neurogenesis has not been explored in following diffuse brain injury, and therefore this study aims to characterise the neurogenic response in a traumatic axonal injury (TAI) model of diffuse TBI. **Methods:** Adult SD rats were subjected to TAI or sham-operation. BrdU (200mg/kg i.p.) was administered twice-daily for 4d beginning 1d post-injury, to label proliferating cells *in vivo*. Brains were collected 1,2,4 and 8w post-trauma (n=2-5). BrdU-labelled cells were quantified in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) at 1w to assess proliferation of precursor cells, whereas neuronal differentiation and survival in the DG granule cell layer (GCL) was analysed by quantifying co-labelled BrdU/NeuN (neuronal marker) cells at 4 and 8w. Proliferation in the lateral ventricle subventricular zone (SVZ) and subsequent neuronal differentiation/survival in the cortex were assessed similarly. **Results:** At 1w post-TBI, BrdU-labelled cells in the DG increased 2-fold compared to sham ($p<0.05$). At later time-points, a 120% increase in BrdU-labelled cells was maintained in the GCL, with 90% co-labelling with NeuN. In the SVZ, a 163% increase in BrdU-labelled cells was observed at 1w post-TBI ($p<0.05$); however, very few new neurons (<10% of BrdU-labelled cells) were evident in the cortex. **Conclusion:** Diffuse TBI induces proliferation in the SVZ and SGZ, leading to increased neurogenesis in the DG. Future studies will investigate stimulating neurogenesis with specific growth factors to enhance neuronal differentiation and survival in the cortex, potentially aiding recovery following diffuse brain injury.

ORAL-16-03

METALLOTHIONEIN IS AN INJURY DEPENDENT, ASTROCYTE-DERIVED PROMOTER OF AXONAL REGENERATION IN THE CNS

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Mice that cannot express the astrocytic metallothionein (MT) proteins exhibit significantly impaired recovery following traumatic brain injury. It is thought that MTs promote brain healing via intracellular free radical scavenging and heavy metal regulation, in particular zinc. However, we now have significant evidence for a fundamentally different mode of action of MT, relying upon intercellular transfer from astrocytes to neurons which in turn leads to uptake-dependent axonal regeneration. First, we show that MT can be detected within the extracellular fluid of the injured brain by western blotting. Furthermore, we demonstrate that while cultured astrocytes secrete low basal levels of MT (approximately 200pg/100µl), secretion could be induced by co-treatment with 10µM zinc and interleukin-1 (10U/ml) resulting in an approximate 5-fold increase in levels within culture media (from 4 different cultures). Second, using co-immunoprecipitation and a competitive ligand we identify that MT interacts with the low-density lipoprotein receptor megalin resulting in neuronal uptake of MT. We also demonstrate using pharmacological inhibitors and western blotting that MT-stimulated axonal regeneration is dependent on its receptor-mediated uptake and acts via a MAPK-dependent pathway. Finally, we directly demonstrate for the first time the transfer of MT from astrocytes to neurons over a time-course of 4 days in vitro. Our work suggests that the protective functions of the astrocytic protein MT in the CNS includes both extracellular and intra-neuronal roles. This unsuspected action of MT represents a novel paradigm of astrocyte-neuronal interaction after injury. Such interactions may explain the protective and/or regenerative roles of other astrocytic proteins after CNS insult and may have implications for the development of MT-based therapeutic agents.

ORAL-16-04

CONTRIBUTION OF ENDOGENOUS NEURAL PROGENITORS AND ASTROCYTES TO GLIAL SCAR FORMATION IN EXPERIMENTAL POST-TRAUMATIC SYRINGOMYELIA

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CNS injury induces tissue damage creating barriers to neural regeneration. One of the main obstacles is the glial scar which consists predominately of reactive astrocytes and proteoglycans. Reactive astrocytes are important for neuroprotection and self-repair whereas the proteoglycans they produce inhibit axonal regeneration. Post-traumatic syringomyelia (PTS) is a cystic degenerative condition occurring in up to 25% of spinal cord-injured patients, frequently causing progressive neurological deficit. In PTS, a prominent glial scar surrounds the cyst but it is unknown if this is primarily beneficial or detrimental regarding ongoing neural damage. **Purpose**: To examine endogenous progenitor cell and astrocytic responses to PTS and their contribution to subsequent glial scar formation. **Methods**: A model of PTS was induced in adult rats (n=48) by injection of intraparenchymal quisqualic acid and subarachnoid kaolin at C7-T1. Controls included sham-operated (n=48) and intact (n=12) animals. Animals received bromodeoxyuridine (BrdU) by either single injection 24 hrs post-syrinx induction or daily injections for 12 days. Spinal cords were examined immunohistochemically up to 56 days post-syrinx induction for BrdU and glial fibrillary acidic protein (GFAP). **Results**: Cell proliferation was >35-fold increased in syrinx animals by 7 days post-injury, peaking at >100-fold at 14 days post-injury. By 56 days post-injury 55% of BrdU-labeled cells expressed GFAP, many located in the region bordering the syrinx. Reactive astrocytes, the majority of which were not BrdU-labeled, also contributed significantly to the glial scar. **Conclusion**: PTS injury induces proliferation of spinal cord neural progenitors, some of which differentiated into astrocytes but, additionally, significant hypertrophy and migration of pre-existing astrocytes occurred to form the glial scar.

ORAL-16-05

MICROGLIAL ACTIVATION CONTRIBUTES TO HEPATIC ENCEPHALOPATHY AND BRAIN EDEMA IN EXPERIMENTAL ACUTE LIVER FAILURE: BENEFICIAL EFFECT OF MINOCYCLINE

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Purpose: Encephalopathy and brain edema are serious complications of acute liver failure (ALF). The precise pathophysiologic mechanisms responsible have not been fully elucidated but a previous report (Hepatology 44:366A, 2006) provided evidence that brain-derived proinflammatory cytokines are involved. Therefore, the effects of the anti-inflammatory drug minocycline were investigated on the progression of hepatic encephalopathy and brain edema in rats with ALF resulting from hepatic devascularization (portacaval anastomosis followed by hepatic artery ligation). **Methods**: ALF rats(n=6 per group) were administered saline or minocycline (22.5mg/kg) on days -2, -1 and day 0 of surgery. ALF rats were sacrificed at precoma (loss of righting reflex) and coma (loss of corneal reflex) stages of encephalopathy along with their appropriate sham-operated controls. Minocycline-treated animals were sacrificed in parallel with saline-treated comatose rats. IL-1β in serum and brain was measured by ELISA. IL-1β mRNA in the brain was assessed by real-time PCR. Microglial activation was assessed by immunohistochemistry using anti-OX42 antibody. **Results**: Minocycline delayed the onset of coma and significantly reduced brain water content in ALF rats. Minocycline treatment also significantly attenuated the increase of IL-1β protein in serum, together with IL-1β mRNA expression and IL-1β protein in cerebral cortex of ALF animals. Furthermore, induction of OX-42 immunoreactivity observed in the frontal cortex of ALF rats was suppressed by minocycline treatment. **Conclusion**: These results further support a role for brain-derived cytokines in the pathogenesis of encephalopathy and brain edema in ALF and suggest that minocycline may be beneficial in the prevention of these neuropsychiatric complications in ALF patients.

ORAL-16-06

NEURONAL PROTECTION BY NDFIP1 FOLLOWING INJURY IN TRAUMA AND STROKE

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Purpose: We have discovered a novel mechanism for preventing neuronal cell death following injury. Ndfip1 is an adaptor protein for protein ubiquitination of target proteins via the Nedd4 family of ubiquitin ligases. We wish to investigate the biochemical and cellular mechanisms of neuroprotection by Ndfip1. **Methods:** Experimental injury to the brain was conducted in vivo using either the closed head injury model of trauma, or endothelin-induced cerebral ischemia. Neurons in culture were stressed using growth factor starvation or exposure to noxious reagents including cobalt chloride and hydrogen peroxide. Internal levels of Ndfip1 were manipulated by cell transfection or viral infection. To reduce Ndfip1 levels, a knock-out mouse model was used for in vivo experiments, or siRNA mediated knock-down of mRNA levels in cells. **Results:** In the intact brain following trauma or stroke, a consistent association with neuronal survival and increased Ndfip1 expression was noted. Increasing Ndfip1 levels in cultured neurons also protected against cell death following stress, compared to controls. Biochemical pull-downs revealed that Ndfip1 binds to Nedd4 ubiquitin ligases, and increased ubiquitination of target proteins such as DMT1. Besides proteosomal degradation of target proteins, we also observed increased secretion of Ndfip1 and Nedd4 into the extracellular environment, suggesting a new mechanism of depleting unwanted proteins following Ndfip1 binding. **Conclusions**: Ndfip1-mediated ubiquitination and trafficking of protein cargo is an important mechanism for neuronal survival following stress from injury.

ORAL-16-07

THE INVOLVEMENT OF SIGMA RECEPTOR AGONISTS IN NEUROPROTECTION

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The sigma-receptor is a novel receptor which has been discovered in the recent two decades and whose function is still being elucidated. Although their endogenous ligands are yet to be determined, various studies have identified sigma-receptors to be potentially neuroprotective. This study addressed two sigma agonists, PRE-084 and 4-PPBP and their potential as neuroprotectants and possible mechanism of action. Immunohistochemistry and Western blot analysis of dose response studies carried out in primary cultured mouse neurons stimulated with PRE-084 and 4-PPBP elicited extracellular signal related kinase (ERK) phosphorylation in a concentration dependent manner. This phosphorylation was blocked by the sigma-receptor antagonist BD1047 and the ERK inhibitor PD98059. Pre-treatment of neurons with 10μM PRE-084 induced neuroprotection in a model of glucose deprivation whereas 4-PPBP did not. This neuroprotection was blocked by both BD1047 and PD98059 suggesting that the neuroprotective effect elicited by PRE-084 may be mediated by ERK phosphorylation. This study suggests that selective sigma-receptor agonists could be valid targets for neuroprotection therapy.

ORAL-16-08

IMPROVED FUNCTIONAL RECOVERY AFTER HUMAN BONE MARROW STROMAL STEM CELLS (HBMSCS) TRANSPLANTATION INTO THE ACUTE AND CHRONIC INJURED SPINAL CORD

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Purpose: Multipotent hBMSCs from spinal cord injured (SCI) patients were used to stimulate sparing and regeneration of descending neural pathways following *acute* (1wk) and *chronic* (1mo) moderate contusive SCI. **Methods:** The therapeutic potential of retrovirally transduced donor cells encoding GFP ($hBMSC^{GFP}$) transplanted into immunodeficient (Nude) rat hosts (n=6 for each time point) after acute and chronic SCI (10g,12.5mm, NYU impactor) was assessed both behaviourally (recovery of function) and anatomically using immunohistochemistry and retrograde tracing. **Results:** In both acute and chronic SCI; $hBMSC^{GFP}$ initially survive well in the injured host spinal cord (SC) 1wk after transplantation, induce axonal growth, and co-exist with host glial cells (eg astrocytes/Schwann cells) within the lesion site. At 1wk post-transplantation, immunostaining of SC sections show the presence of RT97+/β-III tubulin+ axons, GFAP+ astrocytes, S100+ profiles and p75+ Schwann cells intermingled in close proximity to the transplanted $hBMSC^{GFP}$, which also produced large quantities of extracellular matrix (laminin & fibronectin) in vivo. Such immunoprofiles remained high from 4-8wks post-transplantation, although no donor hBMSCs were present in the lesion site from 4 wks. $hBMSC^{GFP}$ treated rats showed marked, statistically significant tissue sparing, c.f. controls. Extensive behavioural analysis of $hBMSC^{GFP}$-transplanted rats showed a marked improvement in open field BBB scoring (15) c.f. controls (13) 8wks post transplantation. Additional computer generated Catwalk gait analysis revealed patterns of behavioural recovery. Immunosuppression with CsA (acute study) did not further improve functional recovery after SCI. **Conclusion:** This compelling evidence strengthens the argument for clinical use of hBMSCs for repair of the injured mammalian SC.

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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School of Medical Sciences (Anatomy & Histology), University of Sydney, NSW 2006.

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.