ORAL SESSIONS

ORAL-01-01

FEED-FORWARD AND INTRACORTICAL MECHANISMS IN STRIATE CORTICAL ORIENTATION SELECTIVITY: NEUROPHYSIOLOGY AND COMPUTATIONAL MODELING

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Purpose: The mechanism of orientation selectivity of striate cortical cells has been debated for over 40 years. The highly influential model of excitatory convergence from geniculate afferents proposed by Hubel & Wiesel (*J.Physiol.*,160, 106) has received both much support and much criticism. Using both experimental and computational approaches, we tested the possibility whether the well documented biases exhibited by retinal and LGN cells to stimulus orientation (Levick & Thibos, *Nature* 286, 389; Vidyasagar & Urbas, *Exp Brain Res* 46, 157) could lead to the sharp selectivity seen in the cortex when acted upon by non-specific intracortical inhibition. **Methods:** (1) We applied a variation of a novel protocol of electrical stimulation in the LGN (Kara et al. (*PNAS*, 99, 16261) that strongly activated inhibitory circuits in both LGN and the cortex to study their effects on orientation selectivity of both LGN and striate cells. (2) We studied the activities of simultaneously recorded LGN cells and those in cortex after identifying their locations in optical imaging maps of orientation domains. (3) We simulated a geniculocotrical connectivity scheme that assumed excitatory inputs from LGN cells with orientation biases acted upon by untuned and tuned suppression. **Results:** (1) We found that the inhibition caused by the electrical stimulation significantly improved the orientation selectivity of LGN cells, with the orientation sensitivity index increasing from 0.37±0.04 to 0.51±0.04 (n=19; Wilcoxon sign ranked test, p<0.001). (2) Simultaneous recordings from 15 pairs of LGN and striate cells showed a relationship in their coherence and the degree to which the optimum orientations of the cortical and geniculate cells were similar. (3) The simulations showed that our model could account for the full range of orientation and spatial frequency selectivities and length-response functions of first order striate cells. **Conclusions:** These results suggest that even non-specific inhibition can sharpen geniculate orientation biases to levels seen in the striate cortex and support the model that intra-cortical inhibition could usually provide such sharpening leading to the narrowing of the post-synaptic excitation in the cortex from biased geniculate inputs (Vidyasagar et al, *Trends Neurosci*,19, 272).

ORAL-01-02

INFORMATION PROVIDED BY CORRELATIONS BETWEEN PAIRS OF NEURONS IN AREA MT OF MARMOSET

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Purpose: Correlations in the firing activity of neurons can increase or diminish the information they provide. Here we used the method of Montani et al. (J Neurosci, 27, 2338-48, 2007) to quantify the information provided by the correlations between pairs of motion-sensitive neurons in the visual cortex. **Methods:** Extracellular recordings were made with tetrodes from neurons in the middle-temporal area of anaesthetized (sufentanyl forte, 9 µg/kg/hr) male marmosets (n = 7). The stimulus was a drifting sinusoidal grating, or the sum of two gratings (a plaid) 120 degrees apart, presented at each of 12 directions. We estimated the total information content and its 4 components: information provided by neurons independently, information loss due to the similarity of their mean response, information in stimulus-dependent correlations and information in stimulus-independent correlations. **Results:** Fourty-four pairs of neurons responded over 10 impulses/s and were used for analysis. Most information was carried by neurons independently (85%) and stimulus-dependent correlations provided 20% of the information; information was lost through stimulus-independent correlations (2%), and the similarity in tuning curves (4%). This loss is expected if both reflect common synaptic inputs. Total information was significantly higher for plaids than gratings, primarily because the independent information provided by neurons was higher. **Conclusion:** While correlations are prevalent and informative, coding in area MT is weakly synergistic and might be approximated by an assumption that neurons provide independent sources of information.

ORAL-01-03

PHOSPHORYLATION OF SYNDAPIN I F-BAR DOMAIN IN NERVE TERMINALS REGULATES MEMBRANE TUBULATION

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Syndapin I (PACSIN I) is a synaptically enriched member of the F-BAR (FCH-BIN amphiphysin RVS) family of proteins. It consists of two functional domains; an N-terminal F-BAR, which can bind to and deform phospholipid membranes, and a C-terminal src homology 3 (SH3). Syndapin I is an important regulator of activity-dependent bulk endocytosis (ADBE) of synaptic vesicles (SV), neuron morphogenesis, and links endocytic vesicles with the actin cytoskeleton. Its function in ADBE is regulated by a phospho-regulated interaction with dynamin I. Although syndapin I is an *in vitro* phospho-protein, it is not known to be phosphorylated in nerve terminals. Using phosphopeptide analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS), we identified two phosphorylation sites (Ser-76 and Thr-181) in the syndapin I F-BAR domain in rat brain nerve terminals. Ser-76 and Thr-181 phosphosites are part of N-CAP motifs of α-2 and α-4 helices respectively in the syndapin I F-BAR structure which are important for regulating F-BAR homodimer curvature and dimer-dimer filament assembly. Single amino acid mutations of the phosphosites to glutamic acid (Glu), which mimics phosphorylation, reduce or block phospholipid binding and membrane tubulation *in vitro*, and in cells supporting that these two phosphosites regulate F-BAR function. Surprisingly, these two phosphosites do not regulate syndapin I's role in ADBE. Rather both sites are essential for the morphological development of primary neurons. To our knowledge, this is the first report of regulation of the membrane tubulation function of a BAR domain of a protein by phosphorylation.

ORAL-01-04

DSCAM PROTEINS REGULATE SYNAPTIC SPECIFICITY

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Purpose: To determine whether the repulsive cell surface proteins, Dscam1 and Dscam2, play a role in synapse formation. **Methods:** Serial section electron microscopy was performed on wild type and mutant *Drosophila melanogaster* and the composition of photoreceptor synapses was quantified. **Results:** Multiple-contact synapses are commonly found in the visual systems of both vertebrates and invertebrates. They are comprised of a single presynaptic terminal that releases neurotransmitter upon a distinct combination of postsynaptic elements. How these complex synapses are specified is not known. *Drosophila* photoreceptor terminals are presynaptic to a tetrad of postsynaptic elements. Dendrites from L1 and L2 neurons form an obligate pair in each tetrad. In flies mutant for both Dscam1 and Dscam2, the pairing of L1 and L2 is randomized. 58% of the double mutant synapses (93/161) contained two L1 or two L2 dendrites from the same cell instead of the L1-L2 pairs observed in wild type ($p \leq 10^{-12}$). **Conclusion:** Dscam1 and Dscam2 are redundantly required for the strict pairing of L1 and L2 at postsynaptic tetrads. Given the repulsive nature of Dscam interactions, it is proposed that Dscam1 and Dscam2 specify postsynaptic composition by excluding two contributions from the same cell..

ORAL-01-05

TISSUE PLASMINOGEN ACTIVATOR ENHANCES THE SORTING OF NEUROSERPIN TO THE REGULATED SECRETORY PATHWAY

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Purpose: Neuroserpin is a serine protease inhibitor that is expressed in the brain and implicated in the regulation of emotional behaviour. Neuroserpin has been suggested to be secreted from neurons through both the constitutive and regulated secretory pathways. The secretion of neuroserpin has also been linked to the secretion of its inhibitory target, tissue plasminogen activator (tPA), which occurs at synaptic sites in an activity-dependent manner. Activity-dependent secretion of tPA and neuroserpin is likely to play a key role in modulating synaptic plasticity. The purpose of this study was to investigate the sorting and secretion of neuroserpin in neuronal cells. **Methods:** Nerve growth factor-treated PC12 cells were transfected with tPA-GFP alone or co-transfected with tPA and a range of DsRed-neuroserpin chimeras. The subcellular location of endogenous neuroserpin and fluorescent proteins was monitored by immunocytochemistry and fluorescence microscopy. Protein distribution was quantified using MetaMorph™. **Results:** Neuroserpin accumulated in the growth tips where it co-localised with the regulated secretory pathway marker tPA-GFP. Transfection of tPA-GFP resulted in a significant increase in the levels of both endogenous neuroserpin and DsRed-neuroserpin in the growth tips compared to non-transfected cells ($n=20$, $p<0.001$). Further, analysis of various neuroserpin and tPA mutants suggested a direct interaction between the two proteins within the secretory pathway. **Conclusion:** We have identified an interaction between tPA and neuroserpin that enhances the sorting of neuroserpin to the regulated secretory pathway. This interaction may have implications for the regulation of synaptic structure and neuronal function.

ORAL-01-06

ESTIMATING SYNAPTIC CONDUCTANCE CHANGES DURING UP AND DOWN STATES

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Purpose: Spontaneous cortical activity is often characterised by so-called Up and Down states, where the membrane potential fluctuates between depolarised and hyperpolarised levels. Despite poor space-clamp somatic voltage-clamp has been widely used to assess the nature of the synaptic activity underlying Up and Down states, leading to the conclusion that during Up and Down states excitatory and inhibitory synaptic activity is balanced. Here we use simulations to assess the performance of the somatic voltage-clamp technique to accurately estimate synaptic conductance changes occurring during Up and Down states. **Methods:** Using the NEURON simulation software, we constructed a simplified multi-compartment pyramidal neuron model. Excitatory and inhibitory synapses were spatially distributed and activated by a Poisson process. Voltage-clamp "electrodes" were added to somatic and dendrite compartments. **Results:** Somatic voltage-clamp consistently underestimated the contribution of synaptic activity in dendritic compartments. Dendritic voltage-clamp was better at estimating dendritic synaptic activity, however, was unable to accurately capture somatic activity. By analysing a two-compartment circuit model we derived analytical expressions for these voltage-clamp errors, which were in agreement with simulations based on multi-compartment models. **Conclusion:** Our analysis indicates that somatic voltage-clamp cannot be used to accurately estimate the synaptic conductance changes underlying Up and Down states when those conductances arise from sites distal to the somatic recording location. These results question the interpretation of experimental data suggesting a dynamic balance of excitatory and inhibitory synaptic activity during cortical Up and Down states.

ORAL-01-07

LOCAL FACILITATION OF DRAGONFLY HYPERCOMPLEX NEURONS

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Neurons within the dragonfly (*Hemicordulia tau*) lobula complex respond selectively to the visual presentation of moving targets. These neurons, referred to as 'small target motion detectors' (STMD), have characteristics similar to hypercomplex cells in the mammalian cortex[1]. One surprising attribute of these neurons is their selectivity for small features at the limits of optical resolution. Recent evidence suggests that a form of facilitation may be an important component of reliable target detection. We recorded from the dragonfly centrifugal STMD neuron (CSTMD1) (n=6) and tested for the presence of an underlying facilitation mechanism. We presented the dragonfly with visual stimuli consisting of temporally-continuous yet spatially-discontinuous vertically drifting targets. We recorded neural responses from 5 separate but continuous paths within a region (16° by 40°) of the receptive field of CSTMD1. Additionally, we segmented these trajectories into discontinuous parts over the same region (path segments of 6°, 10° and 20°). That is, we could compare between continuous and discontinuous pathways with the same total motion energy. We observed that increasing segment length (a longer trajectory) resulted in increased neural responses. To elucidate the scale and time course of this facilitation we also drifted targets along a single trajectory, whilst applying a discontinuity in either time, space or both. The results suggest that facilitation is spatially local and the continuity of target motion is exploited to enhance the reliability of CSTMD1 responses. [1]Nordström & O'Carroll (2009) Trends in Neurosciences 32: 383-391.

ORAL-01-08

CHARACTERISATION OF THE EXPRESSION OF NMDA RECEPTORS IN HUMAN PRIMARY ASTROCYTES

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Aims: Astrocytes have long been perceived only as structural and supporting cells within the central nervous system (CNS). However, the discovery that these glial cells may potentially express receptors capable of responding to endogenous neurotransmitters has resulted in the need to reassess astrocytic physiology. The aim of the current study was to characterise the expression of NMDA receptors (NMDARs) in primary human astrocytes, and investigate their response to physiological and excitotoxic concentrations of the known endogenous NMDAR agonists, glutamate and quinolinic acid (QUIN). **Methods:** Primary cultures of human astrocytes were used to examine expression of these receptors at the mRNA level using RT-PCR and qPCR, and at the protein level using immunocytochemistry. The functionality role of the receptors was assessed using intracellular calcium influx experiments and measuring extracellular lactate dehydrogenase (LDH) activity in primary cultures of human astrocytes treated with glutamate and QUIN. **Results:** All seven currently known NMDAR subunits (NR1, NR2A, NR2B, NR2C, NR2D, NR3A and NR3B) are expressed in astrocytes, but at different levels. Calcium influx studies revealed that both glutamate and QUIN could activate astrocytic NMDARs, which stimulates $Ca2+$ influx into the cell and can result in dysfunction and death of astrocytes. Our data also show that the NMDAR ion channel blockers, MK801, and memantine can attenuate glutamate and QUIN mediated cell excitotoxicity. This suggests that the mechanism of glutamate and QUIN gliotoxicity is at least partially mediated by excessive stimulation of NMDARs. **Conclusion:** The present study is the first to provide definitive evidence for the existence of functional NMDAR expression in human primary astrocytes. This discovery has significant implications for redefining the cellular interaction between glia and neurons in both physiological processes and pathological conditions.

ORAL-02-01

TRANSCRIPTIONAL CONTROL OF GLIOGENESIS IN THE DEVELOPING MOUSE BRAIN AND IN GLIOBLASTOMA

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The transcription factor *Nuclear Factor One b (Nfib)* regulates gliogenesis within the developing mouse forebrain, but the mechanism underlying this process remains unknown. Here we show that *Nfib* promotes gliogenesis via repression of *Ezh2* expression. *Ezh2*, which encodes a chromatin modifying protein of the Polycomb complex, contributes to the repression of differentiation-specific genes epigenetically, via the tri-methylation of lysine residues on Histone H3. *Nfib-/-* mice fail to form mature glia within the forebrain, and levels of *Ezh2* are significantly up-regulated in these mice. NFIB binds to the promoter of the *Ezh2* gene *in vivo*, and is able to repress *Ezh2* promoter-driven transcriptional activity *in vitro*. Furthermore, the increased *Ezh2* expression in *Nfib-/-* mice culminates in downstream epigenetic chromatin modifications, including increased repressive histone modifications on tumour suppressor genes such as *CDKN2A*. Finally, alterations in *Nfib* expression may also be relevant to glioblastoma multiforme (GBM), the most aggressive form of brain cancer. Significantly, elevated levels of *Ezh2* are a known hallmark of GBM. *Nfib* is markedly down-regulated in human GBM and over-expression of *Nfib* in GBM cell-lines attenuates their proliferative capacity. Importantly, *Nfib* over-expression also induces a transient reduction in *Ezh2* expression and results in increased expression of glial differentiation-specific genes. Thus, these studies provide a mechanistic insight into how *Nfib* drives gliogenesis via repression of *Ezh2*, a finding with relevance both to the developing mouse forebrain and to human brain cancer.

ORAL-02-02

OLFACTORY ENSHEATHING CELLS PROLIFERATE FROM STEM CELLS AFTER INJURY

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Olfactory ensheathing cells (OECs) support the regeneration of olfactory sensory neurons throughout life. However, it remains unclear how OECs respond to a major injury and whether stem cells give rise to new OECs in those conditions. **Purpose**: To identify where OECs proliferate in the olfactory pathway and the regions to whic they migrate. **Methods**: We examined the proliferation and migration of OECs by surgically removing an olfactory bulb from neonatal mice. The outer layer of the olfactory bulb, the nerve fibre layer, is rich with OECs and thus bulbectomy removed the OECs of the olfactory bulb. The peripheral region of the olfactory nerve within the nasal cavity and the olfactory epithelium where the stem cells are located remained untouched. Proliferating cells were labelled by the thymidine analogue, ethynyl deoxyuridine (EdU) at different days after surgery (days 0-14) and animals (n=3 at each time point) were harvested either 4 hr later or up to 14 days later. **Results**: In the unilateral bulbectomy model, there was a large stimulation of OEC proliferation throughout the olfactory nerve up to 14 days after bulbectomy. Tracking cells that had proliferated revealed that stem cells lining the basal layer of the olfactory epithelium also gave rise to OECs that subsequently migrated along the length of the olfactory nerve. **Conclusion**: These results demonstrate that OECs actively respond to widespread degeneration of olfactory axons and that both local proliferation of OECs as well as stem cells give rise to new OECs that migrate along the olfactory nerve to the regions of need.

ORAL-02-03

THE ACTIVATION OF NEURAL PRECURSOR CELLS DURING PHYSICAL EXERCISE IS DEPENDENT UPON THE INVOLVEMENT OF THE GH ACTIVATION PATHWAY

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Purpose: There is growing evidence that growth hormone (GH) plays multiple roles within the central nervous system. Utilising a mutant mouse model we recently demonstrated a possible function of GH in the activation of neural precursor cells during voluntary exercise in the subventricular zone (SVZ) of aging animals. Here we examine wild-type mice to further define the pathway by which GH activates neural precursor cells in vivo and in vitro. **Methods**: Cohorts of 12 month-old female C57Bl/6 mice were placed into cages with or without access to running wheels. In order to examine changes in precursor numbers along the ventral neuraxis we employed a technique whereby serial coronal sections were collected by vibratome and prepared for the neurosphere assay. **Results**: Addition of GH to dissociated SVZ cells increased neurosphere number (696±14 vs. 958±27 per animal, $p<0.01$) whereas addition of a competitive GH antagonist, G120R, abolished this effect (667±47). To directly examine the effect of blocking the GH activation pathway during exercise, 12 month-old mice were implanted with osmotic pumps containing the GH antagonist. Following 21 days exercise, neurosphere numbers from vehicle only controls increased from 834±7 to 1320±43 ($p<0.01$) whereas GH antagonist infusion prevented this increase (675±67). **Conclusion**: These findings demonstrate that GH is essential for the exercise-dependent activation of SVZ precursor cells within the adult brain. This may present a novel method where neural precursors can be activated within aging and diseased brains to prevent precursor cell loss and demonstrates a mechanism by which activation is possible.

ORAL-02-04

TROPOMYOSINS INDUCE DIFFERENTIATION OF B35 NEUROBLASTOMA CELLS AND CONTROL NEURITE BRANCHING AND GROWTH CONE MORPHOLOGY

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The regulation of the actin cytoskeleton is critical in early mechanisms of neuronal differentiation and neurite formation. The dynamics and structural properties of the actin cytoskeleton are regulated by a number of actin associated proteins, including tropomyosin (Tm). In neurons, products from three different Tm genes (α-, γ- and δ-Tm gene) are expressed. **Purpose**: The current study aims to understand the role of α-, and δ-Tm gene products in early neuronal development. **Methods**: We used B35 neuroblastoma cells to investigate the impact of the actin cytoskeleton on neuronal morphology and early stages of neurite formation using stable clones of B35 cells overexpressing the Tm isoforms TmBr1, TmBr2, TmBr3 (α-Tm gene products) or Tm4 (δ-Tm gene product). **Results**: Our data show that overexpression of Tms is sufficient to induce neurite formation associated with an upregulation of the neuronal differentiation marker MAP2c. Tm gene products differentially control elongation and branching of processes in cAMP stimulated B35 cells. TmBr2 is the only Tm isoform that increases neurite length. TmBr1 attenuates while TmBr3 and Tm4 increase the degree of branching as compared to control B35 cells (n=450). The differential impact of Tm isoforms on neurite branching is associated with an isoform dependent change of growth cone size: TmBr3 and Tm4 induce an increase while TmBr1 induces a decrease in size. **Conclusion**: This association implicates Tms in the regulation of neurite branching by impacting on the actin cytoskeleton in the growth cone compartment. Our work provides strong evidence for a central role for Tms in neuronal differentiation and the establishment of complex neuronal networks.

ORAL-02-05

PROBDNF INHIBITS NEURITE OUTGROWTH BY P75 NEUROTROPHIN RECEPTOR MEDIATED ACTIVATION OF RHOA

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ProBDNF inhibits neurite outgrowth by p75 neurotrophin receptor mediated activation of RhoA Ying Sun, Rainer Haberberger, Xin-Fu Zhou* Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia *Corresponding author Purpose: This study investigated whether the Brain-derived neurotrophic factor (BDNF) precursor (proBDNF) has a physiological role in the neurite growth in vitro, and examined the down streaming signalling pathway. Methods: Dorsal root ganglia (DRG) from adult SD rats (n=6) and the cortex from neonatal C57BL/6 (n=12) mice were cultured. Live images were collected every 6 sec for 30 min by using Nikon BioStation. Primary DRG neurons were incubated in proBDNF at various concentrations and the neurite lengths were measured. Cortical neuron cells were treated with 50 ng/ml proBDNF, and cell lysates were tested for total and activated RhoA and cdc42 by Western blot. Data are mean ± SEM from 3 independent experiments. Results: Live imaging showed the clear collapse of DRG growth cone in response to proBDNF (50 ng/ml). ProBDNF caused a 34% decrease in the extension rate after 6 min and maintained throughout at 30 min treatment. ProBDNF (10, 30, 100 ng /ml) resulted in a 50% decrease in neurite length (58.6 µm vs 118.1 µm) compared with control group. Using ELISA proBDNF and BDNF levels in response to 45mM KCl were determined. After 5 min BDNF pretreatment, RhoA activity was increased by 5 times in cortical neurons, the results were confirmed by immunohistochemistry. Conclusion: Live imaging demonstrated that proBDNF repulsed DRG growth cones and induced the neurite collapse of cortical neurons in neonatal mice. Statistical analysis showed the dose-dependent effect of proBDNF on neurite collapse. ProBDNF were released from DRG neurons. proBDNF rapidly activated RhoA without affecting the cdc42 level. Thus, proBDNF inhibits neurite growth presumably via activation of RhoA.

ORAL-02-06

WHEN DO CORTICAL INTERNEURONS ARREST IN THEIR FINAL POSITIONS?

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Purpose: Cortical interneurons are generated in the medical ganglionic eminence and undergo tangential migration to enter the neocortex. The tangential migratory route is determined by the birth-date, such that early born interneurons [embryonic (E) day 12-14] migrate through the marginal zone, whilst late born interneurons [E15-17] migrate through the subventricular zone. Once within the neocortical domain, interneurons embark on radial migration to reside within the six-layered cortical plate with layer specification corresponding to their contemporaneously-born pyramidal neurons. The question remains of when these interneurons arrive at a final destination? **Methods**: I.P. BrdU injections were conducted on GAD67-knockin-GFP mice at E12.5 (early-), E14.5 (mid-) and E16.5 (late-neurogenesis) and the position of BrdU+/GFP+ interneurons determined at E18.5 (n = 3), postnatal (P) day1 (n = 3) and P7 (n = 3). At each time-point, the cortical-plate was divided into 10 equidistant bins to determine the distribution of the BrdU+/GFP+ cells. **Results**: Our analysis has revealed that mid-born interneurons are the last to position into layer IV and reside for the first postnatal week in the marginal zone. Early-born interneurons reach a final destination in the lower layers V/VI by the end of the first post-natal week. Interestingly, late-born interneurons are the first to position in upper layers II/III with the majority of interneurons positioned by P1. **Conclusion**: This study has demonstrated that the timing of interneuron arrest is not necessarily determined by the order of birth-date. Instead, local factors residing in the different cortical layers may dictate when interneurons should arrive and settle.

ORAL-02-07

GENERATING FLOOR PLATE PROGENITOR CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Generation of mesencephalic dopamine (mesDA) neurons from human embryonic stem cells (hESC) requires several stages of signalling from various extrinsic and intrinsic factors. To date, most methods incorporate exogenous treatment of Sonic hedgehog (SHH) to derive mesDA neurons. However we and others have shown that this approach is inefficient for generating FOXA2+ cells, the precursors of mesDA neurons. Since mesDA neurons are derived from the ventral floor plate (FP) regions of the embryonic neural tube, we sought to develop a system to derive FP cells from hESC. We show that forced expression of the transcription factor GLI1 in hESC at the earliest stage of neural induction, resulted in their commitment to FP lineage. The GLI1+ cells co-expressed FP markers, FOXA2 and Corin, and displayed exocrine SHH activity by ventrally patterning the surrounding neural progenitors. This system results in 63% FOXA2+ cells at the neural progenitor stage of hESC differentiation. The GLI1-transduced cells were also able to differentiate to neurons expressing tyrosine hydroxylase. This study demonstrates that GLI1 is a determinant of FP specification in hESC and describes a highly robust and efficient *in vitro* model system that mimics the ventral neural tube organizer.

ORAL-02-08

MAGNETIC RESONANCE SPECTROSCOPY OF 1.28PPM SIGNAL IN LIVE HUMAN BRAIN REFLECTS NEUROGENESIS-RELATED PATTERN

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Purpose NMR spectroscopy has revealed that neuroprecursors exhibit a fatty-acid related signal in vitro at 1.28ppm. This signal has been advanced for human in vivo investigation using MR spectroscopy, but has generated fierce controversy. On the basis of animal neurogenesis studies we therefore propose three key validation criteria: 1) Topographic specificity in hippocampus and subventricular zone, 2) Age-dependent decline, and 3) Robust response to electroconvulsive therapy (ECT). **Methods** 3 healthy young adults were scanned on a 3Tesla Philips Achieva MRI for protocol optimization and signal topography. A 3D T1-weighted scan was followed by MRS in 3 regions of interest: hippocampus, adjacent to lateral ventricle (LV), and frontal white matter (FWM). 5 older adults were also scanned to assess age-related effects, and 10 patients with major depression were scanned before a prescribed course of ECT as well as after their first ECT session. The 1.28ppm signal quantification used the algorithm of P.D. **Results** 1.28ppm metabolite concentration was significantly higher in LV (0.32 ± 0.3mmol/L) than in the hippocampus (0.02 ± 0.03 mmol/L), and absent in the FWM. There was a significant 87% decrease in signal in our older volunteers compared to younger individuals. 1.28ppm signal increased significantly by 5.5times in the hippocampus after the first ECT session ($F = 2.42$, $p=0.02$). **Conclusion** Human use of the 1.28ppm signal agreed with three main predictions drawn from the animal literature. This signal therefore appears to quantify a neurogenesis-related biomarker, and our study provides the first evidence that a neurogenesis-related response can be measured in humans after psychiatric intervention.

ORAL-03-01

CONE PHOTORECEPTOR DYSTROPHY WITH SUPERNORMAL ELECTRORETINOGRAM: FUNCTIONAL ANALYSIS OF MUTATIONS IN THE KV8.2 VOLTAGE-GATED POTASSIUM CHANNEL PROTEIN

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Purpose: We have previously shown that the unique disorder of cone dystrophy with supernormal ERG arises from mutations in the *KCNV2* gene which encodes the voltage-gated Kv8.2 potassium channel protein. Kv8.2 belongs to a group of electrically-silent modulatory channel subunits that do not produce a current as a homotetramer but interact with other potassium channel subunits to form functional channels. The main objective was to establish the mechanism of mutant gene action at the molecular, cellular and physiological level. **Methods:** A yeast two-hybrid approach was used to establish the partner channel for Kv8.2. Transient transfected mammalian cells were used to examine cellular trafficking of mutant proteins and channel activity using whole cell voltage clamping. **Results:** Kv2.1 was identified as an effective partner for Kv8.2. The location of amino acid substitutions within the Kv8.2 molecule encoded by missense mutations determine whether mutant protein was transported to the cell membrane. Mutant proteins either showed a reduced ability to reduce the current amplitude of Kv2.1 or largely obliterated the current. **Conclusion:** Missense mutations within or close to the tetramerisation domain at the amino terminus of Kv8.2 disrupt the interaction with Kv2.1, resulting in the absence of Kv8.2/Kv2.1 at the membrane. Some mutations in the pore domain allow the formation of heteromeric channels, but these channels are unable to conduct potassium ions. Others allow the formation of heteromeric channels but these channels are retained intracellularly, resulting in reduced heteromeric channels at the membrane.

ORAL-03-02

MODULATION OF SYNAPTIC TERMINAL IN A LIGHT DAMAGE MODEL OF RETINAL DEGENERATION

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Purpose: To assess the effect of modulating neurotransmitter and channel activity at the photoreceptor synaptic terminal on retinal morphology and cell death in a light-damaged model of retinal degeneration. **Method:** Sprague-Dawley rats raised in a 12hour light (300 lux): 12 hour dark cycle and exposed to intense light (2700 lux) for 24 hours were deeply anaesthetized to receive an intravitreal injection of 33U botulinum-A (Btx-A, Dysport). Control samples consisted of non-exposed eyes injected with Btx-A and light-damaged eyes injected with saline. Eyes were collected either at 3 hours or 24 hours after Btx-A or saline treatment. They were subsequently processed for cryo-sectioning and immunocytochemical labelling using macromolecular markers of neuronal types (calretinin, calbindin, PKCα) or markers of the synaptic terminal (synaptophysin, SNAP-25). Cell death was detected using a TUNEL labeling kit. Retinal sections were analysed using high magnification confocal microscopy. **Results:** There was a similar expression pattern of synaptic terminal proteins SNAP 25 and synapthophysin in control retinas and light damaged retinas injected with saline. Light damaged retinas treated with Btx-A showed a reduced expression of synaptic terminal proteins in both the outer plexiform layer (OPL) and inner plexiform layer. Light damage increased the OPL thickness of PKCα labelling signifying changes in rod bipolar cells. The light exposed retina treated with Btx-A showed a reduction in OPL thickness similar to control levels. TUNEL labeling showed a significant 50% reduction ($p < 0.01$, ANOVA) in treated samples at both 3 and 24 hours post- Btx-A treatment. **Conclusion:** Modulation of a photoreceptors synaptic terminal activity is a candidate for intervention strategy in retinal degeneration caused by photoreceptor death.

ORAL-03-03

MOTOR PROTEIN TRANSPORT IN RETINAL HEALTH AND DEGENERATION

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Motor proteins play critical roles in polarized cells, such as neurons, where their failure is often linked to disease. Photoreceptor and the adjacent retinal pigment epithelial (RPE) cells are extraordinarily polarized and functionally specialized. We have used a combination of genetics, molecular biology, and live-cell imaging to study motor transport of proteins along the axonemal connection between the inner and outer segments of the photoreceptor cells, and organelle transport in the RPE. Here, we report our results on the latter. Mutations in MYO7A, which encodes an unconventional myosin, cause Usher syndrome 1B a deaf-blindness disorder. In the RPE of mice lacking myosin-7a, the localization and motility of phagosomes and melanosomes are defective. Analyses of the velocities of the organelles indicate that their motility results from a coordination of myosin-7a and microtubule motors, which are typically faster than myosins. RPE cells that are deficient in kinesin motor activity have impaired ability to digest phagosomes, and develop an histopathology that is very similar to that found in age-related macular degeneration. Interestingly, compromised kinesin-1 function in cholinergic neurons increases amyloid deposits in mouse models of Alzheimer's disease, consistent with a proposed link between these two age-related neurodegenerative disorders.

ORAL-03-04

NEURONAL REMODELLING IN PRIMARY RHEGMATOGENOUS RETINAL DETACHMENT

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Purpose: Rhegmatogenous retinal detachment (RRD) is a prevalent medical emergency that causes photoreceptor loss and is an important cause of retinal remodelling after proliferative vitreo-retinopathy. This study aims to qualitatively characterize the anatomical features of retinal remodelling in inner retina (second-order neurons) from human primary RRD. **Methods:** Three retinal specimens were surgically obtained from patients presenting primary RRD of estimated duration of 2 weeks, 1 month and 3 months. These specimens were obtained as biopsy samples and were fixed in 4% paraformaldehyde for 30 minutes before processing them for immunocytochemistry. Frozen vertical sections were double-labelled for confocal microscopy with antibodies detecting cholinergic amacrine cells and ON bipolar cells (islet-1), cone OFF bipolar cells, amacrine and ganglion cells (calbindin), horizontal cells (parvalbumin) and synaptic terminals (synaptophysin). Specimens were compared to normal control retinas obtained from enucleated eyes from uveal melanoma surgeries. **Results:** RRD causes photoreceptors dysfunction and subsequent loss. Second-order neuronal remodelling in primary RRD was characterized by aberrant expression of synaptophysin in the outer nuclear layer (ONL) associated with dendritic outgrowth and aberrant dendritic morphology from horizontal and bipolar cells. Horizontal cell axons labelled with calbindin presented abnormal projections towards the inner retina. Some Islet-1-immunopositive bipolar cells were found in the ONL. **Conclusions:** Retinal photoreceptor degeneration promotes important morphological alterations in the second-order neurons. Adult human retina in primary RRD responds in a stereotyped manner to photoreceptor degeneration mimicking developmental process in the attempt of re-establishing a neuronal circuitry after deafferentation.

ORAL-03-05

NOVEL NEUROPROTECTIVE THERAPIES FOR RETINAL DAMAGE

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Purpose: Dietary saffron and exposure to 670nm red light have both been shown to have neuroprotective effects. In this study we aimed to identify if genes were differentially regulated by dietary saffron, or pretreatment exposure to 670nm light, using a light-damage (LD) rat model to induce retinal damage. **Methods:** Experimental groups were either pretreated with 9 J/cm2 of 670 nm light daily for 5 days or fed a diet rich in saffron (1 mg/kg/day) for 21 days. RNA from four retina in each of the six experimental groups (control, LD, saffron, 670 nm light, saffron with LD, and 670 nm light with LD) was hybridized to Affymetrix rat genome ST arrays in triplicate (total 18 arrays). **Results:** Of 175 LD regulated entities, pretreatment with 670 nm light reduced levels of expression of 126; dietary saffron pretreatment reduced the expression of 53 entities, of which 50 were downregulated in common with 670 nm light. Pretreatment with 670 nm light regulated expression of 67 entities not regulated by LD, while saffron pretreatment regulated 122 entities not regulated by LD (48 in common with 670 nm light). Ninety percent of the entities regulated by LD were known genes, while non-coding RNAs were prominent among the entities regulated by pretreatment with 670 nm light and saffron in the LD retinas (73% and 62%, respectively). **Conclusions:** These data provide an overview of gene expression induced by two neuroprotectants, and show they have specific effects on gene regulation. The results provide a platform for exploring mechanisms of neuroprotection.

ORAL-03-06

CANNABINOIDS MODIFY VISUAL SIGNALLING IN THE RETINA

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Endocannabinoids and their receptors have been localised to all retinal cells. The endocannabinoid system modulates transmitter release and plays an important role in short term plasticity of excitatory and inhibitory synapses in the central nervous system. Upon depolarisation of postsynaptic neurones, cannabinoids are synthesized on demand and retrogradely travel to activate presynaptic cannabinoid receptors (CB1R), which in turn reduce neurotransmitter release. The physiological roles of the endocannabinoid system in the retina, however, are still unknown. In addition, these modulatory mechanisms of neuronal excitability are likely to be disrupted by the addition of exogenous cannabinoids. **Purpose**: To investigate the effects of cannabinoids on synaptic inputs into retinal ganglion cells (RGCs) and on their light responses. **Methods**: Whole cell patch clamp recordings were obtained from RGCs in the whole mount preparation of the dark adapted mouse retina. The effects of a cannabinoid receptor agonist WIN55212-2 (5μM) on spontaneous synaptic currents in both young (P14-P21) and adult mice (>6 weeks) as well as on the receptive field organisation of RGCs was investigated. **Results**: Overall it was found that cannabinoids reversibly reduced the frequency of excitatory and inhibitory spontaneous synaptic currents in RGCs ($P<0.05$, n=31) in both age groups, without affecting their amplitude distribution. The strongest effect was a reduction of GABAergic currents in young mice. Cannabinoids also reduced the magnitude of light-evoked responses (both spikes and light-evoked postsynaptic potentials) in all RGCs classes (9/9 ON-, 9/11 OFF- and 5/6 ON-OFF-RGCs). In most cases, cannabinoids also decreased the inhibitory effect of surround stimulation. **Conclusion**: Our data demonstrates that exogenous cannabinoids modify spontaneous and light-evoked synaptic inputs into RGCs by affecting neurones presynaptic to RGCs and suggest that the endocannabinoid system modulates neuronal excitability in the retina.

ORAL-03-07

CONTRAST ADAPTATION IN RETINAL GANGLION CELLS OF THE MOUSE

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Purpose: Sensory neurones adjust their sensitivity to optimise the signals they provide about their local environment. In the retina, ganglion cells (RGCs) adapt to image contrast -sensitivity is highest in low contrast environments. It is not clear whether this is the product of changes in excitation, inhibition or both. To study this, we recorded from 3 classes of RGCs: ON-alpha sustained (n=6), ON-direction selective (DS, n=5) and OFF-alpha sustained (n=8). **Methods**: Recordings were made in loose patch and whole-cell configurations in the whole mount retina. The visual stimulus was a small sinusoidally modulated spot (3 Hz) of variable contrast presented for 1 sec. Responses were obtained after prolonged exposure to a high contrast stimulus or a blank screen of mean luminance. RGCs were filled with Lucifer yellow for morphological reconstruction. **Results**: Contrast sensitivity of spike rates and excitatory inputs was reduced by adaptation in all cell classes. Inhibition was largely absent in ON-sustained cells. In ON-DS cells inhibition and excitation in the ON phase were reduced by adaptation; in the OFF phase both were less susceptible to adaptation. Inhibition in OFF alpha cells was reduced by adaptation in the ON phase and increased in the OFF phase. **Conclusions**: Excitatory and inhibitory inputs in all RGCs studied are modified by adaptation to high contrast. Reduction of inhibitory inputs in ON-sustained and ON-DS cells is inconsistent with the reduction in spike rate, which may suggest the primacy of excitatory inputs in determining spike output in these cells. In contrast, adaptation in the spike output of OFF-alpha cells is magnified by adaptation of both excitatory and inhibitory inputs.

ORAL-03-08

IRRADIANCE DETECTION IN VERTEBRATES: EVOLUTION OF MELANOPSIN GENES

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Purpose: Vertebrates possess two photosensory systems. The first is responsible for image formation by the eye and the second provides irradiance detection that mediates circadian photoentrainment. In mammals, both systems are restricted to the lateral eye, but in non-mammalian vertebrates, there are many non-ocular sites that possess novel photoreceptors. The melanopsins, an ancient class of opsin pigments, are perhaps the best characterized of the non-visual photopigments found at these sites. They are encoded by two gene lineages: 'xenopus-like' (*opn4x*) melanopsin and 'mammalian-like' (*opn4m*) melanopsin. The two isoforms are found in teleosts, amphibians, reptiles and birds, but mammals retain only *opn4m*. The objective of the present study was to determine the origin of the two isoforms of melanopsin within the vertebrate lineage. To achieve this, melanopsin gene sequences have been isolated from two species taken from the base of the vertebrate radiation, a jawless lamprey, *Petromyzon marinus*, and a jawed chimaerid holocephalan, the elephant shark *Callorhinchus milii*. **Methods:** Bioinformatic and molecular biology techniques were used to identify and isolate melanopsin genes. **Results:** Our results show that the elephant shark possesses both *opn4x* and *opn4m* isoforms, whereas only a single form of melanopsin is found in the lamprey. **Conclusion:** The gene duplication event that gave rise to the two melanopsin isoforms occurred subsequent to the separation of the two major vertebrate lineages around 450-540 million years ago. Additionally, there has been a further duplication of the *opn4m* gene in the elephant shark, thus extending the photosensitive capability of this species.

ORAL-04-01

ELEVATED SPONTANEOUS ACTIVITY IN THE VENTRAL COCHLEAR NUCLEUS AFTER COCHLEAR TRAUMA

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The emergence of elevated spontaneous neuronal firing rates (hyperactivity) after cochlear trauma, has been well documented in a number of central auditory structure, including the auditory cortex, inferior colliculus and dorsal subdivision of the cochlear nucleus. Hyperactivity is of interest as a possible neural substrate of tinnitus. Surprisingly, the ventral subdivision of the cochlear nucleus has never been investigated, despite the fact that like the dorsal division, it also receives direct input from the damaged cochlea, and supplies major ascending inputs to brainstem and midbrain auditory centres. **Purpose**: We therefore investigated spontaneous neuronal firing rates in the ventral cochlear nucleus in a guinea pig model of cochlear trauma in which we have shown that hyperactivity consistently develops in the inferior colliculus (Mulders and Robertson, 2009). **Methods**:Single neuron extracellular recordings were made in anaesthetized animals 2 weeks after a cochlear trauma. Neurons were classified according to the shape of their peri-stimulus time histograms. **Results**:The mean spontaneous firing rate of ventral cochlear nucleus neurons was significantly elevated compared to sham controls. This hyperactivity was confined to primary-like and onset types of neurons. **Conclusions**: The presence of hyperactivity in the ventral subdivision of cochlear nucleus needs to be considered in relation to neural models of the genesis of tinnitus. Mulders WHAM and Robertson D.(2009) Neuroscience. 164:733-46.

ORAL-04-02

TOPOGRAPHIC RELATIONSHIP BETWEEN HEARING LOSS AND INCREASED SPONTANEOUS ACTIVITY IN AUDITORY BRAINSTEM

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A common consequence of hearing loss is tinnitus, a phantom hearing sensation. A proposed neural substrate for tinnitus is increased spontaneous activity, or hyperactivity in central auditory pathways. Recent studies in our laboratory have suggested a correlation between the frequency region of hearing loss and frequency regions in auditory brainstem showing hyperactivity. However, in these studies only high frequency hearing loss was investigated. Purpose: To further investigate the relationship between the frequency region of hearing loss in cochlea and the tonotopic distribution of hyperactivity in guinea pig inferior colliculus. Methods: Adult guinea pigs were exposed unilaterally to a low (5 kHz, 122 dB SPL, n=4) or high frequency (10 kHz, 124 dB SPL, n=4) continuous tone for 2 hours or received surgery (shams, n=4) without sound exposure. After 2 weeks recovery spontaneous firing rates and characteristic frequency of inferior colliculus neurons were obtained using extracellular single neuron recordings. Results: Low frequency and high frequency tone exposure resulted in low and high frequency hearing loss in the cochlea, respectively. Sham surgery did not affect hearing thresholds. Both tone exposure paradigms resulted in a general increase in spontaneous firing rates (hyperactivity) as compared to sham animals. However, hyperactivity was present in the low but not the high frequency regions of the inferior colliculus after low frequency hearing loss whereas the reverse was true for high frequency exposed animals. Conclusion: These data suggest a close correlation between the tonotopic regions of hearing loss and hyperactivity, in line with observations in human patients that perceived tinnitus pitch is correlated with hearing loss frequencies.

ORAL-04-03

SOMATOSTATIN RECEPTOR-1 AND -2 IN THE MAMMALIAN COCHLEA

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Little is known about expression and function of the somatostatinergic system in the mammalian cochlea. In this study, we analyzed the expression of somatostatin receptor 1 (SST1), somatostatin receptor 2 (SST2) and somatostatin in the immature and mature mammalian cochlea. We demonstrate that the SST1 and SST2 are expressed in outer and inner hair cells (HCs) of the organ of Corti (OC) as well as in defined supporting cells. A similar expression of the SST1 and SST2 receptors in inner and outer hair cells was found in cultivated p6 mouse organ of Corti explants. In order to further characterize the localization of somatostatin receptors during development of inner ear, we have done double immunostainings of SST1 and SST2 with the Hes5 expressed only in the cochlea during embryonic development and calbindin, which served as the marker of sensorineural epithelium in the cochlea of postnatal and adult mice. In addition, we detected different SST1 and SST2 mRNA level in developing cochlea and show that functional maturation of the OC involves changes in the expression patterns of these two receptors. Interestingly, somatostatin itself is not expressed in the mammalian cochlea, suggesting that somatostatin reaches its receptors either trough the blood-labyrinthine barrier from the systemic circulation or via the endolymphatic duct from the endolymphatic sac. In order to learn more about the regulation of SST1 and SST2 receptors, we used mice with either a deletion of SST1 or SST2. We demonstrate that in SST1 knock-out mice, SST2 is expressed in outer HCs and Deiter's cells, but not in pillar cells and inner HCs as compared to wild-type mice. In contrast, in SST2 knock-out mice, the expression pattern of SST1 receptor is not altered compared to wild-type mice. These findings provide evidence of a compensatory regulation in the mammalian cochlea as a consequence of a distinct somatostatin receptor deletion. Since compensatory events can be observed after SST1 deletion but not after SST2 deletion, this indicates that the compensatory event is receptor subtype specific.

ORAL-04-04

THE EXPRESSION OF GABAA RECEPTOR SUBUNIT ALPHA1, GLUTAMIC-ACID DECARBOXYLASE-67, CALBINDIN, N-METHYL-D-ASPARTATE RECEPTOR SUBUNIT 2A IN RAT AUDITORY PATHWAY FOLLOWING NOISE-INDUCED HEARING LOSS

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Purpose: Excessive exposure to loud noise or a mechanical insult results in damage to the cochlea, which can lead to a range of neuronal changes in key nuclei in the auditory pathway. Changes that have been observed include plasticity of tonotopic organisation, changes in the pattern of spontaneous activity and in the balance of excitatory and inhibitory transmitter systems. Moreover, cochlear hearing loss is associated with tinnitus in humans. This suggests that these changes may be involved in generating tinnitus, although the mechanisms remain unknown. In an attempt to determine which area(s) may be involved in the generation of noise-induced tinnitus, we are investigating changes at a number of levels of the auditory pathway; auditory cortex (AC), inferior colliculus (IC) and dorsal cochlear nucleus (DCN), primarily focusing on excitatory/inhibitory transmission. **Methods:** Long Evans rats (n = 15) were unilaterally exposed to 16 kHz bandpass (1/10th octave noise (115 dB SPL)) for 1-hour. Rats were euthanased at 0, 4, 8, 16 or 32 days following exposure and were processed for western blot analysis to identify $GABA_A$ receptor subunit α1 ($GABA_ARα1$), Glutamic-Acid Decarboxylase-67 (GAD-67), Calbindin (Calb1) and N-Methyl-D-Aspartate receptor subunit 2A (NR2A). **Results:** Following exposure we saw an immediate increase of NR2A in the contralateral and ipsilateral AC. We observed a significant decrease in GAD-67 in the ipsilateral and contralateral DCN over the 32 days following exposure. An overall decrease of Calb1 was also observed in AC and IC and an overall increase in the DCN. **Conclusion:** These changes may reflect an attempt to rebalance excitatory and inhibitory transmission, and may be related to the perception of tinnitus.

ORAL-04-05

AGE-RELATED HEARING LOSS IN P2X$_2$ KNOCKOUT MICE REARED IN A QUIET ENVIRONMENT FROM BIRTH

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ATP-gated cation ion channels assembled by P2X$_2$ receptor subunits are widely expressed in the cochlear sensorineural tissues and contribute to cochlear electrochemical homeostasis. **Purpose:** Here we investigated whether P2X$_2$ receptor expression affects age-related hearing loss (ARHL). **Methods:** P2X$_2$ knockout (P2X$_2$KO, n=10) and C57/BL6 wild-type (WT, n=11) mice were reared in an acoustically attenuated quiet chamber from birth. At 12 month of age, the mice were anaesthetized (ketemine/ xylazine/ acepromazine, i.p.) and tested for auditory function by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). The latter reflects sound transduction through the cochlear outer hair cells. **Results:** Both strains displayed considerable ARHL across the tested frequency range (4 to 32 kHz) with mean ABR thresholds of 40 –75 dB SPL for WT and 61 – 84 dB SPL for P2X$_2$KO. ABR thresholds for click stimuli averaged 10 dB higher for the P2X$_2$KO (65 dB SPL). Two-way ANOVA indicated that the effect of genotype was highly significant ($p<0.0001$). The DPOAE thresholds were also significantly higher in the P2X$_2$KO (12-20 kHz). **Conclusion:** The lack of P2X$_2$ signalling caused exacerbated hearing loss across all test frequencies with ageing. The higher DPOAE thresholds in the P2X$_2$KO mice implicate outer hair cells in the P2X$_2$ receptor mediated protection against ARHL.

ORAL-04-06

PARCELLATION OF PRIMATE AUDITORY CORTICAL AREAS USING THE DOPAMINE PRECURSOR TYROSINE HYDROXYLASE

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The distribution of modulatory neurotransmitters across the numerous auditory-related areas of the nonhuman primate cerebral cortex is relatively unknown, despite neuropharmacological and functional data suggesting that these substances strongly influence sensory information processing and higher order behaviours. **Methods:** The distribution of the dopamine precursor enzyme tyrosine hydroxylase (TH) across the superior temporal cortex was studied in 5 marmosets (*C. jacchus*) and 3 macaques (2 *M. fascicularis* and 1 *M. nemestrina*). Coronal sections (50 µM thickness) were taken from the temporoparietal junction to the terminus of the temporal lobe, and auditory areas were identified by cyto-, chemo-, and myelo- architectural features. Anti-TH immunohistochemistry and light microscopy were used to identify TH-immunoreactive tissues in each area. **Results:** Fibre density and cell body labelling were compared across the auditory core, belt, and parabelt regions, as well as temporal lobe association areas known to receive strong auditory input, e.g. the temporal pole and insular cortex. TH immunoreactivity in all areas was most prominent in the horizontal fibers of layers 1 and 2. In both genera, a gradient of increasing TH immunoreactivity was observed which followed the hierarchy of the current model of auditory cortex. In all cases, immunoreactive fibres in the middle and deep cortical layers (3-6) were sparse in the core areas, increased somewhat in the belt region, and were most dense in the auditory parabelt region. **Conclusions:** Our data support division of the auditory cortex into a parallel hierarchy of core, belt, and parabelt areas. These findings also suggest that dopamine may exert a greater influence on auditory processing at the level of the parabelt and belt, relative to core areas. Finally, the observed pattern is consistent between New World and Old World primates.

ORAL-04-07

CONNEXIN EXPRESSION IN THE COCHLEA OF MICE WITH ACCELERATED HEARING LOSS

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The C57BL/6 mouse progressively loses hearing after birth and the pathology includes loss of sensory cells and fibrocytes of the lateral wall. Fibrocytes, connected by gap junctions, play an important role in intercellular communication in the cochlea and buffering extracellular potassium. The impact of the progressive loss of fibrocytes on intercellular communication pathways and development of hearing loss is currently unknown. **Purpose:** This study investigated the expression and localisation of connexins (Cx26, Cx29, Cx30, Cx43) in the cochlear lateral wall of the C57BL/6 mouse at different ages. **Methods:** Tissue was collected from C57BL/6 mice at 1 (n=10), 3 (n=9), 6 (n=8) and 12 months (n=8). Real time qPCR was used to quantitate gene expression levels and immunohistochemistry to localise the expression of these gap junction proteins in the cochlea, with a focus on the lateral wall. **Results:** There was no change in gene expression of Cx26, Cx29, Cx30 and Cx43 up to 6 months of age, but they all showed declining expression at 12 months, which was most significant with Cx29. There was a distinct expression pattern within the cochlear lateral wall for each connexin protein as reported previously. Cx26 and Cx30 were highly expressed in the cochlea, while Cx29 and Cx43 were expressed at lower levels. **Conclusion:** Cx expression was confirmed in the C57/BL6 mouse and there was a decline in Cx expression with the progressive development of structural pathology and hearing loss. Approved by the University of Auckland Animal Ethics Committee.

ORAL-04-08

REGULATION OF PURINERGIC SIGNALLING IN THE COCHLEA

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In the inner ear, complex extracellular purinergic signalling pathways regulate cochlear homeostasis, sound transduction and auditory neurotransmission. In other tissues, these pathways are regulated through hydrolysis of extracellular nucleotides by membrane-bound ectonucleotidases (E-NTPDases). **Purpose:** This study investigated expression and distribution of two intracellular members of the E-NTPDase family, NTPDase5 and NTPDase6, in adult and developing cochlea, and their release into cochlear fluids under quiescent and stressed (noise) conditions. **Methods:** Inner ear tissues from Wistar rats were excised. Transcript levels of NTPDase5 and 6 were determined using quantitative RT-PCR and the enzyme distribution analysed with immunoperoxidase and confocal immunofluorescence. Cochlear perfusate was incubated *in vitro* with nucleotide substrates followed by RP-HPLC analyses to assess the presence of soluble enzymes. **Results:** mRNA expression of both enzymes was confirmed in rat cochlear tissues. Adult cochleae (n=5) showed strong NTPDase5 localisation in supporting Deiters cells and spiral ganglion neurons, while NTPDase6 was confined to the inner hair cells. Noise exposure (n=5) upregulated NTPDase5 mRNA and neuronal protein expression ($p<0.05$). NTPDase6 showed prominent expression in the developing hair cell bundles of embryonic and early postnatal cochleae (n=4 for each age point). RP-HPLC analysis provided evidence for the release of soluble NTPDase5 and NTPDase6 into perilymph. **Conclusions:** Spatial and temporal expression of NTPDase5 and NTPDase6 in adult and developing cochlear tissue provides support for their role in regulation of P2Y receptor signalling. Our study also supports a role of NTPDase5 in cochlear response to noise and NTPDase6 in hair cell development.

ORAL-05-01

NEURAL AND MYOGENIC PERISTALSIS IN THE ISOLATED RABBIT SMALL INTESTINE

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Trendelenburg described peristaltic contractions in the isolated rabbit intestine in response to fluid distension (1). Since then little work has been performed in the rabbit to investigate the properties of neural and non-neural responses to distension. Purpose: To investigate if propagating peristaltic contractions can be generated in isolated segments of rabbit intestine and to establish if these involve enteric neurons. Methods: We investigated spatio-temporal features of motor activity in isolated segments of intestine taken from 19 albino rabbits (killed by iv lethabarbital), cannulated and placed in a bath of oxygenated Krebs solution at 37 C. Spatio-temporal maps of changes in diameter were constructed from video recordings (2). Results: Slow distension (2-8 ml/minute) elicited in 13 out of 19 preparations, contractions of the circular muscle which propagated aborally with variable speeds (11.3 mm/s; SD= 4.37). The range was 7-22 mm/s depending on the backpressure of outflow cannula. They resulted in propulsion of contents measured as volume of fluid ejected aborally. TTX 0.6μM abolished the aborally directed propagating circular muscle contractions (neural peristalsis; n=11). Hexamethonium (200μM) blocked the propagating contractions in 2/4 experiments. In 10 out of 19 experiments after blocking neural activity, propagating circular muscle contractions synchronous with the propagating longitudinal muscle pendular contractions were observed. These circular muscle contractions propagated either orally and aborally at a faster speed than neural peristalsis (orally 24.5 mm/s, SD=19.9; aborally 15.3 mm/s, SD=4.1). Conclusions: aborally propagating neurally dependent circular muscle contractions can be elicited by luminal distension in the isolated rabbit small intestine (neural peristalsis). Non-neural orally and aborally propagating circular muscle contractions occurred only after neural activity is blocked, suggesting the presence of ongoing inhibitory neural influence on muscle excitability. The non-neural pattern of contractions propagating aborally corresponds to the previously described "myogenic peristalsis" (3). Whether myogenic peristalsis and antiperistalsis occurs physiologically remains to be established. Fast cholinergic nicotinic transmission is involved in neural peristalsis, indicating an important role of acetylcholine as a major enteric neurotransmitter also the rabbit enteric nervous system. References: (1). Trendelenburg, P. (1917). Naunyn-Schmiedeberg Arch. Exp.Pathol. Pharmakol. 81: 55-129; (2). Hennig et al (1999), J. Physiol., 517, 575-590; (3). Bortoff, A. (1976). Physiological Reviews 56(2): 418-34.

ORAL-05-02

EXTRACELLULAR RECORDING OF IDENTIFIED VISCEROFUGAL NEURONS IN GUINEA-PIG COLON

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Viscerofugal neurons form the afferent arm of reflex circuitry between the gut and prevertebral ganglia that modulate gastrointestinal motility. Previously, we used organotypic culture of guinea pig intestine to cause degeneration of extrinsic sensory axons, thus demonstrating that viscerofugal axons can be extracellularly recorded in mesenteric nerves. Purpose: To make extracellular recordings from identified viscerofugal axons in mesenteric nerves of acute preparations of intestine without prior organ culture. Methods: Flat-sheet preparations of guinea pig distal colon with circular muscle removed (n=8) were studied in vitro. Mesenteric nerve trunks were dissected and recorded with extracellular electrodes. The nicotinic receptor agonist, DMPP (1mM) was locally spritzed onto the tissue (10-30ms, 12psi) from fine micropipettes (5-10um tip). DMPP-sensitive sites were marked on printed micrographs of the preparation. Viscerofugal nerve cell bodies were labelled by biotinamide applied to the recorded nerve and compared to DMPP-responsive sites. Results: On average, DMPP-responsive sites (n=21) were 203±163μm from the nearest biotinamide-labelled viscerofugal cell bodies; significantly closer (p=2.3x10-8, t=5.8, df=229) than randomly generated sites (n=210) to biotinamide-labelled viscerofugal neurons (903±556μm). DMPP was applied to 186 myenteric ganglia in 8 preparations; of these 20 contained DMPP-responsive neurons projecting into the recorded mesenteric nerve. Of these 20, 16 ganglia contained biotinamide-labelled viscerofugal neurons. Of 162 DMPP-insensitive ganglia, 158 lacked viscerofugal cell bodies. Association between DMPP-sensitivity and the presence of retrogradely-labelled cell bodies was highly significant (chi squared p=0.000, 3 d.p.). DMPP-sensitive units were not sensitive to capsaicin (n=8, units=21). Conclusion: Identified viscerofugal axons are readily recorded in mesenteric nerves in freshly dissected preparations and can generally be distinguished from extrinsic sensory neurons by responsivity to locally applied DMPP but not capsaicin.

ORAL-05-03

ENTERIC NEURONS AT EARLY POST-NATAL AGES DIFFER IN ELECTROPHYSIOLOGY AND MORPHOLOGY FROM ADULT NEURONS

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Purpose: To investigate the electrophysiological and morphological development of different classes of myenteric neurons in the duodenum of postnatal mice. ***Methods:*** Preparations of myenteric plexus and attached muscle were dissected from postnatal day (P) 0, P10 and adult mouse duodenum and standard intracellular recordings were conducted. Electrodes contained biocytin so neuron morphology and projections could be determined. Synaptic potentials were evoked by applying 1 pulse or 10 pulses (20 Hz) to internodal strands via focal stimulation. ***Results:*** Myenteric neurons with AH- or S-type properties were present at P0. By P10, all S-type neurons (n=18) displayed fast excitatory postsynaptic potentials. Of note, 69% of P10 AH-type neurons displayed a prominent after-depolarizing potential (ADP) following an action potential evoked by 1 pulse stimulation at -100 mV holding potential (Amplitude: 40.7 ± 5.0 mV, Duration: 2.0 ± 1.0 s, n=11). A significantly smaller and shorter duration ADP was recorded in some adult AH-type neurons (Amplitude: 11.5 ± 0.5 mV, Duration: 77.2 ± 0.8 ms, n=4). Although the gut has not yet grown to its adult size, AH-type neurons (n=16) displayed adult morphology by P10. They had smooth cell bodies with multiple processes that projected up to 3 mm circumferentially. P0 and P10 S-type neurons had single axons that projected anally, orally or circumferentially. Unlike adult S neurons, lamellar dendrites were uncommon in neonatal S neurons, but all possessed filamentous dendrites. ***Conclusion:*** Postnatal AH- and S-type neurons differed in electrophysiology and morphology from their adult counterparts, and therefore undergo significant maturational changes during post-natal development.

ORAL-05-04

ELECTRICAL ACTIVITY OF EMBRYONIC ENTERIC NEURONS

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Purpose: Enteric neurons arise from neural crest cells that migrate into the gastrointestinal tract. A sub-population of enteric neural crest cells expresses pan-neuronal markers at early stages of development (E10.5 in the mouse). Our study examined when these cells become electrically active. Methods: We used calcium imaging and patch clamp electrophysiology to study preparations of dissociated E11.5 and E12.5 mouse gut. Results: A sub-population of neural crest-derived cells isolated from E11.5 mice responded to electrical field stimulation (20mA, 20Hz, 2s) with a sharp increase in intracellular calcium ion concentration ($[Ca^{2+}]_i$) similar to that observed in adult enteric neurons (n = 136). These cells were immunoreactive for the pan-neuronal marker PGP9.5. The proportion of responding PGP9.5+ cells and the amplitude of their responses increased between E11.5 and E12.5, and increased further in older embryos. The calcium transients were dependent on voltage-gated sodium channels and N-type calcium channels, as they were abolished or diminished by tetrodotoxin (TTX) and omega-conotoxin GVIA. Action potentials were recorded from preparations of E12.5 enteric neural crest cells using whole-cell patch clamp (n = 23). These were sensitive to TTX and lidocaine, but were not completely blocked by either or both in combination. Some cells fired tonically in response to a prolonged (500ms) depolarizing current, and others phasically. Conclusion: A sub-population of enteric neural crest derived cells that expresses pan-neuronal markers are electrically active at E11.5. Enteric neurons appear to be some of the earliest in the nervous system to show mature forms of activity.

ORAL-05-05

IMMUNOHISTOCHEMICAL LOCALISATION OF ALPHA-SYNUCLEIN IN MYENTERIC NEURONS OF GUINEA-PIG ILEUM

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Purpose: α-synuclein is a major constituent of Lewy bodies and Lewy Neurites; markers of Parkinson's Disease (PD). α-synuclein promotes SNARE-complex assembly at pre-synaptic release sites [1]; animals lacking the protein exhibit an activity-dependent decrease in SNARE-complex assembly, dysfunction and neurodegeneration [1]. Commonly, gastrointestinal symptoms precede motor symptoms in PD, supporting the Braak Hypothesis that pathology begins in the gut and spreads centrally via α-synuclein-containing projection neurons. However, α-synuclein's distribution in the gut wall has not been described; we studied which enteric neurons expressed α-synuclein immunoreactivity. **Methods:** Preparations immunohistochemically double labelled for α-synuclein and either 5-HT, CGRP, SOM (somatostatin), TH (tyrosine hydroxylase) or VAChT (vesicular acetylcholine transporter) were quantified from 4 guinea-pigs. **Results:** α-synuclein was present in 8.5±2.5% and 8.5±3.5% of CGRP and SOM-immunoreactive varicosities respectively (mean ± SD). It was detected in 32±6% and 33.75±6% of TH and 5-HT-immunoreactive varicosities. However, α-synuclein was found in significantly more cholinergic varicosities (79.75±3.5% of VAChT varicosities, $p < 0.001$). The proportion of 5-HT and TH varicosities with α-synuclein was significantly higher than either SOM or CGRP profiles ($p < 0.001$). α-synuclein is more closely associated with fast cholinergic transmitting varicosities than slower peptidergic varicose release sites in the ENS. **Conclusions:** Pre-synaptic terminals are affected early in PD, before widespread cell death [1]. Fast cholinergic neurotransmission plays a major role in controlling gut motility and probably requires specialised machinery for repetitive fast transmitter release. α-synuclein is preferentially localised in cholinergic terminals. We speculate that mishandling of α-synuclein in cholinergic enteric neurons may lead to defective neurotransmission, which contributes to early gut dysfunction in PD, including constipation. **References:** [1] Burre J et al (2010) Science 329;1663.

ORAL-05-06

INHIBITORY JUNCTION POTENTIALS AND RESPONSES TO EXOGENOUS ATP BY GUT SMOOTH MUSCLE CELLS BOTH REQUIRE AN 'EQUILBRATION' PERIOD

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Focal electrical stimulation evokes prominent purinergic inhibitory junction potentials (IJPs) in circular muscle cells of guinea pig ileum. Responses are absent in the first 20-30 minutes after setting up (referred to as "unequilibrated cells"); they increase to full amplitude over the ensuing 30-90 minutes (in "equilibrated cells"). Concomitantly, an increase in dye coupling and decrease in input resistance are recorded. **Purpose**: we compared the "equilibration period" with a gap junction uncoupler (carbenoxolone) by recording IJPs and responses to exogenous ATP. **Methods**: Intracellular recordings and dye fills were made from circular muscle cells of guinea pig ileum using microelectrodes containing 5% carboxyfluorescein. **Results**: When ATP (10mM) was spritzed onto "equilibrated" circular muscle cells, it evoked a hyperpolarisation of 13.0+/-1.0mV (n=6), compared to an IJP of -18.3.0+/-0.7mV. In "unequilibrated cells" both responses were smaller: the response to ATP averaged -0.3+/-0.3mV and IJP amplitude was -0.1+/-0.1mV (n=6). Addition of carbenoxolone (100μM) to "equilibrated" preparations reduced ATP hyperpolarisations from -13.1+/-2.8mV to -2.8+/-1.3mV ($p<0.01$, n=5) and IJP amplitude from -18.9+/-1.0mV to 5.1+/-0.8mV ($p=0.001$, n=5). Typically, ATP induced hyperpolarisations showed fast and slow components. Addition of tetrodotoxin (0.6uM) blocked the fast component and reduced the slow component (from -16.5+/-0.7mV to -6.1+/-0.6mV, $p<0.0001$, n=6). Subsequent addition of 10μM MRS2179, a P2Y1 antagonist, further reduced the ATP hyperpolarisation in 3 of 4 preparations by 53.1%+/-2.5 but had no effect in one preparation. **Conclusions**: Endogenous and exogenous ATP has little direct effect on most circular smooth muscle cells. It appears to act mostly on a subset of cells that are coupled by gap junctions to smooth muscle cells.

ORAL-05-07

TRANSABDOMINAL ELECTRICAL STIMULATION INCREASES WETNESS OF FAECES IN PIGLETS

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Background: Transcutaneous electrical stimulation (TES) across the abdomen increased bowel motility in children with chronic constipation (Ismail et al 2009 J Pediatr Surg 44: 2388). An animal model is needed to determine the mechanism and optimise stimulation parameters. Aim: to determine if transabdominal TES on healthy young piglets affects transit time or wetness of stool. Methods: Fourteen piglets (3-6 weeks old) had total gastrointestinal transit time assessed by measuring passage of a blue meal before and after 2 weeks of stimulation. Animals were randomly assigned to sham or active stimulation. Four self adhesive electrodes were attached 2 paraspinal and 2 on the abdomen and connected so the currents crossed. Pulsed electrical current (4 kHz carrier, 80-150 Hz beat, <30mAmp) was applied for 30 minutes daily for 2 weeks. Stool samples were collected throughout, frozen, then wet and dry weight measured. Animals were euthanased, samples of intestine were fixed and processed for histology. The thickness of the muscle and mucosa were measured. Results: TES did not affect oral-rectum transit time. Sham animals had no change in stool wetness (mean+/- SEM, 75+/-4% pre to 72+/-2% post stimulation, $p=0.95$). The animals that received active stimulation developed wet stools for a few days and then the stools returned to normal dryness. The highest water content during stimulation was significantly higher than the sham animals (mean 78+/-1% vs 73+/-1%, $p=0.001$). There was no change in thickness of the muscle or mucosa. Conclusion: Healthy intestines are affected by electrical stimulation across the abdomen. TES transiently affects water content of the stools in healthy piglets.

ORAL-05-08

VIDEO ANALYSIS OF NATURAL FAECAL PELLETS IN ISOLATED MOUSE COLON REVEALS A NOVEL PROPULSIVE MOTOR PATTERN

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Purpose: It is widely believed that propulsion of faecal pellets along the mouse colon results from the colonic migrating motor complex (CMMC), a spontaneous contractile pattern that appears in the proximal colon and propagates almost to the anus. We tested this belief. Methods: The whole mouse colon was removed from humanely killed C57/Bl6 mice and placed horizontally in an organ bath superfused with physiological saline at 37oC. The colon, with its natural complement of faecal pellets, was held straight with micro-pins at the proximal and distal ends. Video-recordings of smooth muscle constrictions and the faecal pellets were made with a video camera positioned above the preparation. Spatiotemporal maps of colonic diameter were constructed using in house software. Results: CMMCs were readily recorded once the faecal pellets had been expelled from the colon, but were very rare or completely absent from the 9 preparations examined. Instead two distinct motor patterns were observed. One was clearly propulsive, consisting of a single anally propagating constriction at the oral end of a pellet that moved it about 1.6 colonic diameters anally, and caused it to become longer and narrower, but then died away. The other was a series of short rhythmic constrictions at the oral end of the pellet that had the net effect of propelling it about 1.0 colonic diameters anally (significantly less distance that the propulsive contractions) and caused it to become shorter and fatter. Conclusions: Propulsion of natural faecal pellets in isolated mouse colon does not require CMMCs, but comes from two distinct motility patterns that also change the shape of these pellets.

ORAL-06-01

METALLOTHIONEIN BLOCKS THE METAL-BASED TOXICITY AND AGGREGATION OF AMYLOID-BETA

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Purpose: The aggregation of Aβ into higher order forms is believed to be one of the causative processes in Alzheimer's disease. There are compelling data suggesting that this aggregation is catalysed by reaction with the metals zinc and copper. We hypothesised that Aβ aggregation would be perturbed in the presence of metallothionein, an endogenous protein which has the ability to stereospecifically interchange zinc and copper with other peptides. We further hypothesised that zinc-metallothionein could ameliorate the toxicity of copper-Aβ aggregates to cortical neurons by a direct interaction involving metal exchange. **Methods**: We established primary neurons in culture, and developed direct and indirect analyses of neuronal viability in the presence of Aβ preparations of different composition. We also undertook biochemical analyses of the metal transfer between Aβ preparations and metallothionein. **Results**: We found that the major human-expressed metallothionein (MT) subtype, MT-2A, is capable of preventing the *in vitro* copper-mediated aggregation of Aβ1-40 and Aβ1-42. This action of MT-2A appears to involve a metal-swap between Zn_7MT-2A and Cu(II)-Aβ, since neither Cu_{10}MT-2A nor carboxymethylated MT-2A blocked Cu(II)-Aβ aggregation. Furthermore, Zn_7MT-2A blocked Cu(II)-Aβ induced changes in ionic homeostasis and subsequent neurotoxicity of cultured cortical neurons. **Conclusion**: These results indicate that MTs of the type represented by MT-2A are capable of protecting against Aβ aggregation and toxicity. We believe that MT-2A might represent a viable therapeutic approach as the metal exchange between MT and Aβ leaves the Aβ in a Zn-bound, relatively inert form. We are therefore investigating peptide mimetics of metallothionein for their therapeutic potential and also investigating the activity of agents which might increase the levels of endogenous metallothionein.

ORAL-06-02

SCREENING AND VALIDATION OF PLASMA BIOMARKERS FOR MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

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Purpose: With the move towards development of disease modifying treatments, there is a need for more specific early diagnosis of mild cognitive impairment (MCI) and Alzheimer disease (AD). Plasma biomarkers are likely to play an important role in this. **Methods**: In a cohort of 257 MCI, 19 AD and 407 cognitively normal subjects, we firstly used isobaric tags (iTRAQ) proteomic methods to identify potential biomarkers in pooled plasma. Based on iTRAQ screening data, multiplex bead immunoassay techniques were chosen to validate targeted proteins in individual samples. **Results**: iTRAQ experiments showed that ten proteins significantly deregulated in MCI and AD plasma, including ApoB100, afamin, vitamin D-binding protein, Ig mu chain C region (IGHM protein). A group of seven apolipoproteins was assayed by multiplex assay. ApoA2, and ApoH were significantly decreased in MCI ($p<0.001$), ApoJ and ApoB/ApoA1 were significantly increased in MCI ($p<0.001$). A combination of ApoH and ApoJ significantly predicted the presence of MCI (area under ROC curve =0.66, $p<0.001$). ApoJ was negatively correlated with grey matter volume ($r=-0.15$, $p<0.001$). **Conclusion**: Proteomic approaches are promising techniques to screening for biomarkers, but further validation of proteomic data is necessary. ApoH and ApoJ can be considered as potential clinical biomarkers for cognitive impairment, and further longitudinal study is necessary to determine if they play a major casual role in MCI and AD.

ORAL-06-03

HUNTINGTIN ASSOCIATED PROTEIN 1 ASSOCIATES WITH AMYLOID PRECURSOR PROTEIN AND REGULATES ITS TRAFFICKING AND Aβ LEVELS

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Purpose: Amyloid precursor protein (APP) is a type I transmembrane receptor-like molecule involved in the pathogenesis of Alzheimer's disease. APP is axonally transported and traffics to different cellular compartments where Abeta is produced. The aim of the present study is to examine how huntingtin associated protein 1 (HAP1) may regulate the trafficking of APP in neurons. **Methods:** We used molecular techniques and HAP1 mutant neurons to analyse the localization, interaction, trafficking of APP and and Aβ production. **Results:** HAP1 and APP were highly colocalized in a number of brain regions with a similar distribution pattern in the mouse and human brains. FRET analysis and immunoprecipitation experiments showed HAP1 and APP may co-exist and associate with each other. Immunohistochemical data and sucrose gradient fractionations showed that APP was retained more in cis-Golgi, trans-Golgi complex, early endosomes and ER-Golgi intermediate compartment but was decreased in autophagy vesicles in HAP1-/- mice compared with the control. APP internalization assay showed a significant alteration in APP endocytosis and reduced re-insertion of APP into the cytoplasmic membrane in HAP1-/- neurons. Live imaging analysis and FRAP assay on APP-YFP vesicles in HAP1-/- neurons showed that the trafficking speed was reduced and the number of motionless vesicles were increased. Knock-down of HAP1 protein with interference RNA in cortical neurons of Alzheimer's disease mice increases Abeta levels released from cortical neurons. **Conclusion:** our data suggest that HAP1 associates with APP and regulates APP subcellular trafficking to the non-amyloidogenic pathway and may regulate Abeta production in neurons.

ORAL-06-04

TIME COURSE OF Aβ25-35- AND Aβ35-25-INDUCED BEHAVIOURAL CHANGES IN RATS

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Purpose: Amyloid beta (Aβ) has been proposed to play a central and causative role in the development of Alzheimer's disease. $Aβ_{25-35}$, an 11-amino acid fragment, is the neurotoxic domain of the full-length Aβ and causes memory impairments. The reverse peptide $Aβ_{35-25}$ is considered as an inactive peptide and hence often used to control the effects of $Aβ_{25-35}$. This study aimed to investigate the time-course of $Aβ_{25-35}$- and $Aβ_{35-25}$-induced behavioural changes. **Methods:** Male young adult Sprague-Dawley rats were tested in the elevated plus maze, object recognition memory and water maze tasks at the time points of day 11 and weeks 4, 8 and 12 following a single bilateral intracerebroventricular infusion of preaggregated $Aβ_{25-35}$, $Aβ_{35-25}$ or saline (n = 10 in each group). **Results:** In the elevated plus maze, the $Aβ_{35-25}$ group spent significantly more time on the open arms and less time on the closed arms relative to the other two groups during the first two tests, and there were no performance differences between the three groups during the last two tests. In the object recognition memory task, both the $Aβ_{25-35}$ and $Aβ_{35-25}$ groups spent significantly less time in exploring objects and had reduced percentage of time in exploring the novel object relative to the saline group during the later tests. In the water maze task, both the $Aβ_{25-35}$ and $Aβ_{35-25}$ groups displayed no or very mild impairments during the later tests. **Conclusion:** A single bilateral <$SU_{b>}$intracerebroventricular infusion of preaggregated $Aβ_{25-35}$ and $Aβ_{35-25}$ resulted in behavioural deficits in the time- and task-dependent manners, which raises a significant concern of using the reverse peptide $Aβ_{35-25}$ as a control of the effects of $Aβ_{25-35}$.

ORAL-06-05

A TRANSCRIPTOMICS APPROACH TO PRECLINICAL ALZHEIMER'S DISEASE

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Purpose: The clinical manifestations of Alzheimer's disease (AD) are secondary to a substantial loss of cortical neurons. To be effective, neuroprotective strategies need to be implemented prior to this cell loss, which requires the discovery of both preclinical markers and early pathogenic mechanisms to serve as therapeutic targets. Transcriptomic analyses, that assume no a priori aetiological hypotheses, promise much in elucidating the pathogenesis of complex diseases like AD. Typically the most susceptible brain areas such as the entorhinal cortex (EC) and hippocampus are dominated by neuronal loss, chronic inflammation and secondary compensatory changes, meaning primary pathogenic changes are likely to be diluted out. Conversely belatedly affected areas such as the primary visual cortex (BA17) show little pathological change and may be equivalent to a hypothetical EC transcriptome in preclinical AD patients. Targeting the BA17 is predicated on an underlying transcriptomic similarity between the two regions. **Method:** RNA was extracted from the frozen entorhinal cortex (EC) and BA17 samples from 2 neurologically normal controls from an established Australian brain bank. Regional transcriptomic profiles were generated using a standard RNA-Seq protocol (poly A selection) with the Illumina GXII sequencer. Open source programs (e.g. Bowtie, TopHat, Cufflinks) were used for sequence alignment and processing including the relative abundance of transcripts and changes in splicing and alternative promoter usage within single genes. **Results:** RNA-Seq profiles of the EC and BA17 in neurologically unaffected individuals reveal similar gene expression patterns with the modest differences attributable to known cytoarchitectural features. **Conclusion:** The transcriptomic similarity between the EC and BA17 suggest that the BA17 disease transcriptome will be contextually relevant to early pathogenic mechanisms in AD. Furthermore the utility of the RNA-Seq platform is demonstrated by the novel gene expression patterns seen in AD-related genes such as MAPT.

ORAL-06-06

ANALYSIS OF A TRUNCATED FORM OF PRESENILIN PROTEIN ASSOCIATED WITH OXIDATIVE STRESS

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The Presenilin proteins, PSEN1 and PSEN2, are directly implicated in inherited early onset Alzheimer's disease. Presenilin proteins are essential for "γ-secretase" cleavage of Amyloid Precursor Protein (APP) to form Amyloidβ peptides in Alzheimer's disease. "PS2V" is a naturally truncated form of human Presenilin 2 that is induced by hypoxia and oxidative stress in human neurons. It is expressed at elevated levels in the brains of those with late onset Alzheimer's disease, bipolar disorder and schizophrenia. Expression of human PS2V protein in HEK293 cells is stringently controlled since, under normal oxygen conditions, it is rapidly ubiquitinated and degraded. We have discovered that a truncated form of zebrafish Presenilin1 equivalent to PS2V (zfPS1V) appears to dramatically up-regulate cleavage of APP. zfPS1V, human PS2V and an equivalent truncation of human PSEN1 ("hPS1V") all significantly increase notch signaling in zebrafish embryos as shown by decreased *neurogenin1* expression ($p<0.0001$). However, zfPS2V does not. These differences open up opportunities for dissection of the critical regions that allow human PS2V to stimulate Notch signaling and APP cleavage under hypoxia/oxidative stress. There is considerable evidence supporting that oxidative stress is important in late onset Alzheimer's disease. Therefore, our discovery provides evidence that changes in Presenilin function may be central to sporadic, late onset forms of Alzheimer's disease.

ORAL-06-07

MITOCHONDRIAL INHIBITION INDUCES ALZHEIMER-LIKE REDISTRIBUTION OF PHOSPHORYLATED TAU EPITOPES

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Hyperphosphorylated tau aggregates into striated neuropil threads and neurofibrillary tangles in the Alzheimer's disease (AD) brain. Neuropil threads are one of the earliest and most prominent pathological lesions to appear in AD brain, accounting for >85% of tau pathology and correlating closely with cognitive decline. Recent studies have demonstrated that mitochondrial inhibition in primary neuron and organotypic brain slice culture triggers rapid activation of the actin-associated proteins cofilin and actin depolymerizing factor (ADF), which subsequently aggregate and recruit phosphorylated tau. The resulting rod-shaped neuritic inclusions bear striking resemblance to tau neuropil threads observed in human AD brains. Here, we have further characterized the phosphorylation patterns and distribution of tau during mitochondrial inhibition with emphasis on the Alzheimer-related phospho-epitopes, using organotypic slice cultures of tau knock-out mice and primary neuron culture. We report that ADF/cofilin accumulates into rod structures independently of tau during ATP-depleting conditions and probably act to enhance or mediate early AD tau pathologies. Further, we show that tau recruited to ADF/cofilin rods is phosphorylated at key epitopes, including the Microtubule-Binding Domain (KXGS motifs), which are widely regarded as early appearing epitopes hyperphosphorylated in AD. These data further our understanding of tau phosphorylation and redistribution during the initiation of AD pathologies and may thus represent useful targets for the development of AD therapeutics.

ORAL-06-08

DECREASES IN SOLUBLE CLUSTERIN OCCUR EARLY IN ALZHEIMER'S DISEASE

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Purpose: Recent GWAS studies have identified clusterin (CLU) or apolipoprotein J as a significant risk factor for late-onset sporadic Alzheimer's disease (AD). The gene encodes a multi-functional protein that is processed into three main alloforms of different molecular weights, cytoplasmic (cCLU), nuclear (nCLU) and an excreted soluble form (sCLU) expressed by neurons and astrocytes. The role of these alloforms in AD remain unclear. **Methods:** Brain tissue from 6 AD patients, 6 patients with mild cognitive impairment (MCI) and 6 disease-free controls was obtained from the Sydney Brain Bank and NSW TRC following institutional approval for the study. Immunohistochemistry and semiquantitative western blotting for CLU, NeuN, glial fibrillary acidic protein (GFAP), monocyte chemoattractant protein-1 (MCP-1) and ApoE were performed. **Results:** A significant decrease in the levels of sCLU was observed in both MCI and AD cases compared to controls, with a greater reduction in MCI (posthoc $p = 0.004$). The levels of sCLU negatively associated with NeuN neuronal cell loss and ApoE, with AD cases demonstrating a significant retention of neuronal CLU (both cCLU and nCLU, posthoc $p < 0.011$), a finding confirmed by peroxidase immunohistochemistry. The levels of sCLU also negatively correlated with changes in GFAP but not MCP-1, suggesting that as astrocyte reactivity increases in AD, the levels of sCLU remain relatively constant per cell. **Conclusion:** This data supports a growing body of literature proposing a neuroprotective role for sCLU (reduced in AD) and a pro-apoptotic role for neuronal nCLU in AD pathogenesis, thus suggesting that therapeutic strategies aimed at increasing the levels of sCLU and alleviating the nCLU burden are likely to have dramatic consequences for the pathological progression of the disease.

ORAL-07-01

LOW BLOOD GLUCOSE INCREASES ABSENCE SEIZURE SUSCEPTIBILITY

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Purpose: Absence epilepsies are a common disease with a strong genetic aetiology. Certain environmental factors can influence absence occurrence but a complete understanding of absence precipitation is lacking. Here we investigate if lowering blood glucose increases spike-wave activity in mouse models with varying seizure susceptibility. **Methods:** Three mouse models were used; an absence seizure model based on the knock-in of a human $GABA_A\gamma2$(R43Q) mutation (DBA(R43Q)), the spike-wave discharge (SWD)-prone DBA/2J strain, and the seizure resistant C57Bl/6 strain. Electrocorticogram recordings were made to measure SWDs from mice prior to and following injection of various doses of insulin. Blood glucose was independently measured to determine the reduction in levels following insulin injection. **Results:** A ~45% reduction in blood glucose levels (6.7±0.3 mM to 4.0±0.4 mM, n=10, p<0.05) was sufficient to double SWD occurrence in the DBA(R43Q) model (19.9±5.9 to 50.3±5.9 SWD/h, n=10, p=0.001) and in the SWD-prone DBA/2J mouse strain(1.1±0.5 to 1.8±0.4 SWD/h, n=7, p=0.01). Larger reductions in blood glucose further increased SWDs in both these models. However, even with large reductions in blood glucose no discharges were observed in the seizure-resistant C57Bl/6 mouse strain (n=6). Injection of glucose reversed the impact of insulin on SWDs in the DBA(R43Q) model (48.5±14.2 to 20.5±9.8 SWD/h, n=5, p=0.02), supporting a reduction in blood glucose as the modulating influence. Fasting also precipitated SWDs. **Conclusion:** Low blood glucose can precipitate SWDs in genetically predisposed animal models and should be considered as a potential environmental risk factor in absence epilepsy patients.

ORAL-07-02

ENVIRONMENTAL ENRICHMENT RESTRICTS EPILEPTOGENESIS IN A GENETIC RAT MODEL OF EPILEPSY

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Purpose: Certain genetic abnormalities are recognised to cause epilepsy, but the influence of the environment on disease severity in these types of epilepsy has been little studied. Here we investigate the effects of environmental enrichment in GAERS (Genetic Absence Epilepsy Rats from Strasbourg), a well-validated animal model of genetically-determined epilepsy. Methods: At weaning, male GAERS were group-housed in either enriched environments (large cages with running wheels, toys, tunnels, etc: n=7) or impoverished environments (standard cages with only sawdust bedding: n=8). At 8 weeks, rats underwent epidural electrode implantation. At weekly intervals starting at 9 weeks, all rats underwent 24 hour EEG recordings. The EEGs were then assessed offline by a reviewer blinded to housing conditions to measure seizure frequency and duration. Results: At 9 weeks of age, all of the impoverished GAERS had seizure activity on the EEG, whereas only 3 out of 7 enriched rats expressed seizures, indicating that the enriched environment delayed disease onset. Analysis of the subsequent weekly EEGs revealed that the enriched GAERS spent less % time in seizure (ANOVA: p<0.0001), and experienced significantly fewer seizures (ANOVA: p<0.0001), compared to the impoverished GAERS. For example, at week 14, the time spent in seizure was 1.1±0.2% for enriched GAERS compared to 2.5±0.2% for impoverished rats. The number of seizures experienced at this time was 10.9±1.3/hour for enriched rats compared with 20.8±1.2/hour for the impoverished GAERS. Conclusion: Here we demonstrate that housing rats with genetically determined epilepsy in an enriched environment from weaning delays disease onset and restricts disease progression. Gene-environment interactions are likely candidates mediating this antiepileptogenic effect.

ORAL-07-03

ENDURING EFFECTS OF EARLY-LIFE STRESS ON NEURONAL FIRING PATTERNS IN THALAMIC RETICULAR NUCLEUS: IMPLICATIONS FOR LIMBIC EPILEPSY

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Purpose: Early-life stress (ELS) has been shown to accelerate amygdala kindling-induced epileptogenesis in rats. Recent studies suggest critical involvement of thalamocortical circuits in the progression of kindling by inducing change in neuronal firing patterns of thalamic reticular nucleus (TRN). We hypothesised that ELS aggravates changes in TRN neuronal firing patterns of amygdala kindled rats. **Methods:** ELS was induced using maternal separation (MS) for 3h/day from P2-P14, while control rats were separated for 15min (EH). Kindling commenced at P-56, with two stimulations delivered twice daily until 5 class V seizures were observed. Thereafter, extracellular single neuronal recordings were performed in vivo under neuralept anaesthesia. The location of recorded cells was confirmed by iontophoretic juxtacellular labelling with neurobiotin. **Results:** TRN neuronal firing did not differ between non-kindled MS and EH rats. Kindling reduced interictal firing frequency (p<0.001) and increased percentage burst firing (p<0.001) of TRN cells compared to non-kindled rats, which was more pronounced in MS (31.6±3.3; 36 cells, 8 rats), compared to EH rats (23.7±3.0; 36 cells, 10 rats, p=0.05). **Conclusion:** These data support the hypothesis that neuroplastic changes in thalamocortical circuitries involving the TRN play a role in the progression of amygdala kindling, and that this may be a mechanism mediating the vulnerability to kindling induced by MS. This has implications for how exposure to ELS could play a role in the pathophysiology of mesial temporal lobe epilepsy, particularly the development of secondary generalised seizures.

ORAL-07-04

MUNC18-1 CONTROLS SYNTAXIN1A TRAFFICKING TO THE PLASMA MEMBRANE: MOLECULAR BASIS OF EARLY INFANTILE EPILEPTIC ENCEPHALOPATHY

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Purpose: Munc18-1 regulates exocytosis by promoting SNARE complex formation but the molecular basis underpinning this effect is still unclear. We hypothesised that Munc18-1 chaperone Syntaxin1a traffic to the plasma membrane. We investigate the interaction of Munc18-1-C180Y, a mutant which has been linked to an early infantile epileptic encephalopathy (EIEE), with Syntaxin1a and test whether the mutation prevents delivery of Syntaxin1a to the plasma membrane therefore abolishes exocytosis. Methods: To investigate Munc18-1 wild type (WT) or C180Y binding to Syntaxin1a *in vitro*, we used isothermal titration calorimetry (ITC) and yeast two hybrid (Y2H) assay. We expressed Munc18-1 WT or C180Y in Munc18-1/-2 double knockdown PC12 neurosecretory cells (DKD PC12) and quantified endogenous Syntaxin1a plasma membrane localisation, and human growth hormone release. Results: ITC experiments revealed no significance difference between Munc18-1-C180Y and WT binding to Syntaxin1a. Nevertheless, Munc18-1-C180Y was unable to bind to Syntaxin1a in a Y2H assay and expression of C180Y in DKD PC12 cells did not significantly rescue Syntaxin1a plasma membrane localisation or stimulated exocytosis. For immunocytochemical analyses, >60 cells were quantified per condition (>3 independent experiments) and data reached a significance level at p<0.05. Conclusions: Our results demonstrate that functional binding of Munc18-1 to Syntaxin1a is indispensable for Syntaxin1a traffic to the plasma membrane and stimulated exocytosis. Notably, C180Y mutation instability impinges on its functional interaction with Syntaxin1a in PC12 cells, resulting in impaired trafficking to the plasma membrane. Our data suggest that a defect in Syntaxin1a trafficking might be the underlying molecular mechanism of EIEE.

ORAL-07-05

SUPRESSION OF ABSENCE-LIKE SEIZURES IN THE RAT BY NEUROPEPTIDE Y IS ASSOSIATED WITH INCREASED NEURONAL FIRING IN THE THALAMIC RETICULAR NUCLEUS (TRN)

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Purpose: Neuropeptide Y (NPY) suppresses seizures in genetic rat model of absence epilepsy (GAERS), but the neuronal mechanisms for seizure suppression are unknown. The thalamo-cortical circuitry plays a critical role in the generation of absence-like seizures in GAERS. This study investigates the effect of intracerebroventricular (ICV) administered NPY on neuronal firing patterns in thalamo-cortical structures in-vivo under neuroleptanalgesia (tubocurarine, fentanyl and haloperidol). Methods: Twenty two Male GAERS aged 8-12 weeks were used in this study. Paired juxtacellular single neuronal recordings were performed in the regions of interest. ICV drug infusions and EEG was monitored simultaneously with electrophysiological recordings. After recording, cells were labelled with neurobiotin by juxtacellular iontophoresis and identified histologically. Cortical EEG and single neuronal firing patterns were recorded from 12 TRN, 8 thalamic relay and 8 cortical neurons and analysed ictally and interictally at three time points (baseline, after saline and after NPY). Significance of parameter change was accessed by one way Freedmans ANOVA test. Results: A decrease in % time spent in seizures was observed after NPY infusion (P=0.0001). There was an increase in interictal firing rate of the TRN cells after the application of NPY (P=0.008). No effect was found on the single neuronal firing patterns of thalamic relay nuclei and cortical neurons. Conclusion: NPY increases the firing frequency of the TRN cell and concurrently suppresses seizures. This implicates the TRN as a key structure by which NPY suppresses seizures.

ORAL-07-06

ANTICONVULSANT EFFECTS OF COMPLEMENT C5A RECEPTOR INHIBITION IN VARIOUS SEIZURE MODELS

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Inflammation appears to play an important role in the etiology of seizures and epilepsy. The complement system belongs to the innate immune system and can trigger inflammation. Various complement factors are upregulated in animal models of epilepsy and in patients with human temporal lobe epilepsy (Aronica et al., 2007; Neurobiol Dis 26:497ff). Given the presence of the membrane attack complex in microglia within epileptic brains, it is likely that the anaphylatoxin C5a is produced in epileptic brain. Our hypothesis is that activation of complement with the production of C5a plays a crucial role in the pathogenesis of seizures, such as increasing excitability. Here, we determined the anticonvulsant activity of inhibitors of the C5a receptor CD88 in various seizure models. The CD88 inhibitor PMX53 showed reproducible anticonvulsant activity in some seizure models, but not others. 3 mg/kg PMX53 (s.c.) statistically significantly elevated the CC50 in the 6 Hz model by about 2.5-4.5 mA ($p<0.001$-0.05 in 3 independent experiments). It also protected fully corneally kindled CD1 mice against stage 3-5 seizures ($p=0.04$ and $p=0.027$ for 2 experiments) and inhibited pilocarpine-induced effects (see GL abstract at ANS2011). However, there were no effects of PMX53 in the maximal electroshock seizure threshold test and the pentylenetetrazole (i.v.) model. Preliminary data using CD88-deficient mice suggest that the anticonvulsant mechanism of action of PMX53 is dependent on CD88. In conclusion, it is likely that the complement factor C5a is increasing excitability within the brain. The inhibition of CD88 may become a promising new treatment for generalised and partial seizures, as well as pharmacoresistant seizures.

ORAL-07-07

CHANGES IN BRAIN KYNURENINE PATHWAY METABOLISM DURING AGING

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The kynurenine pathway (KP) of tryptophan catabolism plays an important role in several biological systems affected by aging including energy metabolism, immune function, nuclear transcription and DNA repair. We aimed to measure the content of tryptophan (TRYP) and its metabolites, kynurenine (KYN), kynurenic acid (KYNA), picolinic acid (PIC) and quinolinic acid (QUIN) in the brain of aging female wistar rats. We also measured the activity of the KP enzymes, indoleamine 2,3 dioxygenase (IDO), tryptophan dioxygenase (TDO), and quinolinic acid phosphoribosyltransferase (QPRTase) in the same tissue. We found age-related differences in the TRYP levels in the brain which declined significantly with age. TDO activity was observed to consistently decrease with age for the brain. In contrast, brain IDO activity increased significantly across the age range. Brain KYN and KYNA levels progressively increased with age. QUIN and PIC levels in the brain continued to increase with advancing age. Brain QPRTase maintained a steady decrease in activity at each age group up to 24 months of age. These age associated changes in tryptophan metabolism have the potential to impact upon major biological processes including, lymphocyte function, pyridine (NAD(P)(H)) synthesis and NMDA mediated synaptic transmission, and may therefore contribute to several degenerative changes of the elderly.

ORAL-07-08

DOSE-DEPENDENT PROTECTIVE EFFECT OF CONNEXIN43 MIMETIC PEPTIDE AGAINST NEURODEGENERATION IN AN EX VIVO MODEL OF EPILEPTIFORM LESION

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Purpose: Epileptic seizures typically result in delayed neuronal loss secondary to the initial damage and an up-regulation in connexin43 (Cx43). **Aim:** This study investigated the role of Cx43 gap junctions in lesion spread and cell loss following epileptiform activity. **Methods:** Epileptiform injury in hippocampal slice cultures was induced by 48h exposure to 100µM bicuculline methochloride (BMC) followed by a 24h recovery period. A Cx43 mimetic peptide (concentrations between 5 - 500µM) was applied during either the BMC treatment or recovery periods, and neuroprotection, as determined by propidium iodide (PI) uptake, measured at the end of the recovery period. **Results:** During the 24h recovery period following BMC treatment, lesion spread was observed in the CA1. Application of the mimetic peptide produced a concentration - and exposure time - dependent neuroprotection. During the BMC period, peptide concentrations of 5, 10 and 50µM (sufficient to block hemichannels) had a protective effect as assessed by PI uptake ($p<0.05$) while a substantial gap junction blockade with 500µM peptide exacerbated the lesion ($p<0.05$). By contrast, doses of 50 and 500µM applied during the recovery period protected the CA1 region from further damage ($p<0.05$). **Conclusions:** The results indicate that while the slices are undergoing excessive neuronal firing and epileptic stress, gap junction communication appears to be essential for tissue survival but hemichannel opening may be damaging. Following epileptiform insult, however, gap junction communication plays a crucial role in the spread of neuronal damage. The findings from this study identify gap junction communication as a potential therapeutic target for epilepsy.

ORAL-08-01

ISOFLURANE CAUSES DIFFERENTIAL COGNITIVE DEFICITS IN MIDDLE-AGED AND YOUNG ADULT RATS IN THE MORRIS WATER MAZE TASK

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Purpose: Post operative cognitive dysfunction (POCD) has been reported in young, middle-aged and elderly patients with greater incidence with increasing age. Neurocognitive deficits have been associated with anaesthetic exposure in aged rodents, with inconsistent findings in younger adult animals. We hypothesized that isoflurane will have greater effects on learning and memory in middle-aged compared with young rats. **Methods**: Young adult (3 months; n=25) and middle-aged (12 months; n=20) male Sprague Dawley rats were exposed to isoflurane (1 MAC, 4h) or control conditions. Spatial learning (acquisition phase) and memory (probe trial) were tested in the Morris water maze 1 week post-exposure. Middle-aged rats were re-tested in the probe trial at 4 weeks post-exposure for long-term memory retention. Latency (time) to locate the hidden platform, and time spent in the platform quadrant were compared between ages and treatments. **Results**: Isoflurane did not affect water maze task acquisition in either age group. Irrespective of treatment, middle-aged rats performed worse than young rats in the acquisition phase. Isoflurane exposure in young but not middle-aged rats induced a significant deficit in memory retention in the probe trial 24 hours after acquisition, compared with control. Assessment at 4 weeks however, indicated isoflurane-treated middle-aged rats showed no preference for the target location, in direct contrast to sham-exposed rats. **Conclusion**: Isoflurane exposure had no effect on acquisition in the water maze task but did affect retention memory for platform location. Isoflurane anaesthetic impaired spatial reference memory but the effects of isoflurane differ between young adult and middle-aged rats.

ORAL-08-02

LEAD EXPOSURE DURING EARLY LIFE AFFECTS SYNAPTOGENESIS IN HIPPOCAMPUS AND IMPAIRS WATER MAZE PERFORMANCE IN YOUNG RATS

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Purpose: We tested the hypothesis that chronic exposure to low level of lead (Pb) during early life alters synaptogenesis in the CA3 region of hippocampus of young rats that leads to impaired learning and memory. **Methods**: Wistar rats pups (n=10) were exposed to 0.2% Pb-acetate via their dams' drinking water from postnatal day (PND) 1 to 21 and directly via drinking water from weaning until PND 30. The control group (n=10) was given regular water. Pb in blood and brain tissues of was measured by atomic absorption spectrophotometer. Synapses were counted in electron micrographs of the CA3 region of hippocampus. Spatial learning and memory was tested by Morris water maze test. **Results**: Mean values of Pb in blood, brain and hippocampus in Pb-exposed group were significantly ($p < 0.05$) higher than in control animals at PND21 and PND30. Hippocampus had significantly higher levels of Pb compared to the brain. Pb-exposed rats had significantly lower number of synapses in the molecular layer of hippocampus compared to control animals ($p < 0.01$). Pb-exposed rats learned slower than controls. Short term memory was largely unaffected by Pb, whereas, it significantly affected long-term memory. **Conclusion**: These data suggest that Pb preferentially accumulates in hippocampus that leads to a significant reduction in the number of synapses in the molecular layer of CA3 region. Higher accumulation of Pb and decreased number of synapses in the hippocampus appears to be the cause of the observed impairment of learning and memory in the Pb-exposed rats.

ORAL-08-03

HIGH FAT FEEDING IMPAIRS SPATIAL MEMORY IN RATS

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Purpose: Recent evidence shows an association between obesity and cognitive decline. The present study aimed to determine whether very high fat (HF) or western diet (WD) can affect working or spatial memory in rats. **Methods:** Three groups of male Long Evans rats were fed either normal chow (Con), WD (23% fat, 0.19% cholesterol) or very HF diet (60% fat) for 12 weeks (n=12 per group). Body weight, food intake and blood pressure were measured weekly. Behavioural testing was carried out at 12 weeks investigating working memory performance in the novel object recognition test and spatial memory in the Y maze. **Results:** Analysis at week 12 showed that consumption of the WD significantly increased final body weight compared to the Con group, while the HF diet produced no significant weight gain compared to Con ($F(2,33)=12.2$; WDvCon $p<0.001$). Final blood pressure values did not differ within the three groups. With regards to cognitive testing, consumption of WD and HF diets had a significant negative impact on the performance of the rats in Y maze ($F(2,33)=3.9$; WD or HFvCon $p<0.05$ time spent in novel arm) but not on discrimination scores in the object recognition test. **Conclusion:** These results demonstrate that consuming very high fat diet or western diet for 12 weeks impairs the spatial memory but not the working memory of rats. This effect might be associated with the changes in the areas of the brain that are involved in cognitive regulation.

ORAL-08-04

LEARNING-INDUCED CHANGES AT GLUTAMATERGIC AND AGMATINERGIC SYNAPSES IN THE HIPPOCAMPAL CA1 REGION

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Purpose: Agmatine, a novel putative neurotransmitter, is present in hippocampal neurons and recent research has implicated it in learning and memory. Within the stratum radiatum (SR) of the hippocampal CA1 sub-region, agmatine is localised within axon terminals that form asymmetrical synapses with CA1 pyramidal cells. This prompted us to investigate if agmatine is co-localized with glutamate, the primary excitatory neurotransmitter of hippocampal neurons. We recently demonstrated that agmatine levels are elevated at CA1 synapses following 4 days water maze (WM) training. Therefore, we also investigated whether WM training resulted in changes in presynaptic glutamate and/or the postsynaptic density (PSD) at CA1 synapses. **Methods:** Immunogold double-labelling and electron-microscopy were used to quantify agmatine and glutamate immunoreactivity in SR terminals of six Sprague-Dawley rats. Presynaptic agmatine and glutamate levels and PSD thickness were compared at CA1 synapses (n=300) of WM rats (n=3) and control swim only rats (SW, n=3). **Results:** Agmatine was found to be co-localized with glutamate in 97% of all agmatinergic terminals. Likewise, 92% of all glutamatergic profiles contained agmatine. At these CA1 synapses, WM rats showed significant increases in presynaptic agmatine (~80%, $p<0.01$) and glutamate levels (~40%, $p<0.05$), and PSD thickness (~30%, $p<0.001$), compared to SW rats. **Conclusion:** Agmatine is co-stored with glutamate in axon terminals in the CA1 region. WM training elevates agmatine and glutamate levels, indicating that they may co-participate in learning and memory processes. Furthermore, learning induces structural changes in PSD at synapses between glutamatergic/agmatinergic terminals and pyramidal cell dendrites in the CA1 region. This may indicate changes in receptors and proteins associated with the PSD, although the functional significance of these changes is currently unclear.

ORAL-08-05

RYANODINE RECEPTOR INTRACELLULAR CALCIUM RELEASE CHANNELS PROMOTE THE CONSOLIDATION OF LONG-TERM MEMORY

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Purpose: Calcium signaling is vital for memory processing. However, the contribution of ryanodine receptors (RyRs) and IP_3 receptors (IP_3Rs) which control calcium release from intracellular stores is generally overlooked. We have previously demonstrated that IP_3Rs are required during long-term memory formation in young chicks trained on a single-trial discrimination avoidance task. In contrast, RyRs, nitric oxide and noradrenaline are required for memory processing at a time consistent with a role in triggering the long-term memory stage. The present research investigated whether RyRs promote the consolidation of the long-term memory stage and whether this process is dependent upon nitric oxide and noradrenergic mechanisms. **Methods:** Young chicks (n=14-29 per data-point) were trained using a weakly reinforced variant of the single-trial discrimination avoidance task, yielding a labile memory trace. Drugs were administered intracranially into the intermediate medial mesopallium, an association area important for memory formation. **Results:** The administration of a RyR agonist promoted the consolidation of the long-term memory stage following weakly reinforced training. This facilitation of retention was prevented by a β_{1+2}-adrenoceptor antagonist, but not a nitric oxide synthase inhibitor. The inhibition of RyRs interfered with long-term memory consolidation promoted by a nitric oxide donor, but not noradrenaline. **Conclusion:** These findings demonstrate that RyRs are involved in triggering long-term memory consolidation. Nitric oxide appears to promote memory formation though mechanisms dependent upon RyRs. In addition, RyRs may facilitate memory consolidation through noradrenergic activation of β-adrenoceptors. This role of RyRs appears to be functionally distinct from IP_3 receptors.

ORAL-08-06

THE BRAIN-SPECIFIC MICRORNA, MIR-128B, BELONGS TO AN ACTIVITY-DEPENDENT GENE REGULATORY NETWORK ASSOCIATED WITH FEAR EXTINCTION

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Excessive fear and anxiety are key characteristics of numerous disabling disorders that affect millions of people all over the world. Fear extinction, a vital learning process in animals and humans, is defined as a decline in conditioned fear responses following non-reinforced exposure to a feared conditioned stimulus. Our work is a step in the understanding of the neural mechanisms associated with extinction and in the development of therapies for the treatment of anxiety-related disorders like phobias and post-traumatic stress disorder (PTSD). MicroRNAs (miRs) are a newly discovered family of endogenous small non-coding RNAs (~23 nucleotides long) that regulate gene function either by degrading target mRNAs or by directly binding to the 3'UTR of protein-coding genes, resulting in post-transcriptional silencing. Previous studies have demonstrated that the infralimbic prefrontal cortex (ILPFC) is a brain region responsible for the encoding of fear extinction memories. We have found that a brain-specific microRNA, miR-128b, is highly expressed in neurons innervated by dopamine in the ILPFC. We also have discovered that miR-128b is necessary for the formation of fear extinction memory specifically, compared with miR134 and miR140, which show non-specific or no association with fear extinction memory, respectively. Further experiments revealed that miR128b can negatively regulate the function of several plasticity-related genes associated with the acquisition and retrieval of conditional fear. These results suggest that the transient disruption of fear-related genes by miR-128b at the time of retrieval and during extinction training may provide the molecular switch responsible of moving from reconsolidation of the original fear toward the formation of fear extinction memory by through the calcineurin pathway.

ORAL-08-07

ADVANCED PATERNAL AGE IN MICE IS ASSOCIATED WITH COHORT-DEPENDENT BRAIN STRUCTURAL AND BEHAVIOURAL PHENOTYPES

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Purpose: Advanced paternal age (APA) is associated with an increased risk of neurodevelopmental disorders, such as schizophrenia and autism. We have developed a mouse model to explore the neurobiological correlates of APA. This study investigated the effects of APA on brain morphology and behaviours with relevance to neuropsychiatric disorders. **Methods:** We investigated structure-function relationships in the adult offspring of young (3 month-old, Control) and old (12-18 month-old, APA) C57Bl/6J sires. Mice underwent a behavioural test battery comprising tests for locomotion, anxiety, exploration, learned helplessness, avoidance learning, prepulse inhibition (PPI) of the acoustic startle response and amphetamine (AMPH)-induced locomotion. The brains of these mice were examined ex vivo using a 16.4T animal MRI scanner. We repeated the experiments with a second cohort of mice to examine the stability of the findings. **Results:** We found a variable effect of APA between the two separate cohorts of mice. In the first set of experiments, the APA group had increased anxiety-related and exploratory behaviours and a decrease in ventricular volume, which corresponded to an increase in rostral cortical volume. However, in the second set of experiments, a different pattern of behaviour was seen, which included decreased exploration, enhanced PPI and selective alterations to both spontaneous and AMPH-induced locomotion. This latter group of animals had normal ventricular and cortical volumes, although the volume of the corpus callosum was significantly smaller in APA mice. **Conclusion:** This APA mouse model is not associated with stable brain structural and behavioural phenotypes. We have probed this instability by investigating the effects of breeding programme to demonstrate that length of prenatal sire exposure was not a contributing mechanism to offspring outcomes. In addition, there were no explicit outliers in the outcomes measured for APA animals, nor an increase in variance compared to controls. We are currently exploring genomic and epigenomic correlates of these challenging but thought-provoking phenotypes, and conducting a more detailed approach to brain structural alterations using voxel-based morphometry (VBM).

ORAL-08-08

VTA AND NACC NEURONS' INHIBITION DURING REVERSAL LEARNING: A PHARMACOLOGICAL AND AN OPTOGENETIC APPROACH

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Purpose: The aim of this study was to understand the contribution of VTA and NAcc cells to reversal learning performance by using two approaches. First, inhibition of VTA and NAcc cells using a $GABA\text{-}_A$ agonist (muscimol). Second, inhibition of VTA and Nacc cells using light-sensitive opsins (halorhodopsin). The predictions from this investigation were that neuronal suppression of NAcc neurons would have a greater impact on reversal learning performance than suppression of VTA cells, as task complexity increased. **Methods**: Rats (muscimol group) (n=15) were implanted with bilateral guide cannulae above the VTA and NAcc. Another group of rats (n=15) received injections of lentivirus (halorhodopsin) in the VTA and NAcc, and were implanted with a fiber guide system that would deliver a yellow light to target neurons via an optical fiber. After surgery, rats were trained to complete an FR1 discrimination, and then tested in a between reversal and a within session reversal task. **Results**: VTA (n=5) and NAcc (n=5) implanted rats that received muscimol injections made significantly fewer errors during the between reversal session than the control group (saline, n=5): $F_{(1,14)}=42.885$, $p<0.001$. VTA rats also took significantly longer than the control group to reach criterion: $F_{(1,14)}=4.933$, $p<0.05$. However, NAcc rats made significantly more errors than the control group during the more complex within session reversal $F_{(1,14)}=7.031$, $p<0.05$. **Conclusion**: Our preliminary results suggest that NAcc neurons play an instrumental role in reversal learning performance, especially when task complexity increases. The high temporal resolution provided by the optical inhibition of NAcc and VTA neurons (via halorhodopsin) will tell us about the importance of feedback information during error making.

ORAL-09-01

EXPLORING AN LDH-NANOPARTICLE-BASED DRUG DELIVERY SYSTEM FOR THE TREATMENT OF NEURODEGENERATIVE DISEASE

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Small interfering RNAs (siRNAs) are capable of targeting and destroying specific mRNAs, making them particularly suited to the treatment of neurodegenerative conditions such as Huntington's Disease where the production of abnormal proteins results in a gain-of-function phenotype. Although a variety of nanoparticle formulations are currently under development as siRNA delivery systems, application of these technologies has been limited by their high cytotoxicity, low drug loading capacity and release, and inability to penetrate cell membranes. Layered double hydroxide (LDH) nanoparticles are now emerging as a potential new drug delivery system as they exhibit low cytotoxicity and are highly biocompatible. Purpose: Here we present the first study investigating LDH delivery of siRNAs to primary cultured neurons. Methods: The efficacy of LDH mediated nucleic acid delivery was tested using cultured cortical neurons and comparing uptake efficiency to that of mouse fibroblasts (NIH3T3s). In addition, the ability of LDH-mediated delivery of siRNA to silence neuronal gene expression was tested. Results: We show that internalization by neurons (n = 3 independent experiments) is rapid, dose-dependent and saturable, and markedly more efficient than in other cell types. We also demonstrate that LDH mediated siRNA delivery effectively silences gene expression (n = 3 independent experiments). Conclusions: This study therefore confirms the potential of LDH nanoparticles as a drug delivery system for patients suffering from neurodegenerative disease.

ORAL-09-02

FUNGAL NEUROTOXINS AS PHARMACOLOGICAL TOOLS TO INVESTIGATE THE PHYSIOLOGICAL ROLES OF LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM (BK) CHANNELS

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The lolitrem family of fungal alkaloids originate *in planta* as a result of grass-endophyte symbiosis. These indole diterpene compounds are secondary metabolites that are produced when perennial ryegrass is infected with the endophytic fungus *Neotyphodium lolii*. The most abundant compound is lolitrem B which is the main compound responsible for a neurological condition called ryegrass staggers which impairs motor function in grazing animals. Using BK channel knock-out mice, we have shown that motor function deficits induced by lolitrem B in mice are mediated by BK channels[1]. Lolitrem B is a potent BK channel inhibitor (IC_{50} = 4 nM)[2]. By comparing its BK inhibitory effect with that of structurally related analogues, the moieties that confer its high potency have been identified[3]. In investigating possible cardiovascular effects of lolitrem B in rodents we found an unexpected effect on heart rate that was also observed using an isolated heart preparation[4]. This suggested that BK channels are directly involved in heart rate regulation. A similar effect was found using the specific BK channel inhibitor iberiotoxin, further implicating BK channels. This finding was surprising since functional BK channels are only expressed at very low levels in the heart. 1) Imlach et al (2008) Journal Pharm Exp Ther, 327 (3), 657-664. 2) Dalziel et al (2005) Toxicoly Lett, 155, 421-426. 3) Imlach et al (2009) Eur J Pharmacol, 605, 36-45. 4) Imlach et al (2010) PLoS ONE, 5 (1), e8698.

ORAL-09-03

NUCLEOFECTION IS AN EFFECTIVE TRANSFECTION METHOD FOR PRODUCING LONG-TERM NEUROTROPHIN SECRETING CELLS THAT CAN SUPPORT SPIRAL GANGLION NEURON SURVIVAL *IN VITRO*

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Introduction: Spiral ganglion neurons (SGNs), the target cells of the cochlear implant, undergo progressive degeneration in deafness, which can compromise cochlear implant efficacy. Exogenous delivery of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) has pronounced survival effects on SGNs. However, a clinically applicable method of sustained neurotrophin delivery is required. The implantation of genetically-modified cells designed to over-express these neurotrophins has potential to achieve these requirements. **Aims:** To compare two non-viral transfection methods, lipofection and nucleofection, to determine the most effective method to transfect primary rat fibroblasts to produce BDNF or NT-3 long-term and assess survival effects on SGNs *in vitro*. **Methods:** Fibroblasts were transfected using Lipofectamine or Nucleofection. Neurotrophin secretion was measured by ELISA. Survival effects on SGNs were determined by co-culturing transfected fibroblasts with SGNs from early postnatal rats and quantifying SGN survival after three days *in vitro*. **Results:** Nucleofection was the more effective method, producing fibroblasts that secreted neurotrophins for at least four months at a rate of 588±73pg BDNF/10^6 cells/day and 359±47pg NT-3/10^6 cells/day at four months post transfection. Lipofected fibroblasts secreted neurotrophins for only one month at a rate of 26±8pg BDNF/10^6 cells/day and 3254±45pg NT-3/10^6 cells/day at one month post transfection. Significant survival effects were observed on SGNs using nucleofected BDNF-fibroblasts (197±51.6% survival, p<0.05) and NT-3-fibroblasts (149±15% survival, p<0.01) up to four months post-transfection, compared to control fibroblasts (n=15). **Conclusion:** Nucleofection is an effective technique to produce fibroblasts that secrete neurotrophins long-term at levels that support SGN survival *in vitro*. These cells may represent a clinically viable method of sustained neurotrophin delivery to support SGN survival in deafness.

ORAL-09-04

EDU IN MULTIPLE APPLICATIONS AND CELL TYPES

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Purpose: 5-Ethynyl-2′-deoxyuridine (EdU) is a novel thymidine analogue that can be fluorescently labeled at room temperature. In this body of work we have shown that EdU can be used to label proliferative cells in a variety of research fields/species/conditions. **Methods:** We targeted the following cell types, (1) adult neural stem cells from mice in vivo, (2) Embryonic stem cells in vitro, and (3) adult cancer cells from human biopsy. Proliferative cells labeled with EdU were phenotyped immunohistochemically, isolated using fluorescence activated cell sorting (FACS) and mRNA extracted for molecular characterisation. (1) The olfactory epithelium of young mice was harvested one day (n=46) or seven days (n=48) post EdU exposure (100 mg/kg, i.p.). (2) Mouse embryonic stem cells were exposed to 10mM EdU for 4 hours prior to harvest. (3) Resected glioblastoma multiforme tissue from human patients was exposed to10mM EdU for 12 hrs prior to harvesting from culture. The cells were dissociated to a single cell suspension, fluorescently labelled using Click chemistry and the EdU positive population isolated using FACS. **Results:** Molecular profiling of the cells at each stage of the experiment allowed RNA viability to be ascertained. Parallel cultures were phenotyped using immunofluorescence. The average RNA yield for each cell type was (1) 0.1 microgram/microlitre; (2) 1 microgram/microlitre; (3) 0.13 microgram/microlitre with the A260/280 absorbance ratio for all measuring 2.0. **Conclusion:** EdU allows effective targeting of proliferative cells in a variety of tissues, across mammalian species. It allows extraction of high quality mRNA from isolated cell populations, which can be used for the molecular profiling of these cells.

ORAL-09-05

AN ALGORITHM FOR QUANTIFYING NEURONAL AND MUSCLE POTENTIALS AFTER REGENERATION FROM NERVE INJURY

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Purpose: Functional evaluation by measuring the electrophysiological integrity of a nerve after injury and recuperation can be challenging due to difficulties in interpreting electrophysiological responses, particularly if responses are fragmented, incoherent and dispersed over various latencies. Our objective was to develop and test an algorithm based on cross-correlation for quantifying compound action potential responses. **Methods:** The algorithm detects and measures events automatically, and works by comparing evoked responses to a generic standardized signal, measuring the magnitude of each event as an arbitrary unit of energy for each point in time. To test the algorithm, we obtained data from regenerated and intact male rat sciatic nerve (n=6) (CNAP) and tibialis anterior muscle (CMAP) responses (n=9) of varying magnitudes that were elicited by altering stimulus intensity (nerve) or following frequency (muscle). We compared these responses evaluated by our algorithm to the most widely used method used for quantifying such signals, i.e., the peak-to-peak amplitude. **Results:** Our algorithm was able to successfully detect and quantified normal as well as greatly attenuated CMAPs and CNAPs, and has several advantages over traditional methods of quantification, such as it is automatic, objective, does not rely on the observer to identify and/or measure peaks, and can detect small responses that occur within background noise levels that is typical of such responses during regeneration. **Conclusion:** This algorithm serves as a useful tool for studying evoked compound action potentials in regeneration studies.

ORAL-09-06

SURFACE PLASMON RESONANCE IMAGING OF NEURAL NETWORKS

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Purpose: To develop a non-invasive imaging system for the long-term monitoring of the structural (and potentially functional) development of small neural networks. **Methods:** Surface plasmon resonance (SPR) is a phenomenon in which a collective oscillation of electrons on a metal surface (known as a plasmon) may be driven by visible light. Plasmons are highly sensitive to refractive index variations adjacent to the surface. This sensitivity has been very successful for chemical and biological assays. Here we describe the development of an SPR imaging system that can be used to image live cells. **Results:** An SPR imaging system has been developed to monitor small networks of cultured neurons. It uses low intensity illumination (1mW/mm^2) without the need for fluorescent dyes or other labels. This allows non-invasive imaging of live cells for an indefinite period of time. High-speed (10kHz) imaging is also possible. The system has a field of view of 300µm and its resolution is sufficient to image individual axons and dendrites. The contrast of the system is excellent and is currently limited only by the dynamic range of the camera. The system has been used to image cultured cardiac cells and small neural networks. **Conclusion:** An optical imaging system has been developed that is capable of label-free, high-contrast, wide-field, high-resolution, long-term and rapid imaging. The high contrast allows automated image processing to occur, which is necessary for high-speed long-term imaging applications. In principle SPR imaging may also be able to detect action potential activity. To date we have only detected functional signals from cardiac cells with our system. Ongoing research may resolve this.

ORAL-09-07

BRAIN BIOENERGETIC RESPONSES TO TRANSCRANIAL DIRECT CURRENT STIMULATION

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Direct current stimulation (tDCS) of the brain is an emerging treatment in a range of psychiatric and other brain disorders. Our interest lies in the mechanism of its efficacy. Here, we subjected nine healthy volunteers (4 males; average age 22 ± 2.45 y) to a blinded, sham-controlled study of the effects of 10 min 1 mA tDCS delivered to the left temporal lobe. Subjects were studied before, during and after tDCS at 3 Tesla with ^{31}P magnetic resonance spectroscopy (MRS) using a 10 cm surface coil placed directly over the left temporal lobe. Spectra were collected every 30 s for 2 min prior to tDCS, during tDCS and for 20 min following cessation of stimulation. Significant changes in brain bioenergetics were seen following active tDCS, with increases in the high energy compounds ATP and phosphocreatine (PCr), decreases in Pi and a significant increase in brain pH. Conversely, there were no significant changes in these variables following sham treatment. Further, there was a significant time dependence of the increase in ATP and pH and the decrease in Pi with active tDCS, but not with PCr. We conclude that active tDCS induces increased ATP synthesis, with a shift in the steady-state equilibrium of the creatine kinase reaction.

ORAL-09-08

THREE-DIMENSIONAL MULTI-SITE TWO-PHOTON PHOTOLYSIS OF CAGED NEUROTRANSMITTERS

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Signal integration in and between dendrites is important to understand network function. However, dendrites are not confined to a single optical plane. **Purpose**: To develop a two-photon microscope capable of achieving simultaneous multi-site photolysis of caged neurotransmitters at specific locations along the three-dimensional (3D) dendritic tree of a neuron. **Methods**: Whole-cell patch-clamp technique was used to record the electrical responses obtained from pyramidal cells in layers II and V in 300 µm slices of rat somatosensory cortex. The 3D morphology of a neuron is visualised by loading fluorescein (10mM) into the cell via a patch pipette and rendered by laser scanning using two acousto-optic modulators. A spatial light modulator functions as a programmable hologram to split-up an incident femtosecond-pulse Ti:S laser to project multiple diffraction-limited focal spots onto dendrites in different planes. Such spots can be used for photolysis of neurotransmitters or activation of optogenetic channels. To perform photolysis, caged glutamate (10mM) is applied via a pico-spritzer near dendrite(s) using short laser pulses (15ms at 730nm). **Results**: From the 3D image of the neuron, the appropriate hologram is generated to project several spots to the chosen dendritic locations. Depending on the strength of the laser pulses (average power 40-80mW/spot) and the number of uncaging locations, EPSCs from 30 pA to several nA could be measured. The latter could generate sequences of action potentials. **Conclusions**: Our results demonstrate how depolarisations evoked at several dendritic locations interact to form depolarisations at the soma. This new technique allows for systematic studies of how excitatory synaptic responses evoked at multiple locations over the entire dendritic tree interact.

ORAL-10-01

MOTOR CORTEX, BUT NOT MOTOR THALAMUS, ACTIVITY IS IMPAIRED IN ANAESTHETISED PARKINSONIAN RATS

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Purpose: Parkinson's disease symptoms are thought to reflect abnormal processing in the basal ganglia, operating via the motor thalamus, to modulate motor cortex activity. The aim of the study was to examine the affect of dopamine depletion on motor thalamus and motor cortex activity. Methods: We recorded ventroanterior-ventrolateral thalamus (VAVL) and sensorimotor cortex (MCx) single-unit and local field potential activities in urethane anaesthetised control and 6-hydroxydopamine (6-OHDA) lesioned parkinsonian rats. Results: Dopaminergic lesion selectively decreased the firing rate of putative pyramidal neurons in layer V of the MCx (0.4 ± 0.1 spikes/s, n=13, p=0.001) compared to control rats (2.3 ± 0.4 spikes/s, n=18). In contrast, dopaminergic lesion did not affect the firing rate of pyramidal neurons in layer VI (control 2.0 ± 0.6 spikes/s, n=20; 6-OHDA 1.2 ± 0.3 spikes/s, n=44), or the overall firing pattern of MCx putative pyramidal neurons. In VAVL thalamus, dopaminergic lesion did not alter the firing rate (control 3.5 ± 0.5 spikes/s, n=63; 6-OHDA 3.8 ± 0.4 spikes/s, n=66), firing pattern or the incidence of neurons with low threshold calcium spike (LTS) bursts (92%). Conclusions: Data show that the activity of putative pyramidal neurons in layer V of the MCx is impaired in Parkinson's disease. However, the lack of pathological activity in VAVL thalamus is not consistent with basal ganglia output driving activity in the motor thalamus in Parkinson's disease, and raises questions about the underlying role of the motor thalamus in the generation of Parkinson's disease symptoms.

ORAL-10-02

THE EFFECT OF CHEWING LOCATION ON JAW MOVEMENT SMOOTHNESS AND CHEWING EFFICIENCY

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The objective was to determine whether changes of chewing location (normal vs. anterior teeth chewing) had an effect on jaw movement smoothness and chewing efficiency. Methods: An accelerometer was attached to the skin of the mentum of 7 asymptomatic subjects and acceleration was recorded during chewing 2-colour (red and blue) chewing gum (Hubba-Bubba Tape Gums, England). During the gum chewing, vertical jaw displacement, acceleration, jerk, and the time differential of jerk, namely jerk cost (an inverse measure of jaw movement smoothness), were obtained as a function of time. Chewing was performed under two conditions: normal chewing on premolar and molar teeth, and test condition (using only canine and 1st premolar teeth). Jerk cost was calculated for the opening and closing phases of each chewing cycle for 5 cycles after an initial 3 cycles, and chewing efficiency was quantified (subjectively) by the colour change in the chewed gum (5 categories). Jerk cost and chewing efficiency were compared between normal chewing and test conditions with Wilcoxon signed rank test. Results: There was a significantly higher chewing efficiency during control chewing (Median Value=5) than during the test condition (Median Value=4; n=7, $p<0.05$). There was no significant difference in jerk cost between conditions in the opening phase (Control 7661.7± 8181.1 m^2s^{-5}, Test 7566.8± 10947.1 m^2s^{-5}, n=7, p=0.87) or closing phase (Control 4940.1± 4994.4 m^2s^{-5}, Test 4012.4± 4225.4 m^2s^{-5}, n=7, p=0.18). Conclusion: The data suggest that anterior tooth chewing decreases chewing efficiency but not jaw movement smoothness.

ORAL-10-03

DEPRESSION OF SOLEUS H REFLEXES IN HUMANS BY REPEATED PAIRED STIMULATION

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In humans, repeated pairs of afferent and cortical stimuli can result in either increases or decreases in excitability of the motor cortex, depending on the precise timing of the stimuli in the pair (Wolters et al. J Neurophysiol. 89, 2339-23451, 2003). Changes outlast the period of stimulation and may reflect long-term potentiation and depression at synapses in the cortex. Here, we investigated whether conditioning with paired stimulation could alter a human spinal reflex pathway. **Methods:** In 8 subjects stimulation of the posterior tibial nerve evoked small H reflexes in soleus before and for one hour after 8 min of conditioning with repeated paired stimuli (0.1 Hz, 50 pairs). In each pair, tibial nerve stimulation evoked a small H reflex and transcranial magnetic stimulation (double cone coil) evoked a small motor evoked potential (MEP). Three interstimulus intervals (ISIs) of +10 ms (H reflex before MEP), 0 ms, and -10 ms were investigated on three days. With <1 ms difference in the latencies of the H reflex and MEP, ISIs also reflect the relative arrival time of the volleys at the motoneurones. **Results:** With ISIs of +10 or -10 ms, there was no consistent change across subjects. However, with an ISI of 0 ms, the H reflex was depressed after conditioning in 7 of 8 subjects. Depression (mean±SD, 48±45%, n=7) was significant from 36 to 60 min after conditioning. This depression was unexpected as simultaneous arrival of two weak facilitatory inputs is generally thought to lead to potentiation of synaptic responses. **Conclusion:** H reflexes are susceptible to plastic change brought about by paired stimulation but the underlying mechanism is uncertain.

ORAL-10-04

EXAMINATION OF MOTOR UNIT POTENTIALS IN THE GENIOGLOSSUS USING AUTOMATED DECOMPOSITION BASED QUANTITATIVE ELECTROMYOGRAPHY (DQEMG)

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Purpose: Multiunit electromyographic (EMG) signal activity is increased in the genioglossus in OSA versus controls during wakefulness (1). This increase may in part be due to peripheral changes in the motor unit, without global increases in single motor unit discharge frequencies (2). We assessed quantitative parameters related to motor unit potential morphology and/or motor unit firing patterns derived from EMG signals in OSA versus controls. **Methods:** Diagnostic sleep studies to obtain Apnoea Hypopnoea Index. A quantitative EMG evaluation was completed using DQEMG to extract needle detected motor unit potential trains (MUPTs). Muscle activity was recorded with concentric needles with a recording area of 0.07mm2. The needle was positioned at >10 sites/subject, after ultrasound measurements. Mean firing rates were compared between the OSA and Controls. **Results:** 868 MUPTs from healthy subjects (n=7; AHI, 3.7/hr) and OSA patients (n=6 to date; AHI, 58.6/hr) were decomposed from the genioglossus muscle. Motor unit potentials in the OSA were longer in duration (12.1ms [9.2-15.1] *versus* 11.4ms [9.1-14.1], [median, 25–75 percentile]; p=0.03) had a larger area to amplitude ratio (1.58 [1.3-2.0] *versus* 1.46 [1.1-1.9]; p<0.001) and discharged at lower mean frequencies (15.2Hz [12.7-17.8Hz] *versus* 16.5Hz [13.0-20.4Hz]; p<0.001) compared to controls. **Conclusion:** These results confirm the need for further, more detailed quantification of neurogenic changes in the pathogenesis of OSA and illustrate the ability of DQEMG to be applied to this setting. [1]Mezzanotte *et al* 1992 *J Clin Invest 89:1571-9* [2]Saboisky *et al* 2007 *J Physiol 585:135-46*.

ORAL-10-05

PLASTICITY IN THE PHRENIC MOTOR SYSTEM FOLLOWING INJURY IN ADULT RAT SPINAL CORD

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Cervical spinal cord injury can compromise phrenic motor pathways impairing diaphragm function and breathing, and such injuries in people often necessitate assisted ventilation. However, experimental studies have revealed plasticity within the phrenic motor system which can mediate functional recovery. Purpose: The present work examines anatomical and functional changes following a high SCI to improve our understanding of post-injury respiratory plasticity and how recovery can optimized. Methods: Adult female rats received a lateral hemisection of the C2 spinal cord. Plethysmography was used to assess ventilation pre- and post-injury. Measurements were made under baseline (breathing normoxic, normocapnic air) and hypercapnic (7% CO2) conditions (n=66). All animals were then left to recover for 1-12 weeks post-injury. At the end of the study, a subset of animals (n=38) were terminally anesthetized to assess diaphragm activity. Bilateral diaphragm EMG recordings were made in spontaneously breathing animals under baseline and hypercapnic conditions. Anterograde (biotin dextran amine delivered to inspiratory cells in the medulla) and transynaptic retrograde tracing (pseudorabies virus (PRV) delivered to the diaphragm) was used to examine the changes in the phrenic circuitry following injury. Results: Despite reduced innervation of PhMNs ipsilateral to C2Hx, spontaneous diaphragm recovery ipsilateral to injury persisted for 3 months post-injury. Transneuronal tracing also revealed anatomical changes in the phrenic pathways mediating diaphragm activity contralateral to injury. Conclusion: These collective results reveal changes in the phrenic motor system that likely reflect restorative and compensatory plasticity. Studies aimed at enhancing PhMN recovery ipsilateral to C2Hx need to consider how these plasticity mechanisms may underscore maintenance of breathing post-injury.

ORAL-10-06

THE EFFECT OF SUBSENSORY AND SUPRASENSORY SACRAL NERVE STIMULATION UPON COLONIC PROPAGATING PRESSURE WAVES

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Purpose Supra-sensory stimulation of the S3 sacral nerve induces pan-colonic propagating sequences throughout the colon in patients with severe constipation. Whether the same response can be induced at a sub-sensory threshold is unknown. **Methods:** Under general anesthesia, in 14 patients with scintigraphically confirmed slow transit constipation, a fibre-optic manometry catheter (90 recording sites @ 1cm intervals) was positioned colonoscopically, with the tip clipped to the cecum to prevent displacement. A temporary electrode (Medtronic, Sydney) was implanted in the S3 sacral nerve foramina. Over 2 days, 3 parameters were tested; 1) sub-sensory; 2). Sham; 3) supra-sensory. Each 2hr hour stimulation period was preceded by a 2 hr basal period. All data are expressed as mean deltas (i.e. stimulation period – basal period). **Results:** In comparison to sham stimulation, sub-sensory stimulation had no effect upon colonic motility. In contrast supra-sensory stimulation induced a significant increase in the PS frequency (Sham; -1 ± 9 v Supra-sensory; 7 ± 5 PS/2hr; p = 0.04) and high amplitude PS frequency (-0.7 ± 1.4 v 0.6 ± 0.6 HAPS/2hr; p =0.04) when compared to sham stimulation. **Conclusion:** In patients with severe constipation, sub-sensory sacral nerve stimulation has no detectable influence upon colonic propagating pressure wave activity. In contrast, as we have shown previously, supra-sensory stimulation is capable of inducing propagating pressure waves throughout the colon. The mechanisms that underlie this does dependant response remain unknown.

ORAL-10-07

RAT STRIATAL CHOLINERGIC INTERNEURONS HAVE MARKEDLY FEWER SYNAPSES ON THEIR SOMA AND PRIMARY DENDRITES COMPARED TO STRIATAL SPINY PROJECTION NEURONS

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Purpose: Understanding the three-dimensional circuitry of neurons is essential for interpreting electrophysiological data as well as computer modelling of neurons and networks. This study measured the absolute number and type of synapses on somas and primary dendrites of rat striatal medium-spiny projection neurons and cholinergic interneurons. **Methods:** Two adult, male, Wistar rats (Rats 1 and 2) underwent perfusion-fixation. Serial 50µm vibratome sections were cut through the striatum. Immunolabelling of sections from Rat 2 with choline acetyltransferase (ChAT) helped identify cholinergic interneurons. One 50µm section per rat was processed for transmission electron microscopy. Subsets of serial 80nm sections per rat were analysed to reconstruct three medium-spiny neurons, two cholinergic interneurons (Rat 1) and two immunolabelled cholinergic interneurons (Rat 2). Neurons were identified by their ultrastructural anatomy, and in Rat 2, their ChAT-immunolabelling. Somas and primary dendrites were mapped for their synaptic input. **Results:** The majority of synapses were symmetrical, thus presumably inhibitory. Medium-spiny neurons had 3.5 times more somal synapses (130 + 36, mean + SD) than cholinergic interneurons (32 + 10, Student's *t*-test, $p < 0.01$). There were four-times as many symmetrical synapses per µm of primary dendrite for medium-spiny neurons (2.08 + 0.42) when compared to cholinergic interneurons (0.45 + 0.03, $p < 0.04$). **Conclusion:** These data suggest that there is relatively weak inhibitory control of the larger soma and proximal dendrites of cholinergic interneurons. This is consistent with individual excitatory inputs generating action potentials in cholinergic interneurons. Greater inhibitory control of medium-spiny projection neurons may contribute to their relative electrophysiological silence on stimulation.

ORAL-10-08

THE RECOVERY TIME COURSE OF HUMAN MUSCLE AND NERVE AFTER SUSTAINED, FATIGUING VOLUNTARY ACTIVITY

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Purpose: Repetitive discharges in both muscle and nerve are required to sustain voluntary muscle contractions. Such sustained activity causes fatigue in the muscle, and activity-dependent changes in axonal excitability in the nerve. This study investigated the different time courses of recovery of adductor pollicis and its ulnar nerve supply. **Methods:** 20 subjects aged 20-41 years sat with the forearm supported and semi-pronated, the thumb was positioned in 75% maximum abduction and securely attached to an isometric force transducer. Surface EMG was recorded from adductor pollicis and percutaneous electrical stimuli were delivered to the ulnar nerve at the wrist. Changes in muscle and nerve properties were monitored for 40 min using brief maximal voluntary contractions (MVC) and supramaximal resting twitches for muscle recovery, and indices of axonal excitability for motoneurone recovery. **Results:** Voluntary force was reduced by 44.8±2.6% after a 1 min sustained MVC ($p<0.001$). This rapidly recovered to 91.7±2.4% of baseline values. Resting maximal twitch force fell by 48.2±4.1% but was still depressed by 24.5±4.0% after 40 min ($p<0.001$). The motoneurone stimulus-response curve shifted to the right after the fatiguing contraction ($p<0.001$) but returned to baseline with recovery. The strength-duration time constant followed a similar pattern as did the 100 ms threshold-electrotonus response to 40% hyperpolarising conditioning pulses (both $p<0.001$). Supernormality showed a small, significant decrease ($p=0.03$) that recovered. The 0.5 ms reduction in the relative-refractory period ($p<0.001$) showed no evidence of recovery. **Conclusions:** These results suggest that activity-dependent changes in axonal excitability are unrelated to the processes that produce muscle fatigue. This evidence supports the hypothesis that the fatigue-induced reduction in the force-generating capacity of a muscle is an intrinsic property of muscle fibres.

ORAL-11-01

HYPOTHERMIA PRIOR TO SURGICAL DECOMPRESSION OF THE INJURED SPINAL CORD: BUYING TIME FOR TREATMENT BY REDUCING INTRACANAL PRESSURE

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Purpose: Human SCI is usually accompanied by persistent cord compression. Experimentally cord compression results in rapid neurological decline over hours. Undertaking decompression in humans within hours is impractical and there is therefore an important need for a therapy to prevent the neurological deterioration of patients prior to decompressive surgery. The aim of this study was to determine if hypothermia limits neurological decline following compressive SCI and reduces raised local intracanal pressure. **Methods:** Rats were subject to a moderate thoracic SCI and spacers inserted to compress the spinal cord by 45%. Canal pressure was monitored via a canulae within the spacer. Decompression was performed 0, 2 or 8 hours post-injury. Hypothermia (33oC) was commenced in half the animals 30mins post-injury and maintained for 7.5 hours, with the other half remaining normothermic. Motor recovery was assessed weekly and the volume of tissue damage determined at 8 weeks. **Results:** Hypothermia significantly improved the behavioural and histological outcome of animals undergoing 8 hours of compressive injury (primary outcome measure). The hypothermia treated group (n=16) regained weight-supported locomotion (BBB score 9.5±0.9) while the normothermia group (n=16) remained severely paraparetic (BBB score 5.3±0.6, P≤0.0005). Hypothermia reduced mean local intracanal pressure from over 30 mmHg to around 13 mmHg (n=9, p<0.001) and neurological recovery was closely linked to the rise in local intracanal pressure. **Conclusion:** Hypothermia significantly slows the rate of neurological deterioration accompanying cord compression by reducing local intracanal pressure and may be a useful bridging therapy to prevent neurological decline prior to decompressive surgery.

ORAL-11-02

USE OF A RAT MODEL OF ISCHAEMIC STROKE TO IDENTIFY MARKERS OF TEMPORAL STROKE PROGRESSION

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Purpose: Stroke is the third most common cause of death in most Western countries and the major cause of disability. Currently, only one thrombolytic drug, tissue plasminogen activator (tPA), is licensed for ischaemic stoke patients, but its use is limited by the need to know the time of stroke onset for treatment to occur within the time limit. This is unknown for many patients who are excluded from tPA treatment. Identification of appropriate biological markers could expand the proportion of patients able to benefit from the existing therapy. We aim to analyse the expression profile of molecules in the blood over time post-stroke to identify those showing changes, with the intention of establishing a biological “stroke clock” to enable determination of time since stroke onset. **Methods:** We induced stroke by thread occlusion of the middle cerebral artery in 8 male Spontaneously Hypertensive Rats, followed by reperfusion after 1.5 hours. Blood samples were taken post-stroke and analysed by ELISA for molecules of interest selected from the literature. **Results:** The level of CINC1 in plasma changed substantially over the first 6 hours post stroke. However, the levels of other molecules (BDNF, fractalkine, ICAM-1 and MCP-1) were uninformative. **Conclusion:** These results validate the strategy of using gene expression profiles to track stroke progression, and identified one informative profile out of five analysed. Expanding this approach to screen many genes could enable identification of individual genes or panels of genes, clarifying the time since the stroke and facilitating the safe use of tPA therapy for stroke.

ORAL-11-03

ALTERATIONS IN SUBSTANCE P AND NEUROKININ-1 RECEPTOR EXPRESSION IN RESPONSE TO TRAUMATIC SPINAL CORD INJURY: HUMAN AND ANIMAL STUDIES

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Purpose: Spinal cord injury (SCI) is a debilitating event that frequently results in permanent physical disability. Following severe SCI, persistent spinal cord oedema is present at the trauma site with delayed spread to adjacent segments. The development of oedema leads to raised intrathecal pressure with subsequent tissue damage and associated neurological dysfunction. Previous studies in our laboratory have shown that the neuropeptide substance P (SP) plays a critical role in oedema formation following brain injury, and that inhibition of the SP neurokinin-1 (NK_1) receptor attenuates oedema formation and improves post-injury neurological outcome. To ascertain whether similar mechanisms might occur following SCI, the current study investigates the expression of SP and NK_1 receptors following SCI in both human and rat spinal tissue. **Methods:** Archived tissue from post-mortem human SCI and a mild experimental model of rat SCI were obtained and stained for SP and NK1 using immunohistochemistry. The tissue was then scanned using a Hamamatzu nanozoomer and the digital images analysed semi-qualitatively using a colour-deconvolution technique. **Results:** Injured human spinal tissue showed an increase in SP expression that was maximal at 3 days post injury. NK_1 expression also increased, however this was maximal immediately following injury. Rat SCI tissue showed a decrease in SP and NK_1 expression at 1-week post-SCI. **Conclusion:** SP expression changes after SCI and may play a role in its pathophysiology. However, the differences in expression between the human and rat tissue may reflect either species differences or differences in mechanical injury mechanisms or severity and requires further investigation.

ORAL-11-04

SUBSTANCE P ALTERS THE PROFILE OF ENDOGENOUS NEUROGENESIS AFTER DIFFUSE AXONAL INJURY

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Purpose: Several research studies over the past 30 years have focused on improving outcome following traumatic brain injury (TBI). With the discovery of endogenous neurogenesis, recent research has been directed at encouraging newly generated cells to effectively integrate and survive. We have previously reported that the substance P antagonist, n-acetyl- tryptophan (NAT), improves outcome in rats following TBI. However, whether such improvement correlates with increased new cell integration is unknown. This question is addressed in the current study. **Methods:** Male Sprague-Dawley rats (360-420 g) were anaesthetized with 3% isoflurane and subjected to acceleration-induced TBI. Rats were treated 30 minutes post-injury with either 2.5 mg/kg NAT or vehicle. Twice daily pulses of 100 mg/kg BrdU were injected on days 1-4. Brains were collected at 1, 2, 4 or 7 weeks post-TBI (n=4-7/treatment/timepoint). Sham animals were anaesthetized but did not receive injury or treatment (n=5 at 1, 4 and 7 weeks). Additional rats were treated as above but received ICV infusion of Substance P from 48-96 hours post-injury and then killed at 1 week post-injury. **Results:** Injury significantly increased the number of BrdU positive cells 7 days post-TBI (p<0.001), with a further increase observed in animals treated with Substance P ± NAT (p<0.001). Only treatment with NAT significantly improved motor performance from day 2-7 (p<0.05 compared to vehicle). **Conclusion:** Current data indicate that Substance P infusion ameliorates the NAT-induced improvement in functional outcome although increases the number of BrdU positive cells. NAT treatment alone is associated with migration of these cells from the subventricular zone to the corpus callosum.

ORAL-11-05

AGE DEPENDANT CELL PROLIFERATION IN THE HIPPOCAMPUS AND SUBSTANTIA NIGRA OF THE LESIONED RODENT BRAIN

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Purpose: Evidence of cell proliferation in the hippocampus and subventricular zone is well documented. However evidence for neurogenesis in the adult substantia nigra is disputed, with majority of data being derived from adult rodents. With emerging evidence of age and injury related differences in rodent neurogenesis, we characterised age and focal injury modulated cell genesis. **Methods:** 3, 16, ≥52, and ≥104 week-old male Sprague Dawley rats with and without a sub-maximal dose of intra medial forebrain bundle 6-OHDA (n=3 per group), were treated post lesion with ethynyl deoxyuridine (EdU), at 25 mg/kg over 10 days to label proliferative cells. Animals were sacrificed at 28 days post lesion. Sections of the dentate gyrus of the hippocampus and substantia nigra were immunostained with multiple stem cell and neural markers to phenotype the EdU-labelled proliferating cells. **Results:** Cell proliferation was maximal in the hippocampus in juvenile animals with a decline in proliferation with age ($p \leq 0.01$, $p \leq 0.05$). An increase in hippocampal cell genesis was observed following lesioning ($p \leq 0.01$), with the majority of the cells co-localising the general neuronal marker NeuN. Elevated cell genesis was demonstrated in the sub-maximally lesioned substantia nigra ($p \leq 0.01$). However none of the newly generated EdU-labelled cells localised the glial markers GFAP and IBA1, the precursor markers SOX2 and PAX6 and the dopaminergic neuronal marker tyrosine hydroxylase. **Conclusion:** Cell genesis declines with age in the rodent brain, with focal lesion causing cell proliferation within multiple brain regions remote to the lesion site.

ORAL-11-06

VEGF AND PDGF MODULATES THE GLIAL RESPONSE TO A CORTICAL STAB INJURY

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Purpose: Increased astrocyte and microglia proliferation due to traumatic brain injury can impede regeneration of axons and produce progressive cavitation at the site of injury. Modulating the astrocytic and microglial response has potential therapeutic benefit in brain repair. We examine the modulatory effect of a single bolus of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) in combination, on the glial, mitogenic, and angiogenic response in a model of acute cerebral injury. **Methods:** A combination of VEGF and PDGF (20 pg) was injected into the striatum of adult male Sprague-Dawley rats (n=3/group). Animals received intra-peritoneal injections of ethynyl deoxyuridine (EdU) up to 5 days post injection, to label proliferative cells. Immunofluorescence was used to qualitatively and quantitatively assay the astrocytes, microglia, proliferating cells, and vasculature across the injury site. **Results:** Treatment caused a decrease in gliosis up to day 5 at the injury site ($p \leq 0.01$). However, an increase in gliosis was observed up to day 60 post treatment ($p \leq 0.01$). An increased infiltration of ramified microglia was observed up to day 60 post treatment ($p \leq 0.01$, $p \leq 0.05$), with phagocytic microglia increasing on day 5 ($p \leq 0.01$). The influence of VEGF and PDGF on cell proliferation and angiogenesis was not statistically significant under the current experimental regime. **Conclusion:** We demonstrate a profound and protracted modulation of the innate immune response of the brain by VEGF and PDGF.

ORAL-11-07

INHIBITION OF BONE MORPHOGENIC PROTEIN SIGNALLING DURING DEMYELINATION ENHANCES MYELIN REPAIR

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Purpose: Oligodendrocyte apoptosis is a key pathological event in CNS demyelination. This leads to demyelination of axons and progressive impairment of nerve cell function. Only limited endogenous remyelination occurs and enhancement of this process by augmenting regeneration of oligodendrocytes is emerging as a promising therapeutic strategy. Our previous work has revealed that Bone Morphogenic Protein (BMP) signalling is elevated during cuprizone-induced demyelination in two populations of cells that are likely sources of replacement oligodendrocytes, namely subventricular zone (SVZ) neural precursor cells and oligodendrocyte progenitor cells within the corpus callosum. We hypothesized that inhibiting BMP signalling during demyelination would enhance repair. **Methods:** We used osmotic mini-pumps to infuse Noggin, an endogenous antagonist of BMP4, or vehicle into the brains of mice during cuprizone-induced demyelination. BrdU was added to drinking water to mark proliferation and BrdU and lineage specific proteins were detected by immunohistochemistry. **Results:** Noggin infusion was effective in reducing BMP signalling as it reduced levels of phosphorylated SMAD1/5/8, a key component of BMP4 signalling. In cuprizone challenged mice, Noggin infusion decreased GFAP+ astrocyte numbers and increased Olig2+ oligodendrocytes in the SVZ compared to vehicle infused mice (n=5,5; $p<0.05$). In the corpus callosum, when assessed after 1-week of recovery following demyelination, Noggin infused mice had increased Olig2+ oligodendrocytes and CC1-BrdU double positive oligodendroglial cells compared with vehicle infused mice (n=5,5; $p<0.05$). In addition, Noggin infused mice had increased myelination compared to vehicle infused mice as assessed by fluoromyelin staining (n=4,5; $p<0.05$) and electron microscopy (n=3,3; $p<0.05$) after 1-week recovery following demyelination. **Conclusions:** Thus, our results suggest that inhibiting endogenous BMP signalling during demyelination promotes mature oligodendrocyte regeneration.

ORAL-11-08

EPO AMELIORATES AXONAL DAMAGE, ATTENUATES MACROPHAGE ACTIVATION AND RESTORES MOTOR FUNCTION FOLLOWING TAI AND HYPOXIA

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Purpose: Diffuse brain injury with secondary hypoxia is a frequent presentation in TBI, and is associated with worsened neurological outcomes. Erythropoietin (EPO) is neuroprotective in focal TBI, however its therapeutic effect has never been investigated in a diffuse TBI-hypoxia paradigm. In this study we examined whether EPO improves neurological outcome, reduces axonal pathology and attenuates neuroinflammation in rats following traumatic axonal injury (TAI) with/without hypoxia. **Methods:** TAI was produced in rats by dropping a 450g weight from 2m, and hypoxia by ventilating rats with 14% O2 in N2 for 30min after TAI. Rats were administered either recombinant human EPO-α (5000IU/kg, i.p.) or vehicle at 1 and 24h after injury. **Results:** TAI+hypoxia+EPO rats showed significant improvement on the Rotarod by 5d (24.3±2.2 rpm) when compared with TAI+hypoxia+vehicle (14.6±1.8rpm) ($p<0.05$). EPO-treated rats continued to improve after 5d, reaching sham levels by 10d post TAI+Hx. No differences were detected between TAI+vehicle and TAI+EPO treatments. Neurofilament staining revealed abundant swollen axons and bulbs in the corpus callosum and brainstem of TAI+hypoxia+vehicle rats, while TAI+hypoxia+EPO rats had less reactivity, with fewer bulbs and swellings at 1d and 7d post-injury. TAI+EPO rats showed no alterations in axonal injury when compared to TAI rats. Accumulation of CD68-positive macrophages was observed at 7d and 14d post-injury in TAI+hypoxia+vehicle rats, with a pronounced decrease detected in their EPO-treated rats. **Conclusion:** These preliminary results suggest that EPO provides neuroprotection following TAI+Hx, which may be due to hypoxia-induced upregulation of the EPO-receptor.

ORAL-12-01

MULTIPLE SPINAL RELAY PATHS CONTRIBUTE TO THE CORTICAL ACTIVATION: A BASIS FOR DISTRIBUTED PROCESSING OR REDUNDANCY?

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Purpose: To determine the contribution of different spinal pathways to the cortical activation evoked by bone (humerus), muscle (biceps or triceps) and a mixed nerve (median) inputs. The contribution of the spinothalamic, dorsal column-lemniscal and spinocervcial pathways was assessed by serial section of the ascending spinal tracts; namely, mid-line myelotomy, dorsal column transection (below C2) and ipsi-lateral dorsal lateral funiculus transection at C2. **Methods:** In anaesthetised New Zealand white rabbit (n=21; alpha-chloralose 70mg/kg) small nerve branches innervating the humerus, adjacent biceps or triceps muscle and the median nerve were exposed for electrical stimulation (2ms pulse, ≤ 2mA). Three 21-element platinum electrodes (courtesy of Cochlear Ltd) were placed over the exposed somatosensory cortex (2 arrays contra, 1 ipsi-lateral). Cortical responses (averaged 20 trails) to bone, muscle and median nerve stimulation were compared before and after spinal lesions. **Results:** Bone, muscle and median nerve stimulation evoked overlapping patterns of cortical activation in which response amplitudes and distribution were largest for whole nerve, intermediate for bone and smallest for muscle inputs. Invariably, the lesion of one, or two tracts, revealed a distributed spinal relay redundancy for bone, muscle and median nerve inputs. In each case the 3rd lesion abolished cortical activation. Stimulation above the spinal lesions evoked consistent cortical responses. **Conclusions:** Cortical responses to inputs arising from bone, muscle and median nerve inputs are relayed over multiple sensory pathways. Despite activation of discrete nerve branches we were unable to resolve any differential somatotopic localisation for inputs arising from adjacent structures such as bone and muscle in the forearm.

ORAL-12-02

ADENOSINE AMINE CONGENER MITIGATES CISPLATIN-INDUCED OTOTOXICITY

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Cisplatin is one of the most commonly used chemotherapeutic agents. The principal side effects of cisplatin include ototoxicity, neurotoxicity and nephrotoxicity. Cisplatin ototoxicity is manifested in most patients as tinnitus and bilateral high-frequency hearing loss. Purpose: Here we present a novel pharmacological intervention to mitigate cisplatin ototoxicity using systemic administration of a selective adenosine A1 receptor agonist adenosine amine congener (ADAC). Methods: Wistar rats were exposed to a two-cycle cisplatin treatment similar to clinical course of cancer chemotherapy. Each cycle comprised 4 days of intraperitoneal cisplatin injections (1 mg/kg twice daily) separated by 10 days of rest. ADAC (100 µg/kg) was administered intraperitoneally for 5 days at 24 hour intervals during the second cisplatin cycle, or immediately upon completion of the cisplatin treatment. Hearing thresholds were measured using auditory brainstem responses (ABR). Results: In control cisplatin-treated animals (n=8), ABR threshold shifts ranged from 12-28 dB across the frequency range used in this study (4-28 kHz). ADAC treatment during the second cisplatin cycle reduced cisplatin-induced threshold shifts by 22-64% ($p<0.05$), whilst the treatment was ineffective if ADAC administration was delayed until after the completion of the cisplatin regime. Functional recovery was supported by increased survival of hair cells and reduced apoptotic activity. Conclusion: These findings indicate that systemic administration of ADAC may protect the cochlea from the cisplatin-induced hearing loss, however its potential interference with antineoplastic effects of cisplatin is yet to be established. This study was approved by the University of Auckland Animal Ethics Committee.

ORAL-12-03

RELATIONSHIP OF DISTORTION PRODUCT OTOACOUSTIC EMISSION COMPONENTS TO HEARING IN HUMANS

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Otoacoustic Emissions (OAE), sounds emitted by the cochlea, are generated by non-linear physiological processes that underpin sound detection. Distortion Product Otoacoustic Emissions (DPOAE) are evoked using two pure tones which interact to cause nonlinear distortion in the cochlea. This distortion generates energy that travels both back to the ear canal (*generator component*), and forward to its characteristic frequency place then is reflected back to the ear canal (*reflection component*). As a result, energy measured at the ear canal is a combination of these two waves.. **Purpose:** We report the use of a novel stimulus and signal-processing paradigm to deconvolve the DPOAE into its two components and the comparison of characteristics of each component with measures of hearing. **Methods:** Each DPOAE component was extracted using swept pure tones and digital signal processing, with and without contralateral acoustic stimulation to evaluate the effect of efferent pathways, and compared with behavioural hearing assessments (n=15). **Results:** The generator component was robust across participants and showed non-linear growth with sound level. The reflection component was more variable and had a different growth function. Vector subtraction of DPOAEs with and without contralateral stimulation showed changes in both the amplitude and phase of each component with contralateral stimulation of efferent, descending auditory pathways. **Conclusion:** The new procedure permits extraction of both DPOAE components and provides evidence that they correlate differently to behavioural measures of auditory function. Extracting the major components of the DPOAE offers a method for investigating the physiological processes underlying normal and abnormal cochlear function.

ORAL-12-04

ROBUST AUDITORY RESPONSES IN THE CORE AND BELT AREAS OF SUFENTANIL ANAESTHETISED MARMOSETS

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Purpose: Obtaining responses in cortical neurons to species-specific vocalisations has generally been performed in awake animals, as anaesthesia is deemed to depress auditory responses severely, in particular when stimuli other than simple tones are used. Although awake preparations ensure the fidelity of the neuronal responses, there is an inherent limitation when the physiological data need to be combined with detailed histological reconstruction and anatomical tracing. Here we tested the efficacy of a Sufentanil-based anaesthetic regime, which is effective in preserving responses in visual association areas, to study stimulus-evoked neuronal responses in the marmoset auditory cortex. **Methods:** Adult marmosets were anaesthetised using a combination of Sufentanil (8 µg/ kg per hour, i.v.) and nitrous oxide (70%) inhalation. Unit activity was recorded throughout the cortical layers to auditory stimuli presented binaurally. Stimuli consisted of simple tones (to determine the cells characteristic frequency) and 3 marmoset calls, namely the Ock, Tsik and Twitter calls. **Results:** Responses from 250 cells showed a range of spiking activity encoding for various components of the calls, including beginning and ending as well as the fluctuations during the call. Both phasic and tonic activity, as well as excitatory and inhibitory components were observed. Furthermore a very late onset component was observed following the cessation of the stimulus, at approximately 150 to 200 msec, which varied according to laminar location of the cell. **Conclusion:** Sufentanil anaesthetic preserves complex response patterns in auditory areas across all laminae. This finding demonstrates the feasibility of combining the study of complex auditory response features in core and belt areas in acute preparations, allowing detailed histological reconstruction of the recording sites and correlations with neuroanatomy.

ORAL-12-05

A COMPARISON OF NEURONAL AND BEHAVIORAL DETECTION AND DISCRIMINATION PERFORMANCES IN RAT WHISKER SYSTEM

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Purpose: The rat whisker-barrel system is structurally well characterised and represents one of the main channels through which rodents collect information about their environment. We used the rat whisker pathway as a model system to investigate the correlation between the response function of cortical neurons and the behaviour of rats in a sensory detection versus discrimination task. **Method:** In Experiment 1, extracellular recordings were made from neurons in the barrel cortex area of anaesthetised rats (n=16) while applying vibro-tactile stimuli of varying amplitudes to the whiskers. In Experiment 2, rats (n=4) were trained in a behavioural task to select the higher amplitude stimulus between two vibrations applied to their whiskers. This combined detection/discrimination task involved stimuli that were identical to those used in Experiment 1 and thus allowed a direct comparison of the behavioural performances with the neuronal findings. **Results:** Neurons (n=235) showed a characteristic sigmoidal input/output function with an accelerating non-linearity at low stimulus amplitudes and a compressive nonlinearity at high stimulus amplitudes. An ROC analysis revealed that for near threshold stimuli, the neuronal discrimination performance surpassed the detection performance despite the fact that detection and discrimination represented identical amplitude differences. Similar to neuronal results, the rats' performance was significantly better for the discrimination task compared to the detection task. The behavioural performance followed a trend that was highly correlated with that of the population of individual neurons. **Conclusion:** Both behavioral and neuronal data are consistent with the "pedestal effect" previously reported in human psychophysics.

ORAL-12-06

FREQUENCY PERCEPTION OF APERIODIC VIBRO-TACTILE STIMULI IN HUMANS CAN NOT BE EXPLAINED BY MEAN DISCHARGE RATE IN ENTRAINED FAST ADAPTING AFFERENTS

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Purpose: Flutter frequency perception has been proposed to depend primarily on afferent firing rate rather than a temporal pattern code (Salinas et al. 2000) based on experiments in the monkey. We have used aperiodic vibrotactile stimuli studied in humans with microneurography and psychophysics to test this conjecture. **Methods:** Stimuli were applied to the fingertip by a computer-controlled mechanical pin-array whose high speed and low mass allowed precise control of the temporal structure of stimulus trains. Human microneurography confirmed that pin protraction readily excited FA afferents, but no activation of slowly adapting (SA) afferents was seen. For three 1s aperiodic stimuli (mean frequencies: 22, 26 and 30Hz, minimum inter-spike interval 8.7ms) the point of subjective equality (PSE) with periodic vibrations was found in a two-alternative forced choice experiment. The study was approved by the Human Ethics Advisory Panel at UNSW. **Results:** In all seven subjects the PSE for each of the three aperiodic stimuli was below the respective mean frequency rendering this effect highly significant ($p<0.05$; Wilcoxon). The PSEs averaged 79% (SD=9%; n=21) of the corresponding mean frequency of the aperiodic stimuli. **Conclusion:** Human flutter frequency perception of aperiodic stimuli can not be explained by mean discharge rate of entrained FA afferents as previously proposed. Alternative neural mechanisms for perception must be considered, most likely assigning different weights to inter-spike intervals depending on their length and distribution within the sample. *Salinas et al. (2000) Journal of Neuroscience 20:5503-5515*.

ORAL-12-07

ACTIVATION OF LOW THRESHOLD STRETCH-SENSITIVE RECTAL MECHANORECEPTORS DURING SPONTANEOUS COLONIC MIGRATING MYOELECTRIC COMPLEXES

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Purpose: Pain arising from the gastrointestinal tract is thought to arise from intense contraction (spasm) of the gut wall, but the classes of afferents activated are unknown. We determined which classes of rectal afferents are likely to be activated by physiological and intense contractions of the rectum. **Methods**: Extracellular recordings were made from rectal nerves in flat sheet preparations of the mouse colorectum. Recording of spontaneous colonic myoelectric migrating complexes (CMMCs) in the rectum and distal colon, and distension of the rectum were performed under isotonic or isometric conditions. **Results**: Spontaneous CMMCs were regularly recorded in the mouse rectum with force amplitude of 50.7 ± 7.1 mN (n=10) or shortening of 1.1 ± 0.14 mm (n=5). Both muscular (n=6) and muscular-mucosal (n=8) afferents were activated during spontaneous CMMCs. The majority (70%) of the low threshold afferents were activated during CMMCs and by low levels of circumferential stretch (1-2 mm). All low threshold afferents studied (n=8) were activated by bethanechol (100 µM)-induced contractions (2.3 ± 0.4 mm, n=5). The nicotinic agonist, DMPP (30 µM) activated robustly all low threshold stretch-sensitive afferents (n=8) and this activation persisted in a zero Ca2+ high Mg2+ solution (n=3). No high threshold serosal afferents were activated during spontaneous CMMCs or intense contractions evoked by bethanechol, even though in majority of rectal nerves, serosal units were activated by intense stretch (threshold: 15 ± 2.1 g, n=10). **Conclusion**: The majority of low threshold stretch-sensitive rectal mechanoreceptors, but not high threshold serosal afferents, are activated during spontaneous CMMCs and by bethanechol-induced contractions in the naïve mouse colorectum.

ORAL-12-08

C-TACTILE MEDIATED ALLODYNIA: A UBIQUITOUS AND ACUTELY REPRODUCIBLE PHENOMONON?

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Purpose: We recently showed that unmyelinated (C) tactile afferents in human hairy skin contribute to the crossover between neutral-touch (vibration) and painful-touch, i.e. allodynia. In hairy skin (anterior leg), allodynia persisted following compression of myelinated afferents, whereas anaesthesia of unmyelinated cutaneous afferents abolished allodynia. Although there is no evidence for the existence of a similar class of fibres in glabrous skin, the *'qualia'* of affective stimuli are comparable in hairy and glabrous skin. **Methods:** In 40 healthy subjects, sustained muscle pain was induced by infusing hypertonic saline (5%) into flexor carpi ulnaris muscle - innervated by ulnar nerve. In order to quantify the spatial extent and relative expression of allodynia across skin types, vibration (200Hz-200µm) was applied to glabrous skin of little and index fingers and hairy skin of dorsal forearm. Pain ratings were recorded using a Visual Analog Scale. The contribution of different fibre classes within innervation territory of ulnar nerve to the vibration-evoked allodynia was determined by myelinated-compression of ulnar nerve and/or anaesthesia of unmyelinated cutaneous afferents (Xylocaine 0.25%) around vibration site. **Results:** During muscle pain, vibration evoked a significant and reproducible allodynia that was comparable across skin types and spinal segments. Allodynia persisted during blockade of myelinated afferents but was abolished by selective anaesthesia of unmyelinated cutaneous afferents. Prior to induction and following cessation of muscle pain, vibration was reported as non-painful. **Conclusion:** This is the *first* study to provide psychophysical evidence for the existence of low-threshold unmyelinated mechanoreceptors in human glabrous skin. These observations suggest a role of these seemingly ubiquitous fibres in sensory-perceptual realignment, a familiar manifestation of clinical-pain states and neurological disorders.

ORAL-13-01

MODELING SCHIZOPHRENIA; CONSIDER THE SMALL BRAIN!

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BACKGROUND: Epidemiological evidence indicates that schizophrenia is a neurodevelopmental disorder. Using rodent models to study brain development and behaviour, we, and others, have shown that various exposures, such as low vitamin D and Poly I:C, during development implicate early abnormalities in dopamine (DA) neuron development. Modeling the ontogeny of DA systems may provide new leads in understanding both the etiology of schizophrenia and abnormal DA signaling observed in patients. AIMS: To transiently alter brain dopamine ontogeny in both zebrafish and Drosophila, and examine resulting behaviour in adults. METHODS: Using the morpholino RNA interference approach we have created a zebrafish (Danio rerio) model where we transiently interfere with dopamine synthesis. In addition using genetic tools available in the fruit fly, (Drosophila melanogaster) we have also developed a model in which we can transiently control the release of dopamine during development. RESULTS: The DA phenotype of embryonic zebrafish mutants reveals reduced DA synthesis and truncated dopaminergic innervation in the CNS. Although locomotion appeared to be grossly normal in the adult morphants, they appeared to show an anxiogenic phenotype. Surprisingly, adult Drosophila males revealed increased visual responsiveness when DA activity was increased specifically during late stages in larval brain development. We are now characterizing DA connectivity in these fly brains. CONCLUSIONS: These "Model Systems" combine conserved DA neurobiology with the ability to study large numbers of animals, as well as increased access to genetic tools, short generation times and more tractable development. These studies confirm that early alterations in DA signaling can permanently affect brain function and may even help to explore the etiology of serious psychiatric disorders, such as schizophrenia. We plan to use psychomimetics and antipsychotics in future experiments to further characterize the DA ontology phenotypes we have uncovered.

ORAL-13-02

THE SUBCHRONIC PHENCYCLIDINE-TREATED RAT: MODELLING THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA AND REVERSAL WITH RISPERIDONE

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Purpose: The development of animal models for schizophrenia has proven difficult, some symptoms have exclusively 'human' characteristics and thus unsuited, while other behaviours have been modelled but cannot be associated with concurrent brain neurochemical changes. Persistent blockade of NMDA receptor function by repeated phencyclidine (PCP) produces pathophysiological changes that model the cognitive/attentional deficits, social dysfunction and pathophysiological dysfunction of parvalbumin containing GABAergic neurons, observed in schizophrenia. In this study we evaluate the validity of the sub-chronic PCP rat in modelling behaviour associated with the positive symptoms of schizophrenia, and the effect of the antipsychotic, risperidone on the this behaviour. **Method:** Twenty-four (n=8/group) male Lister-hooded rats were administered PCP at a dose of 2mg/kg i.p. bi-daily for 1 week, or vehicle. Half of the phencyclidine group was concurrently treated with risperidone (0.5mg/kg i.p.) twice daily for 15 days, beginning 3 days before the start of PCP administration. Six weeks later all rats received a single PCP (3.2mg/kg i.p.) challenge and were placed in a locomotor box for 20minutes where their activity was recorded. **Results:** The PCP group displayed significantly more activity compared with the CON group after PCP challenge. Co-administration of the antipsychotic risperidone significantly reduced the effect of the PCP challenge after bi-daily PCP administration. Group effect: ($F(2,22)=25.9$; $p<0.0001$); PCP v PCP&Risp $p<0.05$. **Conclusion**: PCP produces a long-lasting behavioural sensitization which may be associated with neuronal toxicity or receptor sensitization. This effect is attenuated by co-administration of the atypical antipsychotic, risperidone, suggesting that risperidone may have some neuroprotective action against chronic PCP treatment.

ORAL-13-03

COGNITIVE IMPAIRMENTS IN DVD-DEFICIENT RATS WITH RELEVANCE TO SCHIZOPHRENIA

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Background: Schizophrenia is a poorly understood but very disabling group of brain disorders with cognitive dysfunction a core symptom of this disease. Based on clues from epidemiology, we have proposed that low prenatal vitamin D may be a risk factor for later development of schizophrenia. There is now robust evidence from in vitro and whole animal studies showing that low vitamin D levels during early life adversely affect brain development and adult behaviour. Methods: Female rats were fed a vitamin D deficient diet from 6 weeks prior to conception until birth, when they were transferred to a diet containing vitamin D. Control rats were fed a vitamin D containing diet throughout the experiment. Six-month old DVD-deficient and control Sprague-Dawley rats (n>6 per group) were assessed on selected cognitive domains using a 5 choice serial reaction time task, a 5 choice continuous performance task, and working memory using a delay match to position task in an operant chamber. Levels of dopamine, serotonin, glutamate and GABA were measured in brain tissue using HPLC. Results: DVD-deficient rats demonstrated mildly enhanced impulsivity and while they were normal on all measures of vigilance on response trials, their lack of inhibition on withhold trials was observed immediately and persisted throughout testing. Reduced glutamate levels in the striatum were correlated with impaired performance on the continuous performance task. No other neurotransmitter abnormalities were found. DVD-deficient rats had normal working memory as demonstrated by the delay match to position task. Conclusion: We show that DVD-deficient rats have impaired behaviour on tasks that assess attention and vigilance, which are analagous to human continuous performance studies, but have normal working memory. One interpretation of these data is that DVD-deficient rats have reduced glutamatergic input from the cortex to the striatum, which results in altered acquisition of a new response while inhibiting a previously learned response. These results provide specific targets to further investigate the influence of low pre-natal vitamin D on brain function.

ORAL-13-04

DEVELOPMENTAL TRAJECTORY OF THE ENDOCANNABINOID SYSTEM FROM NEONATE TO ADULT

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Purpose: Postmortem data implicate the cannabinoid CB_1 receptor (CB_1R) in schizophrenia pathogenesis. Endogenous CB_1R ligands (endocannabinoids) regulate brain development and modulate GABA release in mature brain. Since schizophrenia is a neurodevelopmental disorder we aimed to map the developmental trajectory of the endocannabinoid system in normal human brain. **Methods**: Tissue from the middle frontal gyrus of 63 subjects aged 39 days to 49 years was analysed using quantitative RT-PCR. mRNA targets were in three categories: 1) the cannabinoid CB_1 receptor (CNR1), 2) synthetic enzymes for the two major cortical endocannabinoids (NAPE-PLD for anandamide and DAGLα for 2-arachidonylglycerol [2-AG]), and 3) hydrolytic enzymes (FAAH for anandamide and MGLL and ABHD6 for 2-AG). **Results**: CNR1 mRNA expression peaks during infancy and decreases throughout postnatal life ($r=-0.562$, $P<0.001$). mRNA encoding synthetic and hydrolytic enzymes for anandamide increased from the neonatal period to adulthood (NAPE-PLD $r=0.570$, $P<0.001$, FAAH $r=0.480$, $P<0.001$), suggesting that anandamide takes more prominence as humans mature. Enzymes related to 2-AG show steadier mRNA across the lifespan. The synthetic enzyme DAGLα shows an inverted U-shaped pattern gently peaking at school age. A peak in the hydrolytic enzyme MGLL appears in infancy, while ABHD6 increases steadily until adulthood ($r=0.438$, $P=0.001$), suggesting that different mechanisms control 2-AG signalling depending on the age of the individual. **Conclusion**: These data represent the most comprehensive assessment of human endocannabinoid system development to date. Understanding the functions which presumably underlie the dynamic regulation of this system observed during life will provide an avenue for investigating how cannabis exposure during critical developmental periods may increase the risk for schizophrenia in vulnerable individuals.

ORAL-13-05

NEUROENDOCRINOLOGICAL REGULATION OF INSULIN DYSFUNCTION INDUCED BY OLANZAPINE

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Olanzapine is used to treat schizophrenia and other mental health disorders including bipolar, depression, anorexia and acute psychosis. However, chronic olanzapine treatment is associated with insulin-resistance and diabetes type II. A recent clinical study identified a time-dependent response of insulin to olanzapine treatment, whereby insulin decreased in the short-term, returned to basal levels after 2-weeks, then increased following chronic treatment. Olanzapine potently blocks muscarinic M3 receptors (M3R), which are located in the hypothalamic arcuate nucleus (Arc), dorsal vagal complex (DVC) of the brainstem and on the pancreas where they facilitate the cholinergic pathway for insulin secretion. In addition, neuropeptide Y (NPY) can inhibit insulin secretion. PURPOSE: To examine the effect of olanzapine on central M3R and NPY, and their relationship to insulin. METHODS: Rats were treated with olanzapine (2mg/kg, orally, 3x/day, n=12/group) or vehicle for 14-days. Plasma insulin levels were measured. NPY mRNA expression and M3R binding density were examined in the Arc and DVC (n=6/group). RESULTS: Sub-chronic (14-days) olanzapine decreased insulin (p<0.01) compared to controls. M3R binding density (Arc and DVC) and NPY mRNA expression (Arc) increased. NPY mRNA expression in the Arc positively correlated to M3R in the Arc (r=0.77) and DVC (r=0.89) (both p<0.01). There was a negative relationship between M3R in the Arc and insulin (r=-0.63, p=0.07). CONCLUSIONS: Increased M3R binding density may be a compensatory up-regulation in response to olanzapine antagonism. Sub-chronic olanzapine decreases plasma insulin, possibly through M3R blockade and increased NPY, changing insulin sensitivity, which may lead to insulin-resistance and diabetes type II following chronic drug exposure. Pancreatic M3R may also play a role in olanzapine-induced insulin dysfunction.

ORAL-13-06

NEURODEVELOPMENTAL PATHWAYS ALTERED IN A NOVEL, PATIENT-DERIVED CELL MODEL OF SCHIZOPHRENIA

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Neurodevelopmental pathways altered in a novel, patient-derived cell model of schizophrenia G. Abrahamsen1, Y. Fan1, R. Mills2, J. Cooper-White2, J. J. McGrath1,3, A. Mackay-Sim1 1National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, QLD, Australia 2Australian Institute for Bioengineering & Nanotechnology, The University of Queensland, St. Lucia, QLD, Australia 3Queensland Brain Institute, The University of Queensland, St. Lucia, QLD, Australia Schizophrenia is considered a disease of brain development, based on post-mortem brain and epidemiological analyses. Genetic and environmental risk factors are tested in animal models but their clinical relevance is not clear. Our novel model uses patient-derived cells from the olfactory mucosa, the organ of smell, which regenerates throughout life from a neural stem cell. Olfactory neurosphere-derived (hONS) cells were generated from biopsies of olfactory mucosa from healthy controls and patients with schizophrenia (n=9 in each group). Pathway analysis of the gene expression profiles identified core neurodevelopmental functions that were dysregulated in schizophrenia including cell cycle and focal adhesion. Subsequent functional studies demonstrated a faster rate of proliferation in schizophrenia hONS cells due to altered cell cycle regulation. After cell cycles were synchronised the schizophrenia cells had faster and larger increases in cyclin D, cyclin A, and phospho-ERK expression preceding S-phase and a larger proportion of cells entering S-phase in the first 30 hr after synchronisation. Focal adhesion kinase protein levels were significantly lower in the schizophrenia hONS cells and there was significantly lower adhesion of the schizophrenia cells to fibronectin. In addition, schizophrenia hONS cells migrated faster than control hONS cells. This difference was eliminated by inhibition of focal adhesion kinase, and by blocking of integrins. This novel approach identifies for the first time significant alterations in neurodevelopmental signalling pathways in patient-derived cells in schizophrenia.

ORAL-13-07

ARIPIPRAZOLE SELECTIVELY AFFECTS AKT/GSK PROTEIN EXPRESSION IN THE NUCLEUS ACCUMBENS AND CAUDATE PUTAMEN

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Aripiprazole has been used effectively to treat schizophrenia in the clinic, however its mechanisms of action are not clear. In vitro studies suggest that aripiprazole is possibly a functionally selective D2 receptor ligand. Our recent in vivo data showed that aripiprazole has selective effects on the mesolimbic vs. the nigrostriatal dopaminergic pathways. This study aimed to examine whether aripiprazole selectively affects the expression of Akt (a serine/threonine kinase) and GSK3 (glycogen synthase kinase-3) in these two dopaminergic pathways. Methods: Sprague-Dawley rats were treated orally with haloperidol (0.1 mg/kg), aripiprazole (0.75 mg/kg), or vehicle 3 times per day for either 1 week or 12 weeks. Western blotting was performed to examine the expression of Akt and GSK 3α/3β in the nucleus accumbens (NAc) and caudate putamen (CPu). Results: In the NAc, 12-weeks treatment with aripiprazole and haloperidol significantly increased the protein level of Akt and GSK3α and 3β, but 1-week treatment had no significant effect. In the CPu, 12-weeks treatment with haloperidol significantly increased the expression of GSK3β, while aripiprazole had no effect on both GSK3α/3β. Both antipsychotics had no effect on Akt expression in the CPu. Conclusion: These results suggest that, compared to the effects of haloperidol on both the NAc and CPu, chronic treatment with aripiprazole selectively acts on Akt/GSK pathway in NAc but not CPu. This selective effect may contribute to the long-term efficacy of aripiprazole in controlling schizophrenia symptoms with reduced extrapyramidal side-effects.

ORAL-13-08

CANNABINOIDS: RISK FACTORS FOR A NEUREGULIN 1 MOUSE MODEL OF SCHIZOPHRENIA?

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Purpose: Heavy cannabis consumption, particularly during adolescence, appears associated with an increased risk of developing schizophrenia (SZ) in susceptible individuals. However, cannabis is a mixture of cannabinoids, including the psychotomimetic cannabinoid receptor 1 (CB1) agonist Δ9-tetrahydrocannabinol (THC) and the potentially antipsychotic-like cannabidiol (CBD). To clarify the role of cannabinoids in the development of SZ, we investigated the effects of chronic CB1 stimulation (i.e. THC and CP 55,940 treatment) in adolescent/ adult mice mutant for the SZ candidate gene *neuregulin 1* (i.e. *Nrg1* HET). We also characterized the impact of adult CBD exposure in these mice. **Methods:** Adolescent male *Nrg1* HET mice and their wild type-like (WT) littermates received vehicle or THC (10 mg/kg i.p.) for 21 days, whereas adult cohorts were treated chronically with vehicle, CP 55,940 (0.4 mg/kg; 15 days) or CBD (1, 50, 100 mg/kg; 21 days). Behavioural tests (N = 10/cohort) were performed to assess SZ-related behavioural domains. **Results:** Adolescent mice were equally sensitive to the locomotor suppressant effects of THC. Neither treatment nor genotype had any impact on prepulse inhibition. THC impaired cognition and suppressed social interaction in WT mice. However, *Nrg1* mutants developed behavioural tolerance to chronic CB1 stimulation more readily than WTs. Exposure to CBD attenuated the hyperlocomotor activity and prepulse inhibition deficit observed in vehicle-treated *Nrg1* HETs. **Conclusion:** *Nrg1* mutants appear less sensitive to behavioural effects of adolescent CB1 stimulation but more susceptible than WT mice in adulthood. Importantly, chronic CBD rescued partially some of the behavioural abnormalities of *Nrg1* mice.

ORAL-14-01

AUTONOMIC MARKERS OF EMOTIONAL PROCESSING IN THE BRAIN: CONCURRENT FMRI AND RECORDINGS OF SKIN SYMPATHETIC NERVE ACTIVITY

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Purpose: The sympathetic innervation of the skin primarily subserves thermoregulation, but the system has also been commandeered as a means of expressing emotions. In order to understand the central neural processes involved in emotional processing and the generation of autonomic markers of emotion we recorded skin sympathetic nerve activity (SSNA) concurrently with functional magnetic resonance imaging (fMRI) of the brain while showing subjects neutral or emotionally-charged images from the International Affective Picture System (IAPS). **Methods:** SSNA was recorded via tungsten microelectrodes inserted into the peroneal nerve in 12 subjects. Gradient echo, echo-planar fMRI was performed at 3T. 200 volumes (46 axial slices, TR=8 s, TE=40 ms, flip angle=90 deg, raw voxel size =1.5x1.5x1.5 mm) were collected in a 4s-ON, 4s-OFF protocol. Total sympathetic burst amplitudes were measured during the period between scans. Blood Oxygen Level Dependent (BOLD) changes in signal intensity (SPM5, uncorrected $p<0.001$) were measured during the subsequent period to account for neurovascular delays. **Results:** Images of erotica or mutilation caused significant increases in total SSNA. Using this as the input model, we found covariation in BOLD signal intensity within multiple areas. By subtracting fMRI data obtained during presentation of neutral images, images of erotica specifically activated the left orbital and ventromedial prefrontal cortex, the left amygdala and nucleus accumbens. Conversely, images of mutilation specifically activated the right amygdala, right anterior insula and right dorsolateral prefrontal cortex. **Conclusions:** We have identified structures in the brain differentially engaged in the processing of emotionally-charged images, and in the generation of autonomic markers of emotional arousal.

ORAL-14-02

CONCURRENT RECORDING OF SPONTANEOUS FLUCTUATIONS IN SKIN SYMPATHETIC NERVE ACTIVITY AND WHOLE-BRAIN FMRI SIGNAL INTENSITY IN AWAKE HUMANS

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Introduction: We have previously used intraneural microelectrodes to record muscle sympathetic nerve activity (MSNA) while performing functional magnetic resonance imaging (fMRI) of the brain to identify regions responsible for the generation of MSNA (Macefield & Henderson, 2010). In the present study we attempted to identify cortical areas involved in the generation of spontaneous skin sympathetic nerve activity (SSNA). **Methods**: SSNA was recorded via a tungsten microlectrode inserted into the peroneal nerve in 9 subjects. Gradient echo, echo-planar fMRI was performed using a 3T scanner (Philips Achieva). Two hundred 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 2nd and 3rd second of the 4s-OFF period and correlated with Blood Oxygen Level Dependent (BOLD) changes in signal intensity (SPM5: random effects, minimum cluster size 10 voxels, corrected false discovery rate $p<0.05$). **Results**: SSNA was positively correlated to signal intensity in the dorsolateral prefrontal cortex and precuneus, and negatively correlated to signal intensity in the medial prefrontal and perigenual anterior cingulate cortex and the left hippocampus. **Conclusions**: We have shown that signal intensities in several cortical areas covary with spontaneous fluctuations in SSNA, suggesting that these areas may contribute to the generation of SSNA at rest. **Reference**: Macefield VG & Henderson LA (2010) Real-time imaging of the medullary circuitry involved in the generation of spontaneous muscle sympathetic nerve activity in awake subjects. Human Brain Mapping 31: 539-549.

ORAL-14-03

'STRESS' IS ASSOCIATED WITH SYMPATHETIC ACTIVITY IN PEOPLE WITH THE METABOLIC SYNDROME

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Purpose: Stress pathways, including the sympathetic nervous system, have been demonstrated to be activated and involved in generating the metabolic abnormalities that characterise the metabolic syndrome (Metsyn). In this study we aimed to determine whether comorbid anxiety and depression contributed to the sympathetic activation evident in people with the Metsyn. **Methods:** Forty-seven untreated subjects, meeting criteria for the Metsyn according to the International Diabetes Federation (2005) guidelines, were recruited. Sympathetic activity was measured using muscle sympathetic nerve activity (MSNA) of the peroneal nerve. Blood pressure was measured using radial arterial tonometry and heart rate was determined using a lead III ECG recording. Anxiety and depression levels were assessed with the Trait section of Spielberger's State and Trait Anxiety Inventory and Beck Depression Inventory II (BDI-II), respectively. **Results:** In people with the Metsyn, higher scores on the BDI-II were significantly associated with higher MSNA compared with lower scores, 43±2 vs 34±3 bursts/min (mean ± SEM; $P=0.02$), respectively. Similarly, higher anxiety scores were associated with higher MSNA, 43±2 vs 33±3 bursts/min (mean ± SEM; $P=0.007$). Those subjects with higher depression and anxiety scores also exhibited a worse "metabolic profile"; higher scores were associated with elevated insulin, triglycerides and cholesterol. Age, gender and blood pressure were comparable in each group. **Conclusion:** Our data indicate that people with the Metsyn with higher depression or trait anxiety scores exhibit significantly elevated sympathetic nervous activity and a worse metabolic profile. The central nervous system pathways responsible for the sympathoexcitation in these subjects remain to be determined.

ORAL-14-04

STRESS-INDUCED HYPERTHERMIA IS NOT MEDIATED BY BROWN ADIPOSE TISSUE IN MICE

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Purpose: Psychological stress leads to increases in body temperature that are sympathetically mediated. Brown adipose tissue (BAT) is the only organ known to produce heat in response to sympathetic activation. We have previously shown in rats that during conditioned fear, this hyperthermia is not the result of increased thermogenesis in the interscapular area of the back, where the largest deposit of BAT is found. Stress-induced hyperthermia is widely used as an anxiety indicator in mice. We thus sought to verify if this response can be attributed to BAT thermogenesis in mice. **Methods:** Eight C57BL/6 mice were handled and shaved at interscapular and lumbar back skin areas prior to testing. Animals received injections of 20 mg/kg dl-propranolol or saline and placed in either an open field or 4°C enclosure for 30 min. Infrared pictures were taken each minute to record interscapular, lumbar and tail temperatures. **Results:** Propranolol reduced the stress-induced hyperthermia observed during open field exposure ($p<0.01$), as indicated by the interscapular and lumbar back temperatures, but did not change the 2°C difference between these two variables ($p>0.05$), which is a measure of BAT thermogenesis. There was no observable effect of propranolol on behaviour, as animals remained active throughout the test. In contrast, the difference between interscapular and lumbar back was increased by 2°C during cold exposure, and this increase was abolished after propranolol ($p<0.0005$), indicating BAT thermogenesis during this challenge. **Conclusion:** Just as rats exposed to conditioned fear, mice exposed to an open field display a stress-induced hyperthermia that is not mediated by BAT.

ORAL-14-05

SPECIFIC SYMPATHETIC NERVOUS SYSTEM-TARGETED NEUROPEPTIDE Y EXPRESSION IN MICE IMPROVES GLUCOSE TOLERANCE

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Neuropeptide Y (NPY) plays critical roles in the regulation of glucose homeostasis and energy balance. One pathway that NPY exerts these functions may be via regulating sympathetic nervous system (SNS) since NPY is co-stored and co-released with noradrenaline from sympathetic nerve terminals, and since SNS is a well known regulator of energy and glucose homeostasis. **Purpose and methods**: To investigate the specific role of NPY in central noradrenergic neurons and the SNS in the control glucose and energy metabolism, we generated a transgenic mouse model overexpressing NPY selectively in the SNS and brain noradrenergic system by utilizing the promoter of dopamine-β-hydroxylase gene to drive NPY expression in NPY-/- mice. Transgenic and littermate control mice of both genders were examined with 6 -10 mice per group. **Results**: Male and female transgenic mice exhibited similar body weight and body composition, energy expenditure and physical activity compared to gender-matched controls. Daily food intake and faecal output showed an increase in transgenic mice, and significantly so in male group. Interestingly, transgenic mice of both genders displayed lower glycemic excursion during an intraperitoneal glucose tolerance test (1g/kg body weight), with a significantly reduced area under the glucose curve in male transgenic group. This improved glucose tolerance is likely due to an enhanced insulin sensitivity, since insulin secretion in response to glucose challenge was similar between male transgenic and control mice, and was in fact significantly reduced in female transgenic mice in comparison to female controls. **Conclusions**: This study demonstrates an important and specific role of NPY expressed in the central noradrenergic system and SNS in the regulation of glucose metabolism and insulin action.

ORAL-14-06

EFFECTS OF SUSTAINED MUSCLE PAIN ON SPONTANEOUS MUSCLE SYMPATHETIC NERVE ACTIVITY IN AWAKE HUMAN SUBJECTS

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Purpose: We recently showed that acute muscle pain, induced by bolus intramuscular injection of hypertonic saline, causes a sustained increase in muscle sympathetic nerve activity (MSNA) and a modest increase in blood pressure and heart rate. However, the effects of long-lasting pain, which more closely approximates chronic pain, on MSNA are unknown. **Methods**: MSNA was recorded via tungsten microelectrodes inserted percutaneously into the common peroneal nerve from 13 healthy subjects. Tonic pain was induced for ~60 minutes by slow intramuscular infusion of hypertonic saline (7%) into the ipsilateral tibialis anterior muscle. Pain was sustained at a tolerable level (5-6/10 on a visual analogue scale). **Results:** Seven subjects showed progressive increases in mean MSNA amplitude during tonic pain, increasing to 154±17% (SEM) at 40 mins and remaining essentially constant for the duration of the infusion. Conversely, for the other 6 subjects MSNA showed a progressive fall, with a nadir of 67±11% (SEM) at 35 min. **Conclusions:** We conclude that tonic muscle pain has long-lasting effects on MSNA, causing a sustained increase in some subjects yet a sustained decrease in others.

ORAL-14-07

EXERCISE-INDUCED INCREASE IN VAGAL TONE IS MEDIATED VIA CENTRAL INSULIN-LIKE GROWTH FACTOR RECEPTORS

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Our aim was to test whether (IGF) is involved in mediating exercise-induced elevation in cardiac vagal tone and increased resistance to cardiac arrhythmias. For this purpose, using Alzett osmotic mini-pumps connected to icv cannulas, we administered either JB1 (IGF receptor antagonist) or vehicle to adult male Wistar rats that were voluntarily excercising for 5 weeks in running wheels attached to their cages (n=12) or remained sedentary (n=12). Half of exercising and half of sedentary animals received icv JB1 (Ex/JB1 and Sed/JB1 groups); another half received saline vehicle (Ex/Veh and Sed/Veh groups). At the end of exercise training, ECG electrodes were implated sc, and one week later we recorded basal heart rate and assessed cardiac autonomic outflow using atenolol and methyl-scopolamine. On a different day, in acute experiment we assessed sensitivity to proarrhythmic agent aconitine. Exercise training reduced basal heart rate in vehicle-treated animals (331±7 vs 358±9 bpm in Ex/Veh vs Sed/Veh) but not in JB-1-treated rats (374±11 vs 380±10 bpm in Ex/JB1 vs. Sed/JB1). Experiments with sympathetic blockade revealed that exercise-induced reduction in the basal heart rate was mainly due to the increase in the cardiac vagal tone. Exercise training reduced sensitivity to aconitine in both exercising groups (arrhythmogenic dose for aconitine was 266±52 and 585±36 mkg/kg for Sed/Veh and Ex/Veh, respectively 289±66 and 631±46 mkg/kg for Sed/JB1 and Ex/JB1, respectively), without any significant effect of the treatment. We conclude that increase in vagal tone during exercise is mediated via central IGF receptors, and that cardioprotective/antiarrhythmic effect of exercise in our study was not related to the increase in vagal tone.

ORAL-14-08

RAPID ACTIVATION OF RENAL SYMPATHETIC ACTIVITY WITH ONSET OF HIGH FAT DIET IN RABBITS

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Purpose: Short term consumption of a high fat diet (HFD) induces elevated blood pressure (BP), heart rate and renal sympathetic nerve activity (RSNA) which may be the basis for the development of long term obesity related hypertension[1]. In the present study we determined the rapidity of such changes and whether this involved disturbance to other stimuli including airjet stress, hypoxia and baroreflexes. **Methods:** New Zealand White rabbits implanted with telemetry devices for BP and RSNA were placed on a normal or 13.5% HFD. Reflexes and stress responses were examined weekly during the 3-week diet. **Results:** After 1 week on the HFD, rabbits demonstrated 4%, 8% and 30% greater BP, heart rate and RSNA respectively ($P < 0.05$). By the end of 3 weeks of HFD, BP, heart rate and RSNA were elevated by 10%, 5% and 82% (n=9). Baroreflex curve analysis showed that the increase was independent of baroreceptor inputs and similar to the effects of acute stress. Indeed acute airjet stress induced lesser sympathetic responses suggesting that the pathway was already activated. The sympatho-excitatory responses to hypoxia were similar in the HFD and normal diet groups over the 3-week period of diet. **Conclusion:** Together these results suggest that sympathetic activation occurs within several days of a high fat diet and may be associated with a chronic activation of forebrain pathways mediating the sympathetic responses to acute emotional stress. [1]**Prior et al, *Hypertension* 2010; 55:862-868**.

ORAL-15-01

VISUAL RESPONSE LATENCY IN THE LATERAL GENICULATE NUCLEUS OF MARMOSETS

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Purpose: Parallel afferent pathways are customarily described as brisk / fast or sluggish / slow on basis of axonal conduction and visual evoked response latencies. In macaque dorsal lateral geniculate nucleus (dLGN) the difference in visual response latency between magnocellular (MC) and parvocellular (PC) pathway cells is small (MC leads PC by ~10 ms) [1]. Here we asked whether this difference applies to marmosets, and additionally distinguished response latency in koniocellular (KC) cells. **Methods:** Single electrode, extracellular recordings were made in sufentanil-anaesthetised marmosets (Callithrix jacchus, n=20). Response latencies were estimated for achromatic or short wavelength (S) cone-isolating flashed spots covering receptive field centre and surround. Data were binned at 10 ms width and the bin containing the largest number of spikes within 200 ms of stimulus polarity change was taken as peak response latency. **Results:** Peak response latency of PC cells (93±25 ms, mean ± s.d., n=128) was close to MC cells (92±34 ms, n=45) and Blue-on KC cells (96±25 ms, n=32) whereas blue OFF cells peaked at 128±30 ms (n=5). Other cells recorded in the KC layers included in this analysis had a peak latency of 112±45 ms (n=10). The OFF-centre PC and MC cells had longer latencies than their ON-centre counterparts (PC ON, 86±24; PC off, 100±25; MC ON, 74±30; MC OFF, 102±32). **Conclusions:** With exception of blue-off KC cells visual evoked response latencies are similar across cell classes in marmoset dLGN. The assumption that KC pathway is characterised by sluggish visual evoked response is not consistent with our data. **Reference:** Maunsell, J. H. et. al., (1999) *Vis Neurosci* **16**, 1-14.

ORAL-15-02

NEURONAL EVIDENCE FOR A HIERARCHICAL MODEL OF COMPLEX CELL RECEPTIVE FIELDS

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Purpose: Cells in primary visual cortex are often classified into two distinct cell types – simple and complex. Hubel and Wiesel's (1962) hierarchical model proposed that the luminance-phase tuned simple cells are created by combining and aligning the centre-surround receptive fields (RFs) of cells from the thalamus along the preferred orientation of the resulting simple cell. They also proposed that complex cells, that are not sensitive to luminance-phase, could in turn be formed by combining the output from multiple simple cells with similar orientation preferences but with spatially offset RFs. Visual responses of complex cells presented with moving sine wave gratings are known to become phasic (more simple like) as contrast is reduced. This behaviour is best predicted by a hierarchical model in which complex cells are created from multiple simple cell RFs exhibiting a range of contrast response functions. In such a model, as contrast is reduced afferent input to complex cells is biased towards simple cells with low contrast thresholds. In the limiting case, a complex cell's input could be reduced to a single simple cell, thus resulting in the phasic responses observed at low contrasts. **Methods:** To examine this we recorded visual responses from 74 cells in cat visual cortex (areas 17 and 18) while presenting contrast reversing sine wave gratings at multiple spatial phases and contrasts. We then compared the phasicity of complex cell responses at different spatial phases as stimulus contrast was reduced. **Results:** We found that there was a significant negative correlation between log contrast and phasicity of the cells' responses. **Conclusion:** Reducing contrast below the threshold of a subset of a complex cell's inputs increases the phasicity of the complex cell due to the phasicity of the remaining supra-threshold inputs as predicted by the hierarchical model.

ORAL-15-03

VISUAL RESPONSES IN AREA PROSTRIATA, A PROISOCORTICAL FIELD LOCATED NEAR THE ROSTRAL TIP OF THE CALCARINE SULCUS

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Area prostriata is a thin strip of cortex, which in primates forms a transition between the peripheral representation of striate cortex (V1) and the hippocampal formation. In terms of cytoarchitecture, *prostriata* resembles retrosoplenial area 30, having proisocortical features such as poorly defined layer 4 and weak myelination. Although visual responses near this region were demonstrated more than 40 years ago, its role in visual processing has received little attention. We used single-unit electrophysiological recordings to study the visual response properties of prostriata neurons in marmoset monkeys anaesthetised with intravenous sufentanil (4-8 µg.ml-1.h-1) and nitrous oxide (70% in O2). Neurones showed robust responses to simple visual stimuli, such as flashed spot of light, but, in contrast with those in adjacent V1, showed weak orientation selectivity. The median response latency was 53ms (N=94), comparable to that of V1 neurones tested with similar stimuli (50ms, N=38). The receptive fields tended to be very large: while neurons with receptive fields 40°-50° wide were typical, some responded to stimuli virtually anywhere in the contralateral hemifield. Remarkably, receptive field centres were concentrated in the far periphery (eccentricity >50°), although some receptive fields encompassing the fovea were also observed. Spatial frequency selectivity peaked at low frequencies (~0.05c/°), and there was no obvious sensitivity to optic flow fields. In light of its widespread anatomical projections not only to visual areas, but also to medial prefrontal, cingulate motor and auditory parabelt areas, our data indicate that prostriata is at the core of an association pathway that can fast-track visual information to other brain systems, bypassing the traditionally recognised hierarchy of visual processing isocortical areas.

ORAL-15-04

INTER-HEMISPHERIC INHIBITORY CONNECTIONS OF A FEATURE-DISCRIMINATING NEURON IN THE DRAGONFLY

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The 'centrifugal small target motion detector' (CSTMD1) neuron in the dragonfly (*Hemicordulia tau*) responds to the visual presentation of moving features in a size-selective manner. Using electrophysiological techniques, we examined characteristics of CSTMD1 and revealed intriguing higher-order properties. For example, the neural response to a moving target can be completely suppressed by the presence of a second distracter target moving in the visual field of the opposite eye[1,2]. Due to the alignment of the underlying morphology, a reasonable hypothesis to explain this result is that the mirror symmetric CSTMD1 inhibits its counterpart version. This would mean that the strength of the inhibitory region of CSTMD1 would itself be a size-selective phenomenon and that the transfer of visual information from one hemisphere to the other is involved in multiple target computations. To test this hypothesis, we changed the height (0°, 0.8°, 1.6°, 4.2°, 8.3°, 16.6°, 83°) of a distracter target (with a fixed width of 0.8°) and examined the inhibitory effect on the primary target (as it traversed the excitatory receptive field). This experiment was repeated with an average of 20 trials at each height over 4 CSTMD1 neurons. Contradictory to our expectations, as we increased the height of the distracter target we observed that its inhibitory strength was maintained. This was evidenced by either (a) suppression of activity below spontaneous rate (irrespective of distracter size) or (b) that the strength of the inhibition was actually increased with increasing target height. This surprising result reveals that a reassessment of both the inhibitory mechanisms and the functional role of CSTMD1 are required.
[1]Geurten at al. (2007) JEB, [2]Bolzon et al. (2009) J Neurosci.

ORAL-15-05

REPRESENTATION OF A COMPARISON STIMULUS IN THE MIDDLE TEMPORAL AREA (MT) DURING A MEMORY FOR MOTION TASK

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Visually guided behavior often involves decisions that are based on evaluating stimuli in the context of those observed previously. Successful execution is likely to depend on complex interactions between areas traditionally associated with sensory processing, working memory and cognitive control. This study examines the role of MT neurons, more commonly associated with visual motion processing, in the comparison phase of such tasks. **Methods**: Two macaque monkeys were trained to perform a memory for motion task in which they compared the directions of two moving stimuli, sample and test, separated by a temporal delay of 1.5 sec. Monkeys indicated whether the two stimuli moved in the same or different (always opposite) directions via button press. While animals were performing the task, we recorded the activity of 171 neurons in area MT. **Results**: We found that MT activity during the test not only reflected the current direction of motion, but also carried comparison signals that depended on the direction of the preceding sample. Three independent groups of cells, which carried comparison signals, were identified: One group (17%) showed early response suppression during same trials. Two groups of cells showed response enhancement later in the response, one associated with same (20%) and the other with different trials (13%). Cells carrying comparison signals were also more likely to have responses predictive of perceptual decisions. In a subset of trials, sample stimulus was presented in the opposite hemifield to the receptive field, with the exception of early suppression, comparisons signals remained present, however the underlying mechanisms became less clear with more late suppression involved. **Conclusions**: These results demonstrate that during the memory for motion task, MT neurons have access to the remembered sample, although the nature and the temporal dynamics of these modulations depend on the bottom-up and top-down influences involved in the generation of comparison signals.

ORAL-15-06

VISION IN SEA SNAKES

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Purpose: The anatomy of the snake retina has been studied in considerable detail, largely due to the use of morphological characters such as photoreceptor complement to resolve ophidian phylogeny. However, the spectral identities of the different photoreceptor types is only known in eight of the more than 2900 extant species. Of these eight species, the spectral properties are only partially known and there is no information on any marine species. Methods: We used microspectrophotometry and light- and scanning electron-microscopy to characterise photoreceptor visual pigments and ultrastructure in the spine-bellied sea snake (Lapemis curtus, n=5) and the horned sea snake (Acalyptophis peronii, n=5). Results: We show that there are three spectrally distinct pigments with wavelengths of maximum absorbance at 428, 496, 555 (L. curtus) and 430, 496 and 559nm (A. peronii). Light- and scanning electron-microscopy of the L. curtus retina reveals three morphological subtypes of photoreceptor: large single cones, small single cones and double cones. Double cones and one type of large single cone contain the 555nm pigment; another type of large single cone contains the 428nm pigment and the small single cones contain the 496nm pigment. The topographic distribution of neurons in the retinal ganglion cell layer of L. curtus, the olive sea snake Aipysurus laevis and the olive-headed sea snake Disteira major shows there are localised peaks in neuronal density (areae centrales) in the nasal, temporal and ventral regions of the retina. Conclusion: We relate the spectral tuning of the photoreceptor visual pigments and retinal topography in sea snakes to their visual ecology.

ORAL-15-07

INTERHEMISPHERIC CONNECTIONS AFFECT CONTRAST RESPONSE FUNCTIONS OF NEURONES IN CAT'S AREA 18

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In visual mammals such as the domestic cat, the transition zone at the border between visual areas 17 (V1) and 18 (V2) is strongly interhemispherically interconnected. However, the interhemispheric interconnections between the primary visual cortices are not entirely restricted to the regions in the vicinity of the representation of the vertical meridian (VM)[1]. **Purpose and Methods:** We examined the effect of reversible inactivation (cooling to 10°C) of contralateral area V1/V2 border region (cV1/V2 border) on the contrast response functions of single neurones recorded from area 18 of anaesthetized adult cats. The stimuli were optimised, achromatic sine-wave modulated gratings of different contrasts restricted to cells' classical receptive fields (CRFs). **Results:** In most cells (16/19) with CRFs located < 5° from the VM, inactivation of cV1/V2 border resulted in significant reductions of the maximum responses (R_{max}) accompanied by increases in the contrasts generating 50% of R_{max} (C_{50}). However, in three cells, inactivation of cV1/V2 border resulted in significant increases in R_{max} (response gain) accompanied by decreases in C_{50} contrasts (contrast gain). Finally, in three cells with RFs 15-20° from the VM, inactivation of cV1/V2 border resulted in significant reductions in R_{max} (response loss) accompanied by decreases in C_{50} contrasts (contrast gain). **Conclusion:** While the majority of area 18 cells with CRFs located in the vicinity of the VM receive excitatory input from the contralateral hemisphere, a minority receives suppressive input (presumably via inhibitory interneurones). The contralateral primary visual cortex appears to exert contrast dependent suppressive/excitatory effects on area 18 cells with CRFs located some distance from the VM.
[1]Milleret C & Houzel J-C (2001) *Eur J Neurosci* 13, 137-152.

ORAL-15-08

SLEEP DEPRIVATION INCREASES VISUAL RESPONSIVENESS IN DROSOPHILA MELANOGASTER

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Purpose: The effect of sleep deprivation on performance is an increasing concern in modern societies. Typically, sleep deprivation leads to cognitive deficits in humans. We investigated the effects of sleep deprivation on visual responsiveness in the fruit fly model Drosophila melanogaster. Methods: Adult flies (3-7 days old) were sleep-deprived by mechanical stimulation for 12 hours during their subjective night. The effectiveness of this treatment was monitored by quantifying fly locomotion, where sleep-deprived flies displayed a sleep rebound the following day. Groups of 30 flies were tested 1-4 hours following treatment for behavioural responsiveness to moving gratings in a visual choice maze paradigm, and these were compared to non-sleep deprived controls. Responsiveness to the moving grating was quantified by a weighted average of fly distributions among nine collection points at the end of the maze, where positive scores indicated a response to the moving visual. Results: Mechanical sleep deprivation increased visual responsiveness of wild-type Drosophila from 0.47 ± 0.12 to 1.14 ± 0.17 when examined 3 hours after the end of sleep deprivation (N=180 flies per condition). Analysis of sleep rebound data of night-time sleep deprived flies compared to day-time handled controls confirmed that increased visual responsiveness at this time point was indeed the effect of sleep deprivation and not stress. Further experiments providing visual competition revealed an attention-like defect in sleep-deprived flies. Conclusion: We propose that increased visual responsiveness following sleep deprivation in flies reflects a suppression defect. By impairing attention-like mechanisms in the fly brain, sleep deprivation results in a failure to suppress a visual reflex. We are studying this phenomenon using genetic constructs and electrophysiology to better understand the effect of sleep deprivation on cognition in our fly model.

ORAL-16-01

TYPE-1 INTERFERON SIGNALLING PLAYS A DELETERIOUS ROLE IN THE OUTCOME AFTER STROKE

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Type 1 Interferons (IFNs) are a super-family of pleiotropic cytokines that induce pro-inflammatory gene transcription via the classical JAK/STAT pathway. Increasingly, there has been evidence in the literature to suggest that neuroinflammation plays a key role in the progression of neural injury seen in stroke. To address this IFNAR1-/- mice underwent mid cerebral artery occlusion (MCAO) surgery and demonstrated a decreased infarct size (24.9±7.1mm3 n=8) compared to wild-type controls (65.1±4.8mm3 n=8). Western blot and immunohistochemistry showed alterations in the Stat-1 and 3 phosphorylation profiles in the IFNAR1-/-. Neuroprotection conferred by the absence of IFN signaling was confirmed in IFNAR-deficient primary cultures that were protected from cell death when exposed to oxygen glucose deprivation (OGD). Co-culture experiments using IFNAR1-/- glia and WT neurons and WT glia and IFNAR1-/- neurons were carried out in the OGD model. IFNAR1-/- neurons in the presence of WT glia no longer displayed a neuroprotective phenotype suggesting the glia are a major driver of the neuroinflammatory response. In an attempt to block IFNAR signalling in vivo a blocking monoclonal antibody targeting the IFNAR1 receptor was injected into WT mice via the tail vein (0.5mg) 30 minutes prior to MCAO. This resulted in a 60% decrease in infarct size when compared to the IgG control. Collectively these results indicate signalling through the IFNAR1 subunit is deleterious in stroke. Furthermore, our findings suggest that therapeutic agents targeting the IFNAR1 subunit may be beneficial in reducing the severity of a neuro-inflammatory event following stroke and in doing so limit infarct size.

ORAL-16-02

PTEN PHOSPHORYLATION AND NUCLEAR SHUTTLING IS IMPORTANT FOR NDFIP1-MEDIATED NEUROPROTECTION

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Purpose: Tumor suppressor PTEN has multiple roles in neurons including the regulation for apoptosis and proliferation. Recently we have found that Ndfip1, an adaptor protein for the Nedd4 family of E3 ubiquitin ligases, can interact with PTEN. This interaction results in enhanced ubiquitination of PTEN, and is essential for PTEN nuclear shuttling during brain injury. Here we investigate the role of PTEN phosphorylation in regulating Ndfip1 mediated ubiquitination in stroke. **Methods:** *In vivo* studies used a hypoxia-ischemia model of stroke to observe the location and phosphorylation of PTEN and upregulation of Ndfip1. *In vitro* studies were used to study the interaction between Ndfip1 and both phospho-mimic (PTEN 4D) and dephosphorylated (PTEN 3A) forms of PTEN. **Results:** Using a hypoxia-ischemia model of stroke we observed lower levels of phosphorylated PTEN in the ipsilateral cortex (injured) compared to the contralateral cortex. In addition, nuclear shuttling was observed for PTEN in the ipsilateral cortex in neurons upregulated for Ndfip1. *In vitro* binding assays indicate that Ndfip1 has a lower affinity for PTEN 4D (phospho-mimic) compared to PTEN 3A. Similar results were observed in ubiquitination assays were PTEN 4D has decreased levels of ubiquitination in the presence of Ndfip1 compared with PTEN 3A. **Conclusion:** Our results suggest that during stroke stressed neurons decrease the levels of phospho-PTEN which promotes the interaction with Ndfip1 and results in PTEN ubiquitination and nuclear accumulation. We suggest that nuclear PTEN has neuroprotective functions.

ORAL-16-03

MYD88-DEPENDENT CONTROL OF INFLAMMATION FOLLOWING STROKE

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The inflammation that follows stroke can be both detrimental and beneficial; therefore, understanding its regulation could lead to therapeutic advances. The Toll-Like Receptors acting through the adaptor protein MyD88 control ischemic inflammation in the heart and kidneys however their role in stroke is yet to be investigated. The aim of this study was to understand the contribution of MyD88-dependant signaling to neuro-inflammation following stroke. This study used middle cerebral artery occlusion (MCAO) as a model of stroke. MCAO was applied to WT, MyD88-/-, WT mice reconstituted with WT and MyD88-/- hematopoietic cells and MyD88-/- mice reconstituted with WT hematopoietic cells. Infarct size was measure with TTC staining, and cellular infiltration into the brain was investigated with imunofluorescence. Primary WT and MyD88-/- neuronal cultures were exposed to oxygen glucose deprivation (OGD) and survival was measured with an MTT assay. MyD88-/- mice suffer a larger infarct than WT mice following MCAO (WT: 54.29 ± 7.743 MyD88-/-: 84.48 ± 8.579 n=11-13 $P<0.05$), however, MyD88-/- neurons exhibited no greater injury than their WT counterparts following OGD (n=7-11, $P>0.05$). Interestingly, following MCAO WT mice reconstituted with MyD88-/- haematopoietic cells were found to have an infarct size similar to MyD88-/- mice (n=8, $P>0.05$ vs MyD88-/-), whilst both WT and MyD88-/- mice with WT haematopoietic cells suffered an infarct the same size as the WT (n=5-8, $P>0.05$ vs WT). Immunofluorescence revealed that cellular infiltration in the ipsilateral cortex was dependant on the genotype of the haematopoietic cells with regards to MyD88. Both cellular infiltration and infarct size following stroke has been found to be dependant on MyD88 expression in the hematopoietic cells, this study has shown MyD88 dependant signalling in these cells alone contributes to neuro-protection by diminishing the infarct size following stroke.

ORAL-16-04

THE NEURAL BASIS OF EMOTIONAL WORD PROCESSING IN PEOPLE AFTER STROKE

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Purpose: Find the neural basis of emotional words processing, in people with aphasia after stroke. **Methods**: People with aphasia (n=8), and matched controls (n=8) participated in the study. Interviews were taken before and after EEG recordings, in order to assess the emotional value of words. Validated questionnaires were used for that. EEG was recorded from 64 electrodes cap, using the Neuroscan system. Evoked potentials were computed separately for emotional and neutral words. Peak amplitudes and latencies of auditory evoked potentials were measured. The Comprehensive Aphasia Test (CAT) was administered to participants. **Results**: The emotional value effect on the brain response, or the difference between the response to emotional and neutral words, was less in people with aphasia. In general, auditory evoked potential components were delayed, with smaller amplitudes in people with aphasia, compared to the controls. Normal auditory evoked responses were found for controls. **Conclusions**: Even though the auditory cortex was not known to be damaged, auditory evoked responses were compromised in people with aphasia. Those results are interesting regarding our understanding of the effect of a local stroke on the brain as a whole. This study may have significance for the design of speech language therapy for people with aphasia as it suggests the involvement of primary auditory processing. In addition, brain activity was found in areas known to be damaged by the stroke, supporting residual functioning in these areas and the design of therapy directed at the utilisation of these brain areas. Emotional processing of the words was found to be less in people with aphasia, which also has implications for therapy.

ORAL-16-05

DISTRIBUTION AND QUANTIFICATION OF THE NEUROSTEROID ALLOPREGNANOLONE IN RESPONSE TO STROKE

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The neurosteroid and progesterone metabolite, allopregnanolone, is neuroprotective in models of brain injury including stroke. Allopregnanolone acts at $GABA_A$ receptors by increasing inhibition and limiting excitotoxicity. We aimed to determine whether (i) endogenous allopregnanolone was upregulated following stroke, (ii) this occurred in excitotoxic regions (penumbra), and (iii) further increases occurred following exogenous allopregnanolone administration. Male spontaneously hypertensive rats (SHR) were subjected to 90min middle cerebral artery occlusion (MCAo). Treatment groups received allopregnanolone (8mg/kg) 20min post-reperfusion. Distribution of allopregnanolone was assessed in groups sacrificed at 3 and 6h post-MCAo (n>3/group). Allopregnanolone positive cells were identified by immunohistochemistry. Brain allopregnanolone was quantified by gas chromatography mass spectrometry (GCMS) in striatum and sensory cortex at 3h post-MCAo (n=4). A dramatic increase in allopregnanolone immunoreactivity was seen between 3 and 6h in penumbral regions (sensory, insular, and piriform cortices). Little change was seen in either unsalvageable stroke core (striatum) or anterior cerebral artery supplied motor cortex. This suggests a gradual upregulation of allopregnanolone within areas of excitotoxicity. GCMS quantification revealed 2- to 3-fold higher levels of brain allopregnanolone in animals administered allopregnanolone compared to non-injected controls. This finding was consistent in infarct core, penumbra, and both stroke and contralateral hemispheres. These results indicate an upregulation of allopregnanolone within stroke regions. Increased immunoreactivity may result from increased binding within these areas. The endogenous allopregnanolone upregulation following stroke is slow (significant increase from 3–6h) and hence may not be capable of significant neuroprotection post-stroke. However, this can be significantly augmented by exogenous administration at a standard dose.

ORAL-16-06

LOWER SAFETY LEVEL OF BLOOD PRESSURE IN THE PATIENT OF ACUTE CEREBRAL INFARCTION

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Purpose: At the acute phase of cerebral infarction (CI), the autoregulation of cerebral blood flow (CBF) is compromised, and the appropriate BP level has been still unbiguous. Therefore, for revealing the lower BP safety level in the treatment of acute CI, the relation between CBF and BP was explored using the data observed by SPECT. **Methods:** Patients of acute internal carotid artery or middle cerebral artery (MCA) territorial infarction were screened between January 2008 and January 2010. The cases with SPECT performed within 7 days following stroke onset were consecutively enrolled. Then, included cases were classified into moderate stenosis (n=9), severe stenosis (n=7), occlusion (n=10) and occlusion with stenosis (n=4) groups based on the MRA findings. CBF at the border zone of MCA was measured and the ratio (ischemic lesion / intact region) was compared among these 4 groups. **Results:** The average ratio was significantly reduced in the occlusion group compared to the moderate and severe stenosis groups. Significant correlation was observed between CBF ratio and systolic BP in the moderate stenosis group. The CBF ratio was significantly correlated to both systolic and diastolic BP in the occlusion with stenosis group. According to the linear regression analysis, the safety level (the ratio of CBF is higher than 0.7) of lower BP was 120/70 mmHg in the moderate stenosis group and 150/85 mmHg in the occlusion with stenosis group. **Conclusion:** It may not need to keep high BP even in the acute CI patients. However, BP control should be carefully managed in the acute stroke patients with occlusion and stenosis in main arteries of the brain.

ORAL-16-07

ASSESSMENT OF MAGNESIUM AND MILD HYPOTHERMIA IN RAT FOCAL AND GLOBAL CEREBRAL ISCHAEMIA MODELS

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There are, at present, few neuroprotective treatments available following stroke and cerebral ischaemia. To address this, we have assessed the neuroprotective efficacy of magnesium and mild hypothermia (35°C) alone and in combination in experimental stroke/cerebral ischaemia rat models. Methods: Permanent and transient (90min) intraluminal thread middle cerebral artery occlusion (MCAO) was used to model focal ischaemia (SD or SH rats). Transient carotid occlusion (8min) with hypotension was used to model global ischemia (SD rats). Mg treatment: IV bolus 360µmol/kg + infusion 120µmol/kg/h for 24h (focal) or 48h (global); hypothermia treatment: core body temperature of 35°C or 33°C for 24h. Results: Combined Mg and mild hypothermia was effective when commenced 2h or 4h after permanent MCAO, but ineffective at 6h. Combined treatment was ineffective when administered 2h or 3h after transient MCAO. Following global ischaemia, treatment was effective when commenced 1.5 to 2h after ischaemia, but ineffective at 3h or 4h. Mg alone was ineffective. Mild hypothermia was effective following global ischaemia, and reducing the level of hypothermia to 33°C did not improve outcomes following either focal or global ischaemia. Conclusions: Mg and mild hypothermia can reduce ischaemic brain injury when administered early, however to further improve the therapeutic window and efficacy it may be necessary to combine the treatment with other neuroprotective agents.

ORAL-16-08

CHARACTERISATION OF A NEW MODEL OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE SHEEP

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Purpose: More than 60,000 Australians suffer a stroke each year, with devastating consequences. Many animal models of cerebral ischaemia, including those in rodents, have been developed to mimic clinical stroke although novel treatments characterized in these models have failed to translate to the human condition. Rodents have a small lissencephalic brain with a small amount of white matter. In contrast, sheep have a large gyrencephalic brains with large white matter domains. Accordingly, we have developed a novel large animal model of ischaemic stroke in the sheep. **Methods:** After mapping the anatomy on post mortem tissue we have developed a novel surgical approach to middle cerebral artery occlusion (MCAO). Merino sheep (n=16) were subject to either sham surgery or MCA occlusion achieved by either diathermy (permanent), ligature (2h occlusion) or the application of an aneurysm clip (2h occlusion). Brain tissue oxygenation (Licox®), intracranial pressure, blood pressure and blood gases were recorded. Animals were monitored for 4h after the induction of stroke and killed by perfusion fixation. **Results:** MCA occlusion by diathermy or ligature was commonly associated with complications such as bleeding. Accordingly, the aneurysm clip approach was found to be the superior method. Aneurysm clip application produced a 52% reduction in brain tissue oxygenation, which recovered upon reperfusion. Cerebral perfusion pressure and blood gases remained stable. **Conclusion:** The sheep model of MCAO, in particular, the aneurysm clip approach, may represent a new method for investigation of the pathophysiology associated with ischemic stroke and provide an appropriate vehicle for pre-clinical testing of therapeutic agents.

ORAL-17-01

PROGRESSION OF PLURIPOTENCY TO NEURONAL LINEAGES

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Purpose: Differentiation of pluripotent stem cells to mature lineages requires multiple temporal stages whereby extrinsic and intrinsic signalling factors direct cell fate. We describe different methods of neural induction of human embryonic stem cells (hESC) and how each method differs with respect to when patterning and commitment to specific neural progenitors occurs. **Methods:** HESC were differentiated to neural progenitors using three different systems of neural induction; (a) noggin treatment of hESC, (b) hESC co-culture with PA6 feeder layer, (c) hESC cultured on laminin substrate in defined media. Neural progenitors were then harvested and analysed for their expression of markers of neural stem cell populations. **Results:** It was found that hESC neural induction by commonly used methods, including noggin treatment or co-culture with PA6 feeder cells, defaults to a dorsal forebrain/midbrain population of neural progenitors. To bias the differentiation of neural progenitors towards ventral and caudal populations requires very early exposure of extrinsic signals, such as Sonic hedgehog and retinoic acid. **Conclusion:** These studies form framework models for deriving specific neuronal lineages of the central and peripheral nervous system from human pluripotent stem cells.

ORAL-17-02

THE ROLE OF RAGE DURING NEURONAL DIFFERENTIATION

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Purpose: The Receptor for Advanced Glycation End products (RAGE) is a protein that is thought to play an important role during the process of neuronal differentiation. RAGE is a multi-ligand receptor. Binding to its ligands activates a number of intracellular signalling pathways that leads to diverse downstream effects that are dependent on the type of ligand itself. S100B is a physiological ligand for RAGE, and its binding is known to promote neurite outgrowth and survival of neuronal cells. **Aim:** The primary aim of this study was to identify the spatio-temporal expression patterns of S100B during the process of neuronal differentiation, and correlate these with the known expression pattern of RAGE. **Methods:** Expression patterns were determined by performing semi-quantitative Western blot and immunocytochemistry (ICC) using a cell line derived from human, the embryonic teratocarcinoma cell line known as NT2 cells (n=3). **Results:** The results show that S100B and RAGE have smilar expression patterns during neuronal differentiation of NT2 cells. The Western blot showed that the level of expression of both RAGE and S100B increased in a similar pattern with progress of differentiation. The receptor-ligand interaction of RAGE and S100B was further confirmed by ICC showing the colocalisation of the proteins in various cellular locations throughout the differentiation process. **Conclusions:** These findings have led us to conclude that S100B is the principal ligand for RAGE and that their interaction may have important functions in the process of neuronal differentiation. Further detailed investigations will be required to characterize the role of RAGE during neuronal differentiation in terms of molecules involved in the signalling pathways.

ORAL-17-03

THE ROLE OF TRKB IN OLIGODENDROCYTE DEVELOPMENT AND CENTRAL NERVOUS SYSTEM MYELINATION

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During development, oligodendrocytes extend a multi-lamellar membrane sheath around axons that expresses a number of unique glycoproteins collectively known as myelin that is essential to saltatory conduction and the normal functioning of neurons. The molecular mechanisms required to achieve myelination are yet to be fully elucidated. Brain-Derived Neurotrophic Factor (BDNF) has recently been associated with myelination, due to the hypomyelination observed within the central nervous system of BDNF knockout mice. Using *in vitro* myelination assays, our laboratory has found that BDNF promotes oligodendrocyte myelination (n=3), via activation of oligodendrocyte-expressed TrkB receptors (n=3). To verify these findings, we utilized shRNA to specifically knockdown TrkB from oligodendroglia in the myelination assays. Surprisingly, the expression of myelin markers increased as a result of TrkB knockdown, as did expression of the oligodendrocyte progenitor marker NG2 (n=3). BrdU studies indicated that TrkB knockdown increased proliferation of oligodendrocyte progenitors (n=3). We have also examined these effects *in vivo* using a mouse model of conditional knockout of TrkB in oligodendrocytes. Analyses at post-natal day 30 indicate that these mice exhibited reduced expression of myelin protein in the CNS, compared to littermate controls (n=4). Interestingly, a significant increase in the number of oligodendrocyte precursors was concurrently observed (n=6), consistent with the increased proliferative response observed *in vitro*. In summary, the deletion of TrkB results in a proliferative response amongst oligodendrocyte progenitors, suggesting an innate compensatory response to hypomyelination in this animal model. We are currently investigating the mechanism for the increased oligodendroglial proliferation.

ORAL-17-04

FUNCTIONALLY DISTINCT OLIGODENDROCYTE PROGENITOR POPULATIONS RESIDE IN THE POSTNATAL MOUSE BRAIN

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Oligodendrocyte progenitors (OPCs) are synaptically connected to neurons, and these synapses are thought to permit neuronal regulation of OPC behaviour, and consequently oligodendrogenesis and myelination. However in addition to oligodendrocytes, OPCs continuously produce new projection neurons for the piriform cortex. **Purpose:** Some reports indicate that voltage-gated ion channel expression, and spiking behaviour differs between OPCs, and we have previously shown that OPCs can be divided into two populations based on whether they do, or do not proliferate in vivo. In this study we perform a detailed analysis of OPC heterogeneity in order to determine how this relates to OPC function. **Methods and Results:** Following sustained administration of the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU), which is incorporated by proliferating cells during s-phase of the cell cycle, a plateau is reached in the proportion of OPCs that are labelled, indicating that ~80% of brain OPCs undergo cell division in vivo (n=3 mice per time point). We have conducted detailed profiling of individual OPCs, by whole-cell patch-clamping single GFP+ OPCs, in transgenic mice that were pre-labelled with EdU, to distinguish between the mitotically active quiescent OPCs. Post-recording, slices were labelled with anti-NG2. OPCs fell into two classes: one population proliferated in vivo and expressed INa+, while the other was mitotically quiescent and had a significantly smaller INa (sINa) (p=0.007). Furthermore, by performing in vivo lineage tracing using PDGFRa-CreERT2 / Rosa26-yellow fluorescent protein reporter transgenic mice, we demonstrate that mitotic, INa OPCs generate oligodendrocytes and the mitotically quiescent, sINa OPCs are neurogenic. **Conclusions:** There are two physiologically and behaviourally distinct classes of OPC in the postnatal brain.

ORAL-17-05

REGULATION OF TRK RECEPTOR FUNCTION BY AN INTRACELLULAR DOMAIN FRAGMENT OF P75NTR

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Purpose: The neurotrophin receptors p75 (p75NTR) and tropomyosin-relatated kinase receptors (Trk) are critical regulators of neuronal development and function. We have previously demonstrated that regulated proteolysis of p75NTR via α- and γ-secretases is key to regulating neuronal survival and cell death. The aim of this project was to assess the role of regulated cleavage of p75NTR in facilitating Trk receptor function. **Methods:** p75NTR-Trk interactions were investigated in HEK293 and PC12 cells, and cultured embryonic E13 motor neurons (MNs) using FRET, colorimetric viability assays, neuronal differentiation and immunobloting techniques. *In-vivo* MN death was induced by transection of the ulnar nerves. **Results:** In this study we identified that a monomeric portion of the p75NTR intracellular domain (p75NTR-ICD) interacts with TrkA, and blocking p75NTR-ICD generation inhibits optimal TrkA function (n=7). In addition we found that a peptide of p75NTR (FC29) spanning the TrkA binding site, promotes TrkA-mediated differentiation and survival following growth factor withdrawal in PC12 cells (n=6). Moreover, FC29 promotes survival of cultured MNs under continuous death signaling stimulation, but only in the presence of TrkB co-activation (n=9). Furthermore, FC29 promotes survival of axotomised ulnar motor nerves *in-vivo*. **Conclusion:** We have shown that FC29 is a powerful inhibitor of p75NTR-mediated neuronal death via a Trk-dependent mechanism. We propose that the Free Chopper region of p75NTR-ICD is crucial for the ability of p75NTR to facilitate Trk function in development, and may be a suitable therapeutic target for the treatment of neurodegenerative conditions.

ORAL-17-06

THE ROLE OF NDFIP1 AND UBIQUITINATION IN THE REGULATION OF THE CELL CYCLE DURING NEUROGENESIS

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Purpose The regulation of cell cycle during neurogenesis is critical for proper brain development and function. Many proteins involved in the cell cycle are regulated by ubiquitination. We have identified Ndfip1, an adaptor protein for the Nedd4 family of E3 ubiquitin ligases, as an important member in regulating the G2 phase of the cell cycle during neurogenesis. **Method** *In vitro* we have studied both Ndfip1 inducible and Ndfip1 RNAi cell lines to determine changes to the cell cycle using flow cytometry and MTT proliferation assays. *In vivo* studies have used Ndfip1 knock-out mice and BrdU pulse labelling to identify cell cycle changes during neurogenesis. **Results** Cells induced to express Ndfip1 are found to have a longer cell cycle and proliferate less that non-induced controls due to a stalling of the G2 phase. Cells with RNAi directed towards Ndfip1 have a shorter cell cycle time (4 fold decrease) that can be attributed to a shortening of the G2 phase of the cell cycle. Ndfip1 can interact with PTEN a known tumour suppressor protein, this interaction results in ubiquitination of PTEN and nuclear translocation. BrdU pulse labelling combined with pHH3 labelling of Ndfip1 knock-out mice indicates that neuronal progenitors have a shortened G2 phase compared to wild type litter mates. **Conclusion** During neurogenesis the levels of Ndfip1 can regulate the length of the G2 phase in neuronal progenitor division. The mechanism for this regulation involves the interaction between Ndfip1 and the tumour suppressor protein PTEN. This results in the ubiquitination and translocation of PTEN to the nucleus and a stalling of the cell cycle.

ORAL-17-07

ADULT NEUROGENESIS AND THE PRODUCTION OF CALRETININ-POSITIVE OLFACTORY INTERNEURONS: A ROLE FOR NEOGENIN AND RGMA

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Purpose: The ability of the adult brain to produce neurons is well established. The molecular mechanisms controlling differentiation to a specific neuronal subtype are still poorly understood. The largest proliferative region in the adult brain is the subventricular zone (SVZ). From the SVZ, new neurons migrate along the rostral migratory stream to the olfactory bulb (OB) where they integrate into the granule cell and glomerular layers. The multi-functional receptor, neogenin, is expressed in the SVZ, and one of its ligands, RGMa, is expressed in a complementary pattern. We have previously shown that these molecules have a role in differentiation leading to production of a specific subset of interneurons in vitro. In vivo examination of both the olfactory bulb and the cerebellum found that the density of granule cell layers was reduced in neogenin loss-of-function mice. Expression of RGMa adjacent to these layers is consistent with the hypothesis that RGMa–neogenin interactions induce neuronal differentiation of calretinin-positive interneurons. Method: In vivo analysis was performed in neogenin loss-of-function and wild-type mice (N=5). Results: Our experiments show neogenin loss-of-function mice have significantly fewer calretinin-positive interneurons than wild-type mice; and RGMa appears to regulate differentiation to this neuronal subtype. Further comparisons between these genotypes show a corresponding effect in the granule cell layer of the cerebellum. Conclusion: Together, these data suggest neogenin and RGMa have a role in differentiation, not just to a neuronal fate, but also to a specific subtype of interneuron.

ORAL-17-08

TRACKING CELL PROLIFERATION AND CELL CYCLE KINETICS REVEALS KEY EVENTS IN THE DEVELOPMENT OF MOUSE SYMPATHETIC GANGLIA

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Purpose: During development, neural crest cells (NCC) first colonise and then undergo proliferation and differentiation to form the sympathetic ganglia. We have examined the cell cycle kinetics underlying this growth in the mouse stellate ganglion. **Methods:** We used multiple-label immunohistochemistry to identify neural crest cells (NCC) and neuronal and glial precursors and used two S-phase markers (BrdU and EdU) and Ki67 to calculate the cell cycle length for each cell type (see Hayes and Nowakowski 2002, Dev Brain Res. 134; 77-85). We examined ages from E9.5-E18.5 and looked at a minimum of three embryos from at least two litters in each case. **Results:** NCC (immunoreactive for Sox10 only) proliferate as they migrate to form the sympathetic ganglia. By E10.5 many cells in the ganglia ceased proliferating and have started to differentiate into neural precursors and show immunoreactivity to Phox2b and tyrosine hydroxylase (TH). Only a small proportion of cells continue to express Sox10. Once neuronal precursors have completely down-regulated Sox10 and only express Phox2b and TH, they recommence proliferation. From E11.5, both neuronal and glial precursors have broadly similar cell cycle lengths (E11.5:16.6 h vs 11.6 h, E12.5: 14.9 h vs 14.4 h and E14.5: 12.2 h vs 10.2 h respectively). Only at E18.5 do they diverge significantly (neural precursors 15.4 h and glial precursors 39.6 h). **Conclusion:** The initial expansion in neuronal precursor numbers in the mouse sympathetic ganglion over glial precursors does not reflect major differences in cell cycle length, but rather the relative number of cells committed to each lineage at E10.5. The dramatic slowing of glial precursor proliferation at E18.5 may coincide with a further differentiation step into immature satellite glia.

ORAL-18-01

SEXUALLY DIMORPHIC EXPRESSION OF AROMATASE IN THE MOUSE BRAIN

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Aromatase (Cyp19a1) is the enzyme that catconverts androgens to estrogens. It is expressed mainly in the gonads but it is expressed in other organs such as brain. The aromatase knockout mice (ArKO) displayed obese phenotype and other sexually dimorphic behavioural phenotype. For example, only male ArKo exhibited compulsive behavior whereas only female ArKO mouse displayed depressive-like behavior. The sites of aromatase expression in the brain have remained controversial due to the difficulty to obtain highly specific aromatase antibody. Using the Enhanced Green Fluorescence Protein (EGFP) tagged aromatase transgenic mouse model and immunohistostanning to GFP, we have detected sexually dimorphic expression of the aromatase in several brain regions which may explain the sexually dimorphic behaviors. This study show that the local production of estrogen in the brain is important in regulating the brain functions and normal behaviour.

ORAL-18-02

PUPIL DILATION BETRAYS THE TIMING OF DECISIONS

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The notion of "mind-reading" by carefully observing another individual's physiological responses has recently become commonplace in popular culture, particularly in the context of brain imaging. The question remains, however, whether outwardly accessible physiological signals indeed betray a decision before a person voluntarily reports it. In one experiment we asked observers to push a button at any time during a 10-s period ("immediate overt response"). In a series of three additional experiments observers were asked to select one number from five sequentially presented digits but concealed their decision until the trial's end ("covert choice"). In these experiments observers either had to choose the digit themselves under conditions of reward and no reward, or were instructed which digit to select via an external cue provided at the time of the digit presentation. In all cases pupil dilation alone predicted the choice (timing of button response or chosen digit, respectively). Consideration of the average pupil-dilation responses, across all experiments, showed that this prediction of timing was distinct from a general arousal or reward-anticipation response. Furthermore, the pupil dilation appeared to reflect the post-decisional consolidation of the selected outcome rather than the pre-decisional cognitive appraisal component of the decision. Given the tight link between pupil dilation and norepinephrine levels during constant illumination, our results have implications beyond the tantalizing mind-reading speculations. These findings suggest that similar noradrenergic mechanisms may underlie the consolidation of both overt and covert decisions.

ORAL-18-03

ANXIOLYTIC PROPERTIES OF PLANT DERIVED ODOURS: A MODEL OF SHORT AND LONG-TERM STRESS

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Purpose: Plant-derived essential oils have been described to possess anti-stress effects. In the present study physiological and behavioural examination was conducted in mice after 3 day (short-term) and 21 days (long-term) of restraint stress. In addition we investigated the anxiolytic properties of Praescent™, a novel mixture of plant derived chemicals composed of *cis*-3-hexenol, *trans*-2-hexenal and alpha-pinene. **Methods:** Male Balb/c mice aged 6-weeks old were used. Animals were divided into 4 groups: control (no odour), control (odour), stress (no odour) and stress (odour), induced by restraint stress for 1 hour (telemetry) or 15 minutes (behaviour). Praescent™ (0.2ml) was dispersed onto the bedding material. Telemetry (n=6): A radio telemetry transmitter (TA10ETA-F20, Data Sciences) was used to record continuous heart rate for 21 days. Behaviour (n=10): Animals were exposed to restraint stress for 3 days or 21 days. Immediately after the last restraint or handling procedure, animals were placed on the plus maze apparatus for 5 minutes. **Results:** Telemetric recordings for handling only showed clear circadian rhythms with significantly higher values during the dark period ($p<0.001$). Contrary, circadian rhythms were disrupted by stress. Exposure to Praescent™ during stress did not restore the stress-induced interruption in the circadian rhythm; rather it accelerated the recovery from prolonged exposure to stress. Behavioural measures show that short-term, but not long-term, restraint stress induced clear anxiogenic effect ($p<0.05$). Praescent™ application to the home cages reversed this effect. **Conclusion:** These results show that the physiological and behavioural changes, occurring in response to stress, are attenuated following Praescent™ exposure.

ORAL-18-04

ADULT VITAMIN D DEFICIENCY ALTERS BEHAVIOUR IN BALB/C BUT NOT C57BL/6 MICE

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Purpose: Based on epidemiological findings, adult vitamin D (AVD) deficiency has been proposed as an environmental risk factor for several neurological disorders including schizophrenia, autism and seasonal affective disorder. The aim of this study was to investigate whether AVD deficiency is associated with altered behaviour using two separate inbred mouse strains. Methods: 10-week old male C57Bl/6 and Balb/c mice were fed either a vitamin D deficient diet or a diet containing vitamin D (1,000 IU/kg) for 10 weeks (n=8-10 per group) prior to testing. The mice underwent a comprehensive behavioural screen to examine a range of behavioural domains, with tests including open field, elevated plus maze, holeboard, light/dark, forced swim, social interaction, hot plate, active avoidance, PPI and locomotor response to amphetamine. Results: We found that the AVD-deficient Balb/c mice differed on several measures, with a significant ($P<0.05$) increase in the time spent on the open arms of the elevated plus maze and a significant ($P<0.05$) reduction in the latency to avoid a footshock in an active avoidance test. There were no significant effects of diet on the other behaviours measured in Balb/c mice or on any behaviours assessed using C57Bl/6 mice. Conclusion: Here we show that AVD deficiency had an effect on behaviour in the mouse, but this was dependent on genetic background. AVD-deficient Balb/c mice had an anxiogenic phenotype with altered performance on an emotional learning and memory task (active avoidance). The data supports the hypothesis that vitamin D may be important for brain function and of relevance to human neurological disorders.

ORAL-18-05

ATTENTION PROCESS TRAINING FOR TINNITUS

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Purpose: Determine effectiveness of APT-II (Auditory Process Training-version II) (1, 2) in reducing tinnitus. Tinnitus is associated with abnormal attention processes (3) likely contributing to its resistance to habituation. Reducing tinnitus' attention-capturing properties may facilitate improvement. APT-II is a cognitive rehabilitation program designed to address attention deficits following brain injury. APT-II tasks exercise different attention components; increasing demands on attention control and working memory systems. **Hypothesis:** Aberrant attention processes contribute to tinnitus perception and APT-II should reduce tinnitus-related distress. Previous research showed selective attention to be impaired for tinnitus sufferers (4) therefore this attention domain was primarily trained. **Methods:** Participants (N=9) underwent 3 weeks of APT-II training. Tinnitus minimum masking levels, tinnitus handicap and severity questionnaires were administered pre and post training. **Results:** A decrease in mean tinnitus handicap was evident after 3 weeks APT-II training (P=.050). There was support for training-related reductions in tinnitus annoyance (P=.069) and minimum masking levels (P=.057). **Conclusion:** Results suggest tinnitus is associated with abnormal attention processing and attention training may address such deficits. **References:** 1. Sohlberg, M. M. & C. A. Mateer 1987. Effectiveness of an attention-training program. *Journal of Clinical & Experimental Neuropsychology* 9(2): 117-30. 2. Sohlberg, M. M., K. A. McLaughlin, et al. 2000. Evaluation of attention process training and brain injury education in persons with acquired brain injury. *Journal of Clinical & Experimental Neuropsychology* 22(5): 656-76. 3. Cuny, C., Norena, A., El Massioui, F., & Chery-Croze, S. 2004. Reduced attention shift in response to auditory changes in subjects with tinnitus. *Audiol. Neurotol*. 9: 294-302. 4. Wise, K., Singh, D & Searchfield, G. D. 2009. Auditory attention and tinnitus. Proceedings of 'Tinnitus Discovery': Asia-Pacific Tinnitus Symposium. *The New Zealand Medical Journal* 123(1311): 89-100.

ORAL-18-06

LOSS OF PLEASURE AND MOTIVATION IN NEUROPATHIC PAIN CORRELATES WITH DOWN-REGULATION OF MU-OPIOID AND D2-RECEPTOR MRNA IN THE NUCLEUS ACCUMBENS

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A loss of motivation for, and enjoyment of pleasurable activities characterises chronic neuropathic pain. In rats, the preference for sucrose over water is used as an indicator of reward experience (pleasure & motivation). The nucleus accumbens (NAcc) is pivotal in encoding reward experience. Dopamine binding at D1/D2-receptors is critical for the motivation to drink sucrose, and opioid binding at μ-receptors, is critical for the pleasure derived from sucrose. To determine whether diminished pleasure & motivation in chronic pain reflects adaptations of NAcc neurons, we investigated, in rats, the effects of neuropathic injury on sucrose drinking, and for each rat correlated this with the expression of D1/D2 and μ-receptor mRNAs in the NAcc. Forty-seven rats were singly housed, of these thirty-six had water and sucrose (1%) available, ad libitum, for twenty days, the remainder water only. Nerve injuries were performed on eighteen rats on day 10. On day 21, the rats were rapidly decapitated and the NAcc isolated and RNA extracted for RT-PCR. Un-injured rats, readily consumed sucrose over 20 days, in these animals there was a significant increase ($p<0.05$) in μ-receptor mRNA expression, which was accompanied by a lateralised (left) increase in D1-receptor mRNA ($p<0.05$) when compared to "water only" control rats. In nerve-injured rats, μ-receptor mRNA expression returned to levels seen in "water only" controls, lateralised reductions in D1- and D2-receptor mRNAs were also observed. The degree of reduction of μ- and D1-receptor mRNA expression, correlated significantly with decreased sucrose consumption ($p<0.05$). These changes may well explain in part, the diminished pleasure & motivation of chronic pain patients.

ORAL-18-07

VOLUNTARY RUNNING REVERSES AGE-ASSOCIATED COGNITIVE DECLINE AND RESTORES SYNAPTIC GROWTH AND NEUROGENESIS IN THE AGED RAT

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Background. Brain ageing in the rodent is associated with synaptic dysfunction and memory decline. The aims of this study were to: 1) identify a single-trial recognition memory paradigm sensitive to age-related change; 2) assess whether voluntary wheel running reduces the adverse effects of ageing on cognitive performance; and 3) characterize the underlying neural substrates for such behavioural changes. **Method.** Aged (18 months) and young (7 weeks) Fischer rats were assessed using an Object Recognition (a hippocampal-independent) and Place Recognition (a hippocampal-dependent) memory task. Following baseline testing, animals had free access to a running wheel for 8 weeks. Animals were sacrificed and underwent histological procedures accordingly. **Results.** Results indicate that aged rats are significantly impaired on the Place but not the Object Recognition memory task compared to young rats. However, voluntary running is able to selectively reverse Place task performance, while having no effect on Object task performance in aged rats. Aged control rats also had significantly lower synaptic density across several hippocampal subregions compared to young control animals. Voluntary running increased synaptophysin densitometric measures in all hippocampal subfields in aged animals. Neurogenesis levels were also increased in response to voluntary running in both young and aged animals. However, only synaptic density in the output areas of CA1 and CA2 were strongly correlated to Place performance; running produces a complex series of changes on these brain-behaviour correlations. Exercise-dependent increases in neurogenesis were not, by contrast, linked to increases in memory performance. **Conclusions.** Place Memory is specifically sensitive to age and is stable during repeat testing in rodents. Voluntary running rescues Place Memory performance, restores synaptic density and promotes neurogenesis in aged rats. Physical exercise also alters the functional properties of synaptic networks in the hippocampus.

ORAL-18-08

PRE-SYNAPTIC MECHANISMS OF GENERAL ANAESTHESIA IN DROSOPHILA AND SYNAPTIC COORDINATION ACROSS THE BRAIN

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Background:General anaesthesia is a routine procedure that causes loss of behavioral responsiveness in all animals and unconsciousness in humans. Although this phenomenon has been known for over 150 years, its mechanisms remain unclear. A mutation in Syntaxin1A, a pre-synaptic molecule involved in neurotransmitter release, was found to confer hyper-resistance to isoflurane in the nematode *Caenorhabditis elegans* (van Swinderen, B et al., 1999). This suggested a possible target site for general anaesthetics involving the synaptic release machinery. **Method**:We propose to study pre-synaptic hypotheses of general anaesthesia in *Drosophila Melanogaster*, a model organism where we can progress from designing molecular lesions and genetic constructs, to addressing behavioural and electrophysiological endpoints. We are developing an automated paradigm to measure simple behaviour in flies, which we applied to our studies of isoflurane anaesthesia. In parallel we are making several genetic constructs in Syntaxin1A gene and interacting synaptic molecules. We will be testing these phenotypes such as locomotion and startle under isoflurane anaesthesia. **Results**: Increasing concentrations of isoflurane abolished baseline locomotion and startle-induced locomotion in wild type and mutant flies. Syntaxin1A mutants were four-fold more resistant compared to the wild type when the H3 interaction domain of the molecule was deleted in heterozygous strains (N=8 animals for each genotype across 5 different isoflurane concentations). This result suggests that the anesthetic resistance originally observed in *C. elegans* Syntaxin1A mutants is also shared in Drosophila. Moreover the Syntaxin1A mutants also displayed a faster recovery phenotype, with 5-fold more Syntaxin1A mutants recovering 10 minutes after isoflurane anaesthesia compared to wild type. Based on these preliminary results, we will use a panel of mutations in Syntaxin1A and pre-synaptic molecules and use spatio-temporal gene expression techniques to narrow the isoflurane resistance effect that we have found.

ORAL-19-01

ENKEPHALIN EXPRESSION IN THE TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS OF THE LACTATING MOUSE IS PROLACTIN-DEPENDENT

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Purpose: During lactation the tuberoinfundibular dopaminergic (TIDA) neurons located in the arcuate nucleus of the hypothalamus exhibit a phenotypic switch, with a reduction in dopamine synthesis and induction of enkephalin expression. Evidence suggests that elevated prolactin may contribute to this phenomenon. The current study aimed to test the hypothesis that the induction of enkephalin expression in TIDA neurons during lactation is prolactin-dependent. **Methods:** Lactating or diestrus mice were treated with bromocriptine over a 24h time period (200µg/mouse sc every 8h). In the prolactin-maintained group exogenous prolactin (200µg/mouse) was administered at the same time as the bromocriptine. All animals received 30µg icv colchicine at the 6h time point. After 24h animals were terminally anaesthetized and transcardiacally perfused with 4% paraformaldehyde. A series of 30µm sections were prepared from the arcuate nuclei of each animal and processed for enkephalin immunohistochemistry. **Results:** Data analysis showed that the number of enkephalin positive cells in the arcuate nucleus increased, from 6±2 in diestrus, to 67±7 in lactation (n=5). Suppression of prolactin by bromocriptine administration during lactation reduced the number of enkephalin positive cells to 32±7 (n=5-7). While this number was significantly lower than that in lactating animals it was still higher than observed in diestrus controls. Administration of exogenous prolactin at the same time as bromocriptine treatment prevented the reduction in the number of enkephalin positive cells (91±5, n=5-7). **Conclusions:** This study demonstrates that the number of TIDA neurons expressing enkephalin is increased during lactation and that the maintenance of this enkephalin expression is prolactin-dependent.

ORAL-19-02

NEURONAL ACTIVATION AND CARDIOVASCULAR RESPONSES TO STRESS IN A MOUSE MODEL OF NEUROGENIC HYPERTENSION

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Purpose: Schlager inbred hypertensive mice (BPH/2J) have been suggested to have high blood pressure due to an overactive sympathetic nervous system. The brain nuclei associated with the hypertension are also those involved in the integration of the cardiovascular responses to stress. Therefore, in the present study, we determined whether BPH/2J mice have a greater response to stress that is associated with greater neuronal activation in the limbic system, hypothalamus and medulla in regions known to regulate sympathetic activity. Methods: Male hypertensive BPH/2J and normotensive BPN/3J mice were implanted with telemetry devices and exposed to dirty cage-switch, an acute model of aversive stress. Results: Stress exposure caused a 60% greater pressor response in BPH/2J compared with BPN/3J mice and an increase in activity, by contrast the level of tachycardia was less in BPH/2J mice. Stress-induced cardiovascular responses were also associated with greater neuronal activation, as detected by c-Fos expression, in BPH/2J compared with BPN/3J mice in the amygdala, dorsomedial ($P<0.001$) and paraventricular hypothalamus, nucleus of the solitary tract ($P<0.05$) and rostral ventrolateral medulla ($P<0.001$). Conclusion: Our findings suggest that hypertension in the BPH/2J mice is associated with greater sympathetic vasomotor responses to central pathways mediating the arousal responses to acute aversive stress. The level of activation observed in the medial amygdala was greater than all other sites examined (+63%) and this closely correlates with the increase in blood pressure. Thus, the inappropriate activation of the medial amygdala to aversive stimuli may be key to the neurogenic hypertension.

ORAL-19-03

RESISTIN CAN ACT IN THE BRAIN TO INFLUENCE CARDIOVASCULAR REGULATION

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Purpose: Resistin is a recently discovered adipocytokine with many metabolic effects that are similar to leptin. Resistin, like leptin, can influence dietary regulation and both have been linked to cardiovascular disease. Leptin is known to increase sympathetic nerve activity when administered into the brain and may contribute to the sympathetic overactivity observed in obesity. It is not known whether resistin has similar effects. The aim of this project is to investigate the effects of centrally administered resistin on lumbar sympathetic nerve activity (LSNA). **Methods:** Male Sprague-Dawley rats were fasted overnight. On the day of the experiment, anaesthesia was induced using isoflurane gas (2.5%-3% in O_2); the femoral artery and vein were cannulated and the anaesthetic was changed to urethane (1-1.4 g/kg IV). Left lumbar postganglionic sympathetic nerve activity was amplified using a low-noise differential amplifier, filtered, integrated at 0.5-s intervals and recorded. Resistin (7µg, n=7) or vehicle (artificial cerebrospinal fluid, n=6) was injected into the lateral brain ventricle (ICV). Following the injection, mean arterial pressure (MAP), heart rate (HR) & LSNA were monitored over the next 4 hours. **Results:** ICV injection of resistin increased LSNA significantly by a maximum of 37± 5% ($p<0.01$) but did not significantly affect MAP or HR. Injection of vehicle did not significantly change any of these parameters. **Conclusion:** Resistin can act centrally to increase LSNA suggesting that it can regulate cardiovascular functions. Exogenous resistin, however, did not affect MAP and HR suggesting that resistin may not have a generalised sympathoexcitatory action. Nonetheless, resistin may contribute to sympathetic overactivity observed in obesity.

ORAL-19-04

FLUOXETINE AND METYRAPONE BLOCK BEHAVIOURAL BUT NOT CARDIAC EFFECTS OF SUB-CHRONIC STRESS

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The purpose of this study was to determine whether repetitive aversive episodes can provoke enduring effects on cardiac autonomic control, and if so, to reveal mechanisms that mediate these effects. Adult male Sprague-Dawley rats instrumented for telemetric recording of heart rate (HR) and locomotor activity (LOC) were divided into five groups (n=7 each): no drug-treated (FS-ND), atenolol-treated (FS-ATEN), fluoxetine-treated (FS-FLUOX), saline-treated (FS-SAL) and metyrapone-treated (FS-MET) group. Atenolol and fluoxetine were administered in a drinking water for 3 weeks (pre-stress, stress and post-stress); metyrapone was injected s.c. 2h prior to each footshock (FS) session. Animals of each group were submitted to a footshock session on five consecutive days. Circadian rhythms of HR and LOC were recorded for 1 week before and for 1 week after stress. In FS-ND group, sub-chronic footshock produced significant and long-lasting reduction in HR both during the active phase (ΔHR=-23±3 bpm) and inactive phase (ΔHR=-20±3 bpm) of the light/dark cycle, and a reduction in LOC during the active phase (Δ=-0.9±0.1 cpm). Bradycardic effect of stress was not related to reduced locomotor activity. The fall in HR persisted after sympathetic blockade (atenolol in drinking water; FS-ATEN: active phase ΔHR=-16±3 bpm, inactive phase ΔHR=-19±2 bpm). Vagal blockade (scopomamine) was performed before and after the stress period; in both instances HR increased to the same level suggesting that stress-induced bradycardia was predominately vagally-mediated. Fluoxetine (SSRI) and metyrapone (inhibitor of corticosterone synthesis) treatments did not affect cardiac changes (FS-FLUOX: active phase ΔHR=-31±4 bpm; inactive phase ΔHR=-29±3 bpm; FS-METY: active phase ΔHR=-12±2 bpm, inactive phase ΔHR=-15±2bpm) but prevented the reduction in LOC. We conclude that: i) behavioural effects (LOC) of chronic stress are mediated by stress-induced rises of corticosterone whereas cardiac effects (HR) are not; and ii) locomotor, but not cardiac effects, could be prevented by SSRI treatment.

ORAL-19-05

RESPIRATORY, BUT NOT CARDIAC, RESPONSES TO ACOUSTIC STIMULATION ARE ATTENUATED BY DIAZEPAM PRE-TREATMENT: A NOVEL INDEX OF ANXIETY IN RATS

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Association between the emotional state and the respiratory function is well documented in humans. In contrast, respiratory indices were largely neglected in animal psychophysiology. **Purpose:** We aimed to determine if sudden alerting stimuli of increasing intensity and laboratory stress affect respiratory rate (RR) in rats and to assess sensitivity of RR responses to anxiolytic drug diazepam. Secondly, we aimed to compare changes in RR with heart rate (HR) responses. **Methods:** Experiments were performed in adult male Wistar rats (n=6). On different days they were injected with either saline or diazepam (2.5 mg/kg, i.p.) and subjected to five brief (500ms) acoustic stimuli of increasing intensity (60dB-100dB white noise) followed 15 minutes later by 15-min restraint. RR and HR were continuously assessed via whole-body plethysmography and telemetry methods, respectively. **Results:** Acoustic stimulation evoked transient increases in RR ranging from 142±46 (60dB) to 377±72 cpm (100dB) that were linearly related to the stimulus intensity (p<.001, η_p^2=.95). HR responses to acoustic stimuli were inconsistent (p=.41, η_p^2=.15). Diazepam inhibited changes in RR (p=.002, η_p^2=.86), but not in HR (p=.106, η_p^2=.44), in response to acoustic stimulation. Restraint stress significantly elevated RR and HR (by 83±10cpm and 160±19bpm respectively). Diazepam inhibited only the tachypnoeic (p=.042, d=1.1), but not the tachycardic (p=.08, d=.90) responses to restraint. **Conclusion:** We conclude that RR is a more reliable and consistent index of short-term physiological arousal compared to cardiac indices. Sensitivity of tachypnoeic responses to diazepam indicates that respiratory response is a promising novel index of anxiety in rats.

ORAL-19-06

AUTONOMIC REGULATION OF BLOOD PRESSURE AND HEART RATE IN CHRONIC RENAL FAILURE

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Tonic and reflex control of blood pressure and heart rate is dependent upon the balance of vagal and sympathetic tone, and disruption of this balance, through either decreased parasympathetic and/or enhanced sympathetic activity, is a feature of a number of hypertensive disorders. In patients with end stage renal function (ESRF), cardiovascular disease is the most common cause of mortality. We have used the analysis of heart rate (HR) and systolic blood pressure variability (HRV and SBPV) as well as the determination of spontaneous baroreflex sensitivity (sBRS) to assess autonomic function in an animal model of ESRF (the Lewis polycystic kidney rat - LPK). Lewis (n = 6) and LPK (n = 4) were implanted with telemetry probes for blood pressure measurement at age 8 weeks. Circadian rhythm data was recorded for 72 hours continuously at 12 weeks of age and 24 hour averages were calculated. When comparing Lewis and LPK, HR was not significantly different between the two (360.1±8.76 vs. 374.6±12.7 bpm) and HRV (total power) while showing a trend towards reduction, was also comparable (21.83±4.85 vs 17.03±6.78 ms^2/Hz). Mean arterial pressure was significantly increased in the LPK (101±4.77 vs. 185.5±13.5 mmHg, p≤0.001) as was SBPV (total power, 6.46±1.84 vs. 18.21±1.01, $mmHg^2$/Hz, p≤0.001). sBRS was significantly reduced in the LPK (3.922±0.8 vs. 2.4±0.22, msec/mmHg, p≤0.01). Our demonstration of marked hypertension, increased SBPV & reduced sBRS are factors likely to contribute to cardiovascular disease and ongoing secondary organ damage as part of the disease process. Longitudinal data is being collected from the same animal cohort to reassess autonomic function as the severity of renal disease progresses.

ORAL-19-07

INFLUENCE OF BRAIN ANGIOTENSIN AT1 RECEPTORS ON EMOTIONAL AND CARDIOVASCULAR REACTIVITY IN MICE

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Purpose: Brain AT_1 receptors are implicated in regulating cardiovascular reactivity to emotional stress. However, the role of AT_1 receptors in defensive emotional behavior and its impact on the cardiovascular stress response remains elusive. In this study, we examined the influence of AT_{1A} receptor knockout (KO) on defensive behavior and associated cardiovascular arousal in mice. **Methods:** KO and wild-type (WT) mice (n=10-11) were implanted with blood pressure telemetry devices, and subjected to contextual fear conditioning and extinction. A separate group of mice (n=5-8) was subjected to conflict tests, the elevated plus maze and the novelty-suppressed feeding task. **Results:** In contextual fear conditioning paradigm, KO mice showed increased anxiety-like behavior (thigmotaxis, defensive scanning of environment) during pre-exposure to the context (footshock chamber), whereas their cardiovascular arousal was similar to that in WT mice. Conversely, in response to a discrete aversive stimulus, footshock, KO mice showed decreased flight, freezing and pressor reactions. KO mice also showed decreased freezing and pressor responses during re-exposure to the context, and an enhanced decay of the freezing response over time. Freezing extinction was accompanied by recovery of the pressor response to the context and by an increase in risk assessment behavior. In addition, KO mice showed increased anxiety-like behavior in the elevated plus maze and the novelty-suppressed feeding task. During these tests cardiovascular activation was similar between genotypes. **Conclusion:** These data indicate that AT_1 receptors may exert bimodal influence on the defense reaction by activating neural circuits that regulate the response to discrete, imminent dangers (to prepare body for instant fight or flight), while inactivating those involved in risk assessment (goal conflict) behavior.

ORAL-19-08

BLOCKADE OF OREXIN RECEPTORS REDUCES RESPIRATORY AND SYMPATHETIC RESPONSE TO HYPOTHALAMIC PERIFORNICAL AREA ACTIVATION

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Orexin (hypocretin) neurons within the hypothalamic perifornical area (PeF) are believed to contribute to the cardiorespiratory responses associated with stress or arousal (1). In this study we tested the effect of systemic administration of almorexant (an orexin dual receptor antagonist) on the sympathetic and respiratory response to activation of the PeF. Renal sympathetic nerve activity (RSNA) and phrenic nerve activity (PNA) were recorded in rats (n=5) anaesthetized with urethane. Microinjections of bicuculline (10 pmol in 20 nl) into the PeF evoked increases in PNA burst rate (62 ± 9% of baseline, n=11 sites) and RSNA (38 ± 6% of baseline, n=8 sites). Systemic administration of almorexant (15 mg/kg iv) had no significant effect on baseline PNA burst rate or RSNA, but significantly reduced the increases in these variables evoked by bicuculline microinjection (to 18 ± 5%, P < 0.0001 and to 21 ± 3% of baseline, P < 0.05, respectively). Almorexant administration did not change baroreceptor reflex effects on RSNA in these experiments, and when tested in one experiment had no effect on the sympathetic and respiratory response to stimulation of peripheral chemoreceptors. The results indicate that blockade of central orexin receptors powerfully inhibit the respiratory and sympathetic response evoked by activation of PeF neurons. The orexin neurons may either be essential components of the central pathways mediating sympathetic and respiratory responses from the PeF, or else facilitate these responses via activation of orexin receptors in the brainstem or other central locations. 1) Kuwaki T et al., Autonom Neurosci 142: 11-16, 2009.

ORAL-20-01

RARE AND COMMON *SIGMAR1* DNA VARIANTS AND NEURODEGENERATION

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Purpose: Neurodegeneration genes can harbour rare mutations that lead to single-gene diseases and common polymorphisms that increase susceptibility to more complex disorders. We aimed to determine the genetic cause of Chromosome 9-linked frontotemporal dementia - motor neuron disease (FTD-MND), and whether common variants of this gene are associated with risk of dementia. **Methods:** We performed linkage analysis and mutation screening in a large, multi-generational family with dominant inheritance of FTD-MND. We performed luciferase reporter assays, western blotting and immunohistochemistry to determine the effect of the gene mutation on transcript stability, and the effect of gene overexpression on subcellular localisation of TDP-43 and FUS, two proteins believed to play a major pathological role in FTD and MND. We also examined SNPs either within or adjacent to the disease gene in two elderly community cohorts with extensive phenotype data. **Results:** We have identified a mutation in the Sigma nonopioid intracellular receptor 1 gene (*SIGMAR1*) in a Chromosome 9-linked FTD-MND family. The mutation is in the 3' untranslated region (3'UTR) of the gene and significantly alters gene expression *in vitro* and in patient lymphocyte and brain samples. Modulation of Sigma-1 activity by overexpression, RNA interference knockdown or synthetic ligands leads to intracellular redistribution of TDP-43 and FUS. In the Sydney Older Persons Study cohort (n = 285), we detected a significant association between *SIGMAR1* SNP rs1800866 and dementia in males (p = 0.015). In the Framingham SHARE cohort (n = 911), we detected a significant association between *SIGMAR1*-adjacent SNPs and mini-mental state examination score (p = 0.036). **Conclusion:** Our results suggest that *SIGMAR1* may play a pathological role in neurodegenerative diseases, either via rare mutations leading to a significant alteration of *SIGMAR1* expression, or by more common polymorphisms that may lead to a more subtle change in *SIGMAR1* expression or function.

ORAL-20-02

EXOSOMAL-MEDIATED SECRETION OF ALS-LINKED MISFOLDED PROTEINS IN MOTOR NEURON DEGENERATION

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Purpose: Amyotrophic lateral sclerosis (ALS) is characterised by accumulation of pathological misfolded proteins in affected motor neurons by an unclear mechanism. Key evidence suggests a contribution of the secretory pathway in ALS pathogenesis. However, the predominant pathway(s) underlying secretion of key ALS-linked proteins (SOD1, TDP-43 and FUS) remains undefined. We sought to elucidate the secretory pathway responsible and its involvement in motor neuron degeneration. **Methods:** N-linked glycosylation sites were engineered into ALS-linked proteins expressed in motor neuronal NSC-34 cells to test classical secretion. Non-classically secreted exosomes were purified from conditioned medium of cells and CSF of ALS patients and rodent models and characterised by Western blotting, flotation gradients, protease protection assay and electron microscopy. Expression and localisation of ER stress, endocytic and apoptosis markers was correlated with exosomal secretion in ALS patients and models. **Results:** ALS-linked proteins were principally secreted by exosomes in transfected cell cultures (n=4) and CSF sourced from rats and humans (n=5). Severe depletion of mutant SOD1, TDP-43 and FUS was observed in exosomes, correlating with endosome/lysosome pathway abnormalities and preceding ER stress, inclusion formation and apoptosis in ALS models. Notably, spinal cord induction of endocytic Rabs was shown in presymptomatic $SOD1^{G93A}$ mice (n=5) and sporadic ALS subjects (n=10) compared to non-neurological controls (n=4). **Conclusions:** Based on these multiple lines of evidence, we propose that endocytic pathway dysfunction leading to impaired exosomal secretion, cytoplasmic protein accumulation and autophagic system burden may be an early determinant of motor neuron loss and common denominator of key ALS-linked misfolded proteins. Modulation of endosome transport may therefore provide an innovative potential target for therapeutic approaches in ALS.

ORAL-20-03

TDP-43 PROCESSING IS MODULATED BY C-JUN N-TERMINAL KINASE AND COPPER: IMPLICATIONS FOR THERAPEUTIC TREATMENT OF TDP-43 PROTEINOPATHIES

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Purpose: TDP-43 proteinopathies (FTD and ALS) are characterized by loss of nuclear TDP-43 expression, TDP-43 C-terminal fragmentation, accumulation in the cytoplasm and formation of ubiquitin-positive aggregates. Recent studies have shown that TDP-43 can accumulate in RNA stress granules (SGs) in response to cell stresses. This could be associated with subsequent formation of ubiquitinated aggregates. However, the pathway of TDP-43 accumulation in SGs is not understood. In this study we investigated the mechanism of TDP-43 processing and accumulation in SGs in neurons exposed to oxidative stress. **Methods:** Neuronal cultures were treated with the mitochondrial inhibitor paraquat and examined for TDP-43 and SG processing (n = 6). **Results:** We found that paraquat induced loss of nuclear TDP-43, increased cytoplasmic localization and led to formation of TDP-43 and HuR-positive cytoplasmic SGs. Paraquat-mediated TDP-43 SG accumulation was associated with increased formation of a caspase-dependent C-terminal TDP-43 cleavage product, which was not observed with alternative stress inducers. The co-localization of TDP-43 with SGs could be completely blocked by co-treatment with the c-Jun N-terminal kinase (JNK) inhibitor, SP600125. JNK inhibition did not prevent formation of HuR-positive SGs. Co-treatment of neurons with a copper-bis(thiosemicarbazone) complex (Cu^{II}(atsm)) inhibited JNK activity and TDP-43-positive SG formation, increased expression of key survival proteins and reduced oxidative stress. Cu^{II}(atsm) also inhibited altered TDP-43 processing, delayed disease onset and extended life in a G93A SOD1 murine model of ALS. **Conclusion:** Our studies are the first to demonstrate a specific stress-mediated pathway of TDP-43 accumulation in SGs and inhibition by a potentially therapeutic copper complex. These findings may have important implications for development of treatments for FTD and ALS.

ORAL-20-04

ESTABLISHING AND INVESTIGATING THE CELLULAR AND BIOCHEMICAL BASIS OF PATHOLOGICAL TDP-43 IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) MODELS

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Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the loss of both upper and lower motor neurons. TDP-43, a DNA/RNA binding protein, is a major component of pathological inclusions that are seen in affected neurons. TDP-43 is pathologically modified in ALS. Since 2008, we and other groups have found missense mutations in the gene encoding TDP-43 in familial and sporadic ALS cases, supporting a causative link between TDP-43 dysfunction and neurodegeneration. However, the mechanisms through which TDP-43 causes ALS are poorly understood. **Objectives:** Establish neuronal (NSC-43) and non-neuronal (lymphocyte) cell models of ALS and in these models investigate the possible effect of TDP-43 mutations on cell division, cytotoxicity and post-translational modifications. **Methods:** Lymphocyte cells lines (controls and patients) were treated with various cellular stresses. Both the lymphocytes and mutant TDP-43 transfected neuronal cells were investigated using immunohistochemisty, immunofluoresence, western blot, trypan blue, MTT and annexin V assays. **Results:** In treated lymphocytes and mutant TDP-43 transfected cells, TDP-43 was found to be pathologically modified: redistributed, ubiquinated, cleaved and hyperphosphorylated. In lymphocytes, TDP-43 mutations did not affect the viability and proliferation activity and did not render cells more susceptible to cellular stresses. **Discussion and Conclusion:** Untreated lymphocytes did not show ALS pathology but lymphocytes treated with cellular stresses and mutant TDP-43 transfected neuronal cells showed similar pathology to that of ALS patient cells, such as redistribution, aggregate formation, ubiquitination and phosphorylation, suggesting that these cells may be used to study disease mechanisms.

ORAL-20-05

MUTANT TDP-43 IMPAIRS CYTOSKELETON ORGANIZATION, RESPONSE TO INJURY AND GLUTAMATE TRANSPORTER EXPRESSION IN ASTROCYTES IN PRIMARY CULTURE

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Purpose: Recently the TAR DNA binding protein TDP-43 has been demonstrated an aetiological factor in motor neuron disease (MND). TDP-43 is a DNA and RNA binding protein regulating transcription and splicing. TDP-43 is also involved in transport and local post-transcriptional modification of mRNAs. The protein is abundantly expressed in motor neurons and astrocytes. TDP-43 pathology is triggered by abnormal processing and cytosolic aggregation of the protein or by mutations in the TDP-43 gene. The pathology is similar and the outcome is directly linked to cell death. Mutant TDP-43 causes familial forms of human MND, MND-like disease in transgenic animals and kills motor neurons in primary culture. TDP-43 pathology is also found in astrocytes: a cell type that plays critical roles in the pathology of MND. The mechanisms behind TDP-43-mediated pathology are not known but likely involve non-cell autonomous injury. Thus a clear understanding of normal TDP-43 function and how mutant TDP-43 abrogates this function will provide insight into the basis of MND. Methods: We have established cellular models of TDP-43 proteinopathies by expressing fluorescently tagged TDP-43 (wild-type and mutants) in astrocytes in primary cultures. We have also silenced TDP-43 expression in these cells. We have used these models to investigate the role of TDP-43 and its mutants on normal cell function and on the response of these cells to injury. Results: Presence of TDP-43 mutations, caused reorganisation of the cytoskeleton and lead to impaired wound healing in an in vitro injury model (n=5). Moreover, astrocytes transfected with the Q133k TDP-43 mutation had decreased expression of GLT-1 and GLAST glutamate transporters (n=3). Finally, the presence of mutant TDP-43 increased the activists of the Rho family GTPases Rho A and Rac-1 while significantly reduced Cdc42 activity suggesting a direct role for TDP-43 in the regulation of the Rho-family GTPases.

ORAL-20-06

A BIOMARKER FOR MOTOR NEURON DISEASE

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Purpose: There are no molecular biomarkers of motor neuron disease (MND). An important step in finding effective treatments for MND is to identify biomarkers that could aid in the early detection and progression of the disease. Motor neurons respond to disease by up-regulating proteins on their nerve terminals; which are then shed into body fluids, appearing in both serum and urine. We present evidence that a specific protein is present before the onset of disease in urine of SOD1G93A mice, the mouse model of MND, but not age-matched control mice. **Methods:** Behavioural analysis was performed using neurological assessment, weight, grip strength and hanging wire tests. The protein (60 KDa) was detected in mouse urine via Western blot (WB) and Immunoprecipitation (IP). **Results:** Behavioural testing showed that SOD1G93A mice (n=10) but not aged matched control B6 mice (n=10) develop motor symptoms after 120 days of age, reaching end-stage from around 150 days. The protein was present in urine obtained from SOD1G93A mice as early as 60 days of age with the highest level detected at end stage (n=5). The protein was not detected before 120 days in control mice (n=5). Semi-quantitative WB's showed the amount of the specific protein was highest in SOD1G93A mice urine at end stage of disease (n=5). The identity of the specific protein was confirmed by highly specific IP's. **Conclusion:** A possible biomarker for MND in the SOD1G93A mouse has been identified. Further work is ongoing to develop a more sensitive, quantitative ELISA assay and to determine if this protein is also present in the urine of humans with MND.

ORAL-20-07

ESTIMATION OF MOTOR UNIT LOSS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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Purpose: Muscle function is determined by the number of motor units innervating a single muscle or a group of muscles. We developed a Bayesian Motor Unit Number Estimation (MUNE) algorithm, which provides a statistical estimate of the most probable number of motor units contributing to the nerve-evoked compound muscle action potential (CMAP). We aimed to validate Bayesian MUNE in the $SOD1^{G93A}$ mouse model of ALS. Methods: $SOD1^{G93A}$ and wild-type animals at different stages of disease (30-36 days, presymptomatic; 63-75 days, onset; 150-180 days, end-stage (n=5 for each group)) were anaesthetized with isoflurane (1-2%) in O2 continuously delivered by a nose cone. Stimulus-response curves were acquired by graded electrical stimulation of the sciatic nerve and CMAP recording from the right gastrocnemius muscle. Recordings were analyzed in MUNE10 and motor neuron numbers validated by histological counts of the right gastrocnemius motor neuron pool of lumbar spinal cords. Results: Bayesian MUNE consistently provided conservative motor unit number estimates lying within a 2-fold range (40% to 120%) of histological counts (n=5 for each group). In $SOD1^{G93A}$ mice, a decrease in neuromotor innervation and motor neuron number was evident at disease onset and continued to decline as the disease progressed ($p<0.05$, n=5 for each group, ANOVA), reflecting the loss of motor units compared to wild-type mice. Conclusion: Bayesian MUNE provides reliable conservative motor unit number estimates in mice, implying validation of its clinical use in humans.

ORAL-20-08

CELL SIGNALING AND PRO-SURVIVAL MECHANISMS OF SURVIVAL OF MOTOR NEURON (SMN) PROTEIN

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Spinal muscular atrophy (SMA), a neurodegenerative disorder primarily affecting motor neurons, is the most common genetic cause of infant death. This incurable disease is caused by the absence of a functional *SMN1* gene which leads to a critical reduction in full length SMN protein. The SMN protein has been linked to numerous cellular functions, including a pro-survival role in motor neurons and RNA splicing. However, exactly how SMN protein imparts its cell survival function is still unknown. In this study, the neuronal function of SMN was investigated in primary rat cortical neurons and human SH-SY5Y cells. A recombinant adenoviral vector was used to over-express SMN protein, and its putative pro-survival function was assessed in a novel Akt/PI3 kinase inhibition model, an oxidative stress model (hydrogen peroxide) and an excitotoxic model (glutamate). Over-expressed SMN protein protected against Akt/PI3 kinase pathway inhibition but not oxidative or excitotoxic stress. Using the Akt/PI3 kinase inhibition model I investigated the role of SMN protein in key cellular pathways linked to neuronal survival and apoptosis. Cell homogenates harvested pre- and post- injury were subjected to western analysis to determine caspase-3 and calpain activation levels. Results indicate that SMN protein protects neurons by inhibiting caspase-3 activation via blockade of calpain mediated procaspase-3 cleavage. This study revealed an anti-apoptotic role for the SMN protein in a human neuronal cell death model, which operated by preventing caspase-3 activation.

ORAL-21-01

CORTICAL NEURON RESPONSES TO REELIN IS DEPENDENT ON THEIR POSITIONS

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Purpose: Reelin signalling is required for the generation of a laminated neocortex. When absent in the *reeler* mutant, the cortex is abnormally layered with aspects of partial inversion. Neurons lose the ability to migrate past their predecessors, preventing the formation of inside-out layering. How Reelin exerts its function remains controversial. We have previously shown that Reelin maintains the speeds and trajectories of neurons in the germinal zones, however, this does not address migration towards a source of Reelin in the marginal zone. **Methods:** Time-lapse imaging was used to investigate the behavior of neurons in the presence of ectopic Reelin. These studies were conducted on both wild-type and *reeler* cortical slices, monitoring migration in the germinal zones and cortical plate. Examination of functional Reelin receptors was conducted using a Reelin-Alkaline Phosphatase fusion assay. **Results:** In the presence of ectopic Reelin, neurons in the germinal zones detached from the glial fiber, converted into a multipolar morphology and exhibited a reduction in migratory speeds. Surprisingly, neurons entering the cortical plate showed no deviations in speed, morphology or trajectory. We examined whether ectopic Reelin could alleviate the jamming of neurons entering the cortical plate in the *reeler* cortex, and indeed this was the case. Ectopic Reelin activated migration by increasing both the speed and proportion of neurons migrating. This contrasting response can be attributed to the persistent presence of functional Reelin receptors in *reeler*, but not wild-type, neurons. **Conclusion:** Our findings highlight the differential responses of migrating neurons to Reelin and provide evidence of its function during corticogenesis.

ORAL-21-02

THE RAP1 GUANINE NUCLEOTIDE EXCHANGE FACTOR RA-GEF-1 IS ESSENTIAL FOR THE PROPER DEVELOPMENT OF THE MOUSE DORSAL TELENCEPHALON

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Introduction/Purpose: RA-GEF-1 (also termed RapGEF2) is a guanine nucleotide exchange factor for Rap1. We are studying its *in vivo* role in cortical development using dorsal telencephalon-specific *RA-GEF-1* knockout (cKO) mice. **Methods:** *RA-GEF-1* cKO mice phenotypes are analyzed using immunohistochemistry, retrograde and anterograde tracing. **Results:** *RA-GEF-1* cKO mice showed severe defects in their brain structures including an ectopic cortical mass (ECM) underlying a relatively normal homotopic cortex, the agenesis of the corpus callosum (cc) and the anterior commissure (ac). At postnatal day (P) 0, the ECM was occupied by supragranular and infragranular neurons arranged in an opposite outside-in manner in contrast to the normal lamination observed in the homotopic cortex (*n=3*). Immunostaining for the layer I marker reelin indicated the absence of Cajal-Retzius cells from the ECM. This might explain the aberrant lamination in the ECM and indicates that preplate splitting occurred normally in the cKO mice (*n=3*). Bromodeoxyuridine birthdate labeling indicated a defective migration of the late born neurons (*n=6*). Retrograde tracing in adult mice verified the impairment of the bilateral integration of information via the cc (*n=5*) and the ac fibers (*n=13*). At P0, anterograde tracing of the callosal axons verified the inability of these axons to perform midline crossing, thereby forming Probst bundles in the ipsilateral side (*n=7*). Anterograde tracing of the ac confirmed the misrouting of the ac fibers (*n=8*). **Conclusion:** These results suggest an important role of RA-GEF-1 in neural migration and the formation of midline commissures.

ORAL-21-03

REGION SPECIFIC ACTIONS OF ESTRADIOL AND TESTOSTERONE ON TRKB SIGNALING IN ADOLESCENT C56BL/6 MICE

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Purpose: Sex steroid hormones, as well as neurotrophic factors are involved in the pruning and shaping of the adolescent brain to its adult form. The aim of this study was to determine the effects of altered levels of sex steroid hormones during adolescent brain development, on protein expression and phosphorylation of the neurotrophin receptor TrkB. Methods: We manipulated adolescent sex steroid hormone levels by castration or ovariectomy (OVX) at 5 weeks of age (pre-pubescent). At the same time, silastic implants with placebo, low physiological or high pharmacological levels of testosterone, or estradiol, were administered for three weeks before tissue was collected for Western blot analysis (n = 5-7/group). Results: In female mice, OVX significantly increased TrkB phosphorylation (pTrkB) in frontal cortex ($p<0.05$) and striatal ($p<0.05$) regions, and both low and high doses of estradiol restored pTrkB to intact levels. In the dorsal hippocampus OVX significantly decreased pTrkB ($p<0.05$) and while low estradiol restored pTrkB, high estradiol had no effect. In male mice, castration had no effect on TrkB phosphorylation in all regions analyzed. However, high testosterone treatment significantly decreased TrkB expression in the frontal cortex ($p<0.05$) and phosphorylation in the striatum ($p<0.05$). Conclusions: Results suggest region specific actions of estradiol and testosterone on TrkB signaling. These data provide important insight into how the brain changes in response to altered levels of circulating sex steroid hormones. Further comparisons of adult TrkB signaling following gonadectomy will help shed more light on the impact of sex steroid hormones during development.

ORAL-21-04

RYK REGULATES WNT SIGNALLING IN DEVELOPING CORTICAL NEURONS

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Purpose: The axon guidance receptor Ryk is expressed on cortical axons during development of the mouse corpus callosum, the major interhemispheric forebrain commissure. Our lab has shown that Wnt5a-Ryk interactions are responsible for the chemorepulsive guidance of postcrossing callosal axons away from the midline and into the contralateral hemisphere. The signalling pathway by which Wnt5a-Ryk-mediated callosal axon guidance occurs is currently not well understood. **Methods**: Using immunohistochemistry, we have examined expression of β-catenin, a key component of canonical Wnt signalling, in the cortices of *Ryk+/+* and *Ryk-/-* embryos (n=3) and β-catenin localisation to the nucleus of dissociated cortical neurons in *Ryk+/+* (n=38 neurons) and *Ryk-/-* embryos (n=40 neurons) cultured in the presence or absence of 400ng/ml Wnt5a. **Results**: We observe an increase in intracellular β-catenin in embryonic day 18 (E18) *Ryk-/-* cortex compared to *Ryk+/+* cortex. Cultured cortical neurons from E18 *Ryk-/-* embryos display decreased nuclear localisation of β-catenin compared to E18 *Ryk+/+* cortical neurons ($p<0.001$). Addition of Wnt5a to these cultured neurons induces an increase in nuclear β-catenin localisation in *Ryk-/-* neurons ($p<0.001$) indicating activation of the canonical Wnt signalling pathway. No change in β-catenin localisation is observed in *Ryk+/+* neurons cultured with Wnt5a. **Conclusion**: These data suggest that Wnt5a can activate canonical Wnt signalling only in the absence of Ryk. This indicates that Ryk acts as a gatekeeper controlling activation of canonical versus non-canonical Wnt signalling pathways.

ORAL-21-05

NEUROSERPIN REGULATES AXON AND DENDRITE DEVELOPMENT IN HIPPOCAMPAL NEURONS

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Purpose: Neuroserpin is an inhibitor of tissue plasminogen activator (tPA) that is expressed in the developing and adult nervous system. Mice with altered expression of neuroserpin show increased phobic responses in response to novel situations. Changes in neuronal connectivity may underlie these behavioural effects, as neuroserpin is known to regulate neurite outgrowth in cell lines. The aim of this study was to investigate the function of neuroserpin in neuronal development using a primary hippocampal culture model. **Methods**: Dissociated hippocampal neurons were treated with recombinant neuroserpin and fixed at specific timepoints for immunofluorescent-labelling of axon and dendrite markers. A robust automated analysis of neuronal morphology was carried out on images of these cells to assess the effects of neuroserpin on neurite development (n=1874 neurons, three independent cultures). A follow-up analysis was conducted using forms of neuroserpin lacking anti-proteolytic activity to investigate neuroserpin's mechanism of action (n=2684 neurons, three independent cultures). **Results**: Neuroserpin treatment led to significant changes in the morphology of developing hippocampal neurons. Lamellipodia associated with the cell body, developing neurites and axonal growth cones were strongly reduced in size. Axon formation and axon length were increased in the presence of neuroserpin, while dendrite length and branching were altered in a culture time-dependent fashion. Forms of neuroserpin lacking inhibitory activity also induced these changes indicating that these effects were independent of protease inhibition. **Conclusion**: These results highlight an important role of neuroserpin in modulating the development of both axons and dendrites. We propose that neuroserpin acts as an extracellular signaling molecule that is released in a controlled fashion to regulate neuronal development.

ORAL-21-06

ONTOGENESIS OF THE VITAMIN D RECEPTOR IN THE RAT MESENCEPHALON

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Purpose: Developmental vitamin D (DVD) deficiency is proposed as a risk factor for schizophrenia. We have shown that as embryos, DVD-deficient rats have altered dopamine ontogeny and as adults behaviours associated with aberrant dopaminergic function. The aim of this study was to investigate ontogeny of vitamin D receptor (VDR) in tyrosine hydroxylase (TH) positive neurons throughout development. **Method:** Mesencephalon from Sprague-Dawley rats (n=3) at embryonic day 12 (E12), E15, E18 and postnatal day 0 (P0), P21 and P70 were fixed for immunofluorescence using antibodies against VDR N-terminal (N-20 Santa Cruz) and TH. Western blots were conducted on nuclear and cytosolic fractions using both the N-20 and an antibody against VDR C terminal (D-6). **Results:** Only sparse VDR immunoreactivity was detected within TH positive neurons at E12. Nuclear VDR positive staining in all TH positive cells was apparent from E15 to P70. Somal staining was apparent in post-natal animals. However western blots confirmed that VDR was only present in the nuclear fraction at all ages. Interestingly the N-20 antibody only reacted with VDR in developing mesencephalon whereas D-6 antibody only recognised VDR in adult. **Conclusion:** We can draw four conclusions from this work. All dopamine neurons contain VDR. VDR is strictly nuclear. The timing of VDR appearance coincides the cessation of mitosis in DA neurons. Our western blots data indicate there may be post-translational modification of the VDR after birth. Taken together, our result suggested that vitamin D could play a role in dopamine ontogeny and function.

ORAL-21-07

STEM CELL-DERIVED SENSORY PROGENITORS CAN INNERVATE THE EARLY POST-NATAL SENSORY EPITHELIUM IN VITRO

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The focus of our research is to determine whether stem cells can be used to replace the auditory neurons (ANs) lost following deafness. In order to successfully replace ANs, stem cells must be capable of directed differentiation toward a sensory neural lineage, of organised outgrowth of processes, and of forming functional connections. We have developed an in vitro assay to test these parameters using co-cultures of cochlear explants and human embryonic stem cells (hESCs). Specifically, hESC-derived neurospheres were differentiated toward a sensory lineage using mouse fibroblast feeder cells and the small molecule, Y27632, and then co-cultured for up to 12 days with either cochlear explants isolated from early post-natal rats, or alone (n=10). Untreated neurospheres were set-up concomitantly as controls (n=12). The ENVY line of hESCs was used in all experiments as this line expresses high levels of green fluorescent protein (GFP) in all differentiated progeny, enabling discrimination of stem cells and their processes in co-cultures. hESC sensory progenitors differentiated into neurons which expressed both NF200 and peripherin, and extended processes into the explant. Significantly greater numbers of stem cell-derived processes were observed growing into the explant when neurospheres were pre-treated with fibroblast feeder cells and Y27632 ($p<0.001$), and these GFP positive processes were often observed growing along the endogenous peripheral processes of the explant. In addition, when grown in co-culture with hair cells alone (microisolates; n=16), stem cell processes were capable of locating and growing along the rows of hair cells, however synapse formation occurred infrequently. These data illustrate that hESC-derived neural progenitors primed towards sensory neural differentiation can innervate the sensory epithelium after 12 days in culture, but are likely to require longer periods of culture in order to make mature synaptic contacts with sensory tissues.

ORAL-21-08

THE INNATE IMMUNE COMPLEMENT SYSTEM MEDIATES NEURAL TUBE CLOSURE IN FOLATE-DEFICIENT MICE

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Purpose: There is increasing evidence that components of the innate immune system play novel, non-immune roles during neurodevelopment. However much less is known about the role of the complement system in embryogenesis. This study investigated the expression of complement components in the developing embryo during the period of neural tube closure, and the effect of perturbing C3a- and C5a-receptor signalling on neurodevelopment in folate-replete and folate-deficient mice. **Methods:** Embryos (E7.5-E11.5) were dissected from wild-type mice and expression of complement factors C3, C5 and their receptors (C3aR, CD88) examined using RT-PCR, whole mount *in situ* hybridisation, and immunofluorescence (n=3 / group). In a parallel study, wild-type, $CD88^{-/-}$, or $C3aR^{-/-}$ mice (n=8 / group) were placed on a folate-deficient diet (FDD), prior to mating, and embyros examined at E18 for neurodevelopmental defects. **Results:** We found restricted expression of complement components at proliferative zones in the cephalic regions of the developing neural tube. Specifically, C3 and C5 were present within the neuroepithelium; receptors for their ligands, C3a and C5a, showed focal expression at the apical surface. Intriguingly, no other tissue expression was observed for these factors outside the neural tube. Embryos from FDD mice showed a significant increase in complement factor expression compared to folate-replete breeders. Strikingly, embryos from $CD88^{-/-}$ or $C3aR^{-/-}$ mice on FDD demonstrated a high incidence (~50%) of neural tube-associated defects. **Conclusion:** Complement components are expressed in the developing neural tube. The increased expression of these factors during folate deficiency, and the increased frequency of neural tube defects following FDD and complement receptor deficiency indicates a key role for complement signalling, facilitating neural tube closure with maternal folate stress.

ORAL-22-01

EXPRESSION OF TOLL-LIKE RECEPTORS IN THE CHOROID PLEXUS AFTER HYPOXIA-ISCHEMIA IN NEONATAL MICE

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Hypoxia-ischemia (HI) is a major cause of brain damage in the newborn. Growing evidence suggest that a group of innate immune receptors, Toll-like receptors (TLRs), are involved in the response to ischemia. The choroid plexus is damaged after HI, however, data is lacking on the role of TLRs in the choroid plexus after neonatal brain damage. **The purpose** of this study was to examine the TLR expression and the downstream signaling pathway in choroid plexus after HI alone or in combination with different TLR agonists. **Methods:** C57/Bl6 mice were subjected to HI on postnatal day 9. Animals were sacrificed 24h after HI (n=6-12) and compared to controls (n=5-8). The forebrains or the choroid plexus from the lateral ventricles were dissected out and the mRNA expression was determined by a TLR pathway-specific PCR array (SABiosciences). **Results:** At 24h after HI, TLR 3 (1.64), TLR 5 (1.95) and TLR 6 (1.51) were up regulated whereas TLR1 (-1.73) was down regulated in the choroid plexus. In the brain, TLR 1 (3.35), TLR 2 (2.27) and TLR 7 (2.16) were upregulated, while TLR 5 was down regulated (-1.76). Changes in gene expression in the choroid plexus after stimulation with different TLR agonists with or without HI are under investigation. **Conclusion:** The mRNA of several TLRs is regulated in choroid plexus after HI. However, the response in choroid plexus is different to the changes in the brain. The data suggest that specific TLRs may be functioning in choroid plexus after HI, which differ from those in the rest of the brain.

ORAL-22-02

NEUROPROTECTIVE ACTIONS OF HIF-1 PROLYL HYDROXYLASE (PHD) INHIBITORS IN A NEONATAL RAT MODEL OF HYPOXIC-ISCHEMIC BRAIN INJURY

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Hypoxia-inducible factor-1 (HIF-1) is the key transcription factor regulating the expression of many hypoxia-responsive genes. Under normoxic conditions HIF-1α protein is constantly being degraded due to HIF-1 prolyl hydroxylase enzymes (PHDs) which hydroxylate proline residues on HIF-1α causing ubiquitination and proteosomal degradation of HIF-1α and consequently, constitutive levels of HIF-1α protein are almost undetectable. Hypoxia and drugs that can inhibit PHD activity can cause accumulation of HIF-1 and subsequently increase target gene expression. Previously, we have shown that preconditioning with hypoxia and PHD Inhibitors (cobalt chloride ($CoCl_2$) and desferrioxamine (DFX)) can protect the brain against hypoxic-ischemic (HI) brain injury and this protective effect is largely due to expression of HIF-1 and its target genes. Here we have examined the neuroprotective effects of PHD Inhibitors administered after injury. Sprague-Dawley rat pups (postnatal day 7) were anaesthetised with isofluorane (2-5% in oxygen) and underwent a unilateral common carotid artery ligation and were then exposed to 3 hours of 8% oxygen. A single injection of drug treatment (DFX (200mg/kg, s.c.), $CoCl_2$ (60 mg/kg, s.c.), EDHB (200mg/kg, s.c.) or saline vehicle control (s.c.) was performed immediately after HI procedure. At 1 week post-injury brains were removed for histological analysis. This combined HI procedure results in a significant reduction in volume of the ipsilateral hemisphere. Treatment with DFX (n=12), CoCl2 (n=12) and EDHB (n=10) significantly reduced the degree of damage in the ipsilateral hemisphere by 38%, 42% and 37%, respectively, when compared with vehicle treated littermate controls (n=18). Our findings indicate that modulation of HIF-1 and its target gene expression after HI brain injury is an effective neuroprotective strategy.

ORAL-22-03

RAPID BEHAVIOURAL IMPROVEMENT IN MICE WITH ATTENUATED REACTIVE GLIOSIS AFTER PERMANENT FOCAL CORTICAL ISCHEMIA

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The hallmark of reactive astrocytes is the up-regulation of the intermediate filaments GFAP and vimentin. Mice with astrocytes deficit in intermediate filaments ($GFAP^{-/-}Vimentin^{-/-}$) have larger ischemic infarcts after distal middle cerebral artery occlusion and improved post-traumatic regeneration of neuronal synapses and integration of neural grafts. **Purpose**: To determine whether attenuation of reactive gliosis modifies infarct volume and functional outcome after focal photothrombotic occlusion in adult mice. **Methods**: Permanent focal cortical ischemia over the somatosensory cortex was induced in adult mice carrying a null mutation in the GFAP and vimentin gene (n=28) and wild-type littermates (n=39). Neurobehavioural assessment of motor function was performed. Animals were killed 1 and 3 days post ischemia and infarct volume, serum estradiol concentration and neutrophil invasion of the ischemic core determined. **Results**: Prior to ischemia, animals performed equally in neurobehavioural testing. 1 day after ischemia the $GFAP^{-/-}Vimentin^{-/-}$ had greater motor impairment of the right forepaw than wild-type. 3 days post ischemia $GFAP^{-/-}Vimentin^{-/-}$ mice have a dramatic improvement in neurobehavioural scores. Cortical infarct volumes were similar for all mice independent of genotype, gender or time post ischemia. Estradiol concentration did not correlate with infarct volume. Female mice had significantly less neutrophils within the ischemic core than their male counterparts. **Conclusion**: Mice without intermediate filaments and thus attenuated reactive gliosis had a greater functional deficit in the acute period following stroke but recovered to basal levels along with wild-type animals by 3 days. These results suggest that reactive astrocytes minimise loss of function and inhibit the post ischemic recovery in the acute period.

ORAL-22-04

EVIDENCE THAT CONNEXIN HEMICHANNELS CONTRIBUTE TO INJURY AFTER CEREBRAL ISCHAEMIA

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Purpose: Propagation of ischaemic brain injury shows a characteristic pattern, starting in severely damaged areas and spreading into previously undamaged regions. Gap junctions and connexin hemichannels (undocked connexons) have been implicated in this spread of injury. There is evidence that following ischaemia connexin hemichannels open, forming a conduit between the cytoplasm and the extracellular space, resulting in cell swelling and release of ATP and glutamate. **Methods:** Term-equivalent fetal sheep (0.85 gestation) received 30 min of carotid artery occlusion, followed by either artificial CSF infusion into the lateral ventricle (n=7), or infusion of a mimetic peptide known to block Cx43 hemichannels infused either for 60 min starting from 90 min after ischaemia (n=7) or the same 60 min infusion followed by a further low dose infusion for 24 hours (n=6). **Results**: Mimetic peptide infusion was associated with a graded improvement in EEG recovery, with greater EEG improvement after the longer peptide infusion. This recovery was significant from 5 days post-insult until sacrifice at 7 days ($p<0.05$). Both peptide infusion protocols were associated with a significant reduction in numbers and total duration of seizures and more rapid return of normal sleep state cycling after ischaemia ($p<0.05$). **Conclusions**: Post-ischaemic blockade of Cx43 hemichannels was associated with improved EEG recovery and reduced seizure activity consistent with improved neuronal outcome. This strongly suggests a role for Cx43 hemichannels in the early propagation of global brain injury.

ORAL-22-05

NON-ADDITIVE NEUROPROTECTION IN THE HIPPOCAMPUS FROM EARLY GLUTAMATE RECEPTOR BLOCKADE PLUS DELAYED HYPOTHERMIA IN PRETERM FETAL SHEEP

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Purpose: Induced hypothermia is now established as the first therapy that can significantly reduce neurodevelopmental impairment after perinatal asphyxia. It is critical to appreciate that protection is only partial, likely in part, because for logistic reasons in the majority of infants cooling was not able to be started until 4 to 6 hours after birth. It has been suggested that combination treatment with other putative neuroprotective agents may provide synergistic benefit. Glutamate receptor blockade during the early recovery phase with the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine is partially neuroprotective. In the current study, we tested whether dizocilpine can augment neuroprotection with hypothermia in preterm fetal sheep after severe asphyxia. **Methods**: Profound asphyxia was induced by umbilical cord occlusion for 25 min in 0.7 gestation fetal sheep (equivalent to 28-32 weeks in humans). 15 min after occlusion or sham occlusion fetuses received dizoclipine (2 mg/kg bolus, based on estimated fetal weight, then 0.07 mg/kg/h for 4 h) by i.v. infusion. 5.5 h after occlusion whole body cooling was C, and continued°initiated, and titrated to reduce core body temperature by 3 until 72 h. All animals were euthanized after 7 days of recovery and the brains were perfusion fixed. NeuN immunohistochemistry was used to stereologically quantify numbers of surviving neurons in the CA3 region of the hippocampus. **Results**: 25 minutes of umbilical cord occlusion was associated with severe loss of neurons in CA3 (86±27 vs 317±22 cells/field, mean±SE, $p<0.001$). A similar, modest increase in surviving neurons ($p<0.05$ vs occlusion alone) was seen after dizocipline (157±71), delayed hypothermia (134±24) and dizocilpine-hypothermia (197±11), with no significant difference between these groups. **Conclusions**: Delayed hypothermia and early infusion of a selective NDMA receptor antagonist were associated with modest, and apparently non-additive neuroprotection of the hippocampus after severe asphyxia in preterm fetal sheep.

ORAL-22-06

MATURATION OF MITOCHONDRIAL REDOX RESPONSE TO PROFOUND INTRAUTERINE HYPOXIA

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Purpose: The fetal brain paradoxically becomes more vulnerable to hypoxic damage in the last third of gestation. In this study we examined the hypothesis that this is associated with impaired mitochondrial adaptation to profound hypoxia. **Methods**: Chronically instrumented fetal sheep at 0.6, 0.7, 0.85 gestation were subjected to either 30 min (0.6 gestational age (ga), n=6), 25 min (0.7 ga, n=22) or 12-15 min (0.85 ga, n=10) of complete umbilical cord occlusion. near-infrared spectroscopy (NIRS) derived intra-cerebral oxygenation (DHb = HbO_2-Hb), total haemoglobin (THb) and cytochrome oxidase (CytOx) redox state were monitored continuously. **Results**: After occlusion THb initially increased significantly at 0.6 and 0.7 ga, to a maxima at 7 min, whereas there was no change at 0.85 ga ($p<0.05$). From 7 min THb then fell in all groups. DHb initially fell rapidly in all groups to a plateau from 6 min. The magnitude of this fall in DHb was greatest at 0.7 ga compared to 0.6 and 0.85 ga ($p<0.05$). CytOx initially increased in all groups with the greatest rise at 0.85 ga ($p<0.05$). Strikingly, the 0.85 group showed a progressive fall after 5 min of occlusion, whereas the younger fetuses showed a sustained rise for the remainder of the occlusion periods ($p<0.05$ all groups). **Conclusions**: The rapid rise in oxidized CytOx in parallel with loss of oxygenated haemoglobin after occlusion denotes reduced electron flow down the mitochondrial electron transfer chain due to loss of oxidative metabolism. The greater and more rapid rise in CytOx near-term is consistent with increasing fixed dependence on oxidative metabolism. The late loss of the oxidized CytOx signal near-term is unexplained but speculatively could be mediated by inhibition of cytochrome activity by nitric oxide or by opening of the mitochondrial pores, favouring programmed cell death.

ORAL-22-07

DISRUPTION OF NEURAL PROJECTIONS TO THE DAMAGED CORTEX MAY CONTRIBUTE TO REMOTE LOSS OF RAPHE SEROTONERGIC NEURONS AFTER NEONATAL HYPOXIA-ISCHEMIA

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Purpose: Exposure to a hypoxic-ischemic (HI) insult may lead to life-long neurological deficits in preterm neonates. Unfortunately there is no intervention to ameliorate HI brain injury in neonates and this is primarily because very little is known about the mechanisms underlying neuronal damage after HI. Primary HI damage occurs in the forebrain, however we have recently found that remote brainstem serotonergic neurons are also lost after HI. We hypothesized that disruption of serotonergic fibres innervating the HI-damaged forebrain can lead to loss of serotonergic neurons in the raphé nuclei of neonates. **Methods:** Hypoxia-ischemia was induced in rat pups on post-natal day 3 (P3; right common carotid artery ligation+30 min 6% O_2). Five weeks after injury a retrograde tracer, choleratoxin b (CTb), was injected into the motor cortex to investigate whether neural projections from the brainstem are lost after P3 HI. Brains from control (n=7) and P3 HI (n=8) animals were then immunohistochemically processed to identify retrogradely labelled CTb-positive serotonergic neurons in the raphé nuclei as well as other brain regions. **Results:** We found a significant reduction in numbers of CTb-positive neurons in specific raphé nuclei. Dual immunolabelling revealed that these were almost exclusively serotonergic. Significant losses of CTb-positive neurons were also observed in the ventromedial thalamic nucleus and the basolateral amygdala after P3 HI. **Conclusion:** We propose that disruption of serotonergic neural connections to the damaged cortex may contribute to losses of raphé serotonergic neurons. Thus this study identifies a novel mechanism that may underpin remote neuronal loss in the brainstem after neonatal HI.

ORAL-22-08

POST-INSULT IBUPROFEN TREATMENT ALLEVIATES DAMAGE TO THE SEROTONERGIC SYSTEM FOLLOWING HYPOXIA-ISCHEMIA IN THE IMMATURE BRAIN

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Purpose: Serotonin (5-HT) neurons innervate forebrain regions that are damaged after preterm neonatal hypoxia-ischemia (HI). Early disruption of the 5-HT system contributes to adverse outcomes in adults that match many of those observed in HI-affected neonates. Damage to the 5-HT system occurs after neonatal HI and neuroinflammation plays a key role in this disruption. Ibuprofen is a non-steroidal anti-inflammatory drug that is safely used in the preterm neonate although it is not known if ibuprofen can attenuate neonatal HI brain injury. Here we examined whether ibuprofen administration can inhibit neuroinflammation and prevent damage to the 5-HT neural network in the immature rodent brain following a HI insult. **Methods:** Using a postnatal day 3 (P3) HI rat model (right carotid ligation + 30 min 6% O2) we examined the effects of HI and ibuprofen on neuroinflammatory mediators and brain levels of 5-HT, 5-HT transporter expression and numbers of 5-HT raphe neurons in control (n=8) and HI (n=8) rat pups. Ibuprofen was administered daily for one week (100 mg/kg P3 and 50 mg/kg P4-P9; s.c.) in control (n=6) and HI (n=6) animals. **Results:** Ibuprofen attenuated the HI-induced increase in COX-2, interleukin-1β and tumour necrosis factor-α in the brain. Ibuprofen treatment also prevented the HI-induced decreases in 5-HT levels, 5-HT transporter expression and numbers of 5-HT neurons. **Conclusions:** Post-HI administration of ibuprofen can prevent disruption of the 5-HT system in the immature brain. The associated inhibition of HI-induced COX-2 expression and cytokine production suggests that stemming neuroinflammation using ibuprofen may be a promising avenue to ameliorate damage to the 5-HT network in the HI-affected neonate.

ORAL-23-01

GTF2IRD1, IMPLICATED IN THE WILLIAMS SYNDROME COGNITIVE PROFILE, INTERACTS WITH CHROMATIN-REGULATING PROTEINS

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Williams syndrome is a neurological disorder that results from a hemizygous microdeletion within chromosome 7q11.23 involving 28 genes. Its features involve characteristic physical abnormalities and a set of cognitive and behavioural features collectively called the Williams syndrome cognitive profile (WSCP). Genotype/phenotype correlations in patients implicate the gene GTF2IRD1 and its related homolog - GTF2I - in the main aspects of the WSCP. We generated a Gtf2ird1 knockout/LacZ knock-in mouse line to map its expression in the brain and to examine the consequences of gene inactivation. These mice show cognitive impairment, reminiscent of the WSCP. In order to elucidate GTF2IRD1s molecular function, we performed yeast two-hybrid screens and identified a number of chromatin-modifying proteins, including: Setd6, Zmym5 and Dcaf6. Setd6 contains a SET domain that is involved in histone lysine methylation. Dcaf6 might be important for substrate specificity of the DDB1-CUL4 ubiquitin E3 ligase complex, which has been linked to histone methylation. Zmym proteins associate with a histone deacetylase complex. These interactions suggest a role in transcriptional repression, which is consistent with the reported activity of GTF2IRD1. Our investigation has also shown that GTF2IRD1 is targeted by SUMOylation and that the SUMO-modification of GTF2IRD1 could be mediating its interaction with Zmym5. Indeed, there is growing evidence for the role of SUMOylation in coordinating histone modification and chromatin structure for regulation of gene expression. Epigenetic modifications in the brain have been implicated in learning and memory, as well as a variety of neuropsychiatric disorders. Our findings mark an exciting beginning for investigating the molecular role of GTF2IRD1 in regulating brain function through chromatin modification.

ORAL-23-02

CHARACTERISTICS OF METASTATIC BRAIN TUMOUR MODELS USING WALKER 256 CARCINOMA CELLS FROM TWO DIFFERENT TUMOUR CELL BANKS

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Purpose: The mechanisms underlying development of metastatic brain tumours are poorly understood. Animal models of brain metastases are important for elucidating such mechanisms although little regard has been given to the source of the tumour cells. In the current study, we demonstrate that tumour cells from different sources can give profoundly different results. **Methods:** Walker 256 rat breast tumour cells from either the American Type Culture Collection (ATCC) or the Cell Resource Centre for Medical Research at Tohoku University (CRCTU) were injected into the internal carotid artery of male Wistar rats (N=33), with culture medium injected as controls (N=18). **Results:** Brain tumours derived from the two sources grew at different rates and with different characteristics. 11% of rats inoculated with ATCC-sourced cells developed a single brain tumour in the striatum over ten weeks after inoculation. In contrast, 89% of animals inoculated with CRCTU-sourced cells developed periventricular tumours by 9 days post-injection ($p = 0.003$, Fishers exact test). Albumin immunoreactivity, indicative of blood-brain barrier permeability, was evident in and around the tumours in both groups, although the pattern of staining differed between the tumour populations. Microglial infiltration, as indicated by immunoreactivity for ionized calcium binding adaptor molecule 1, was present in all cases. Reactive astrocytes, detected using glial fibrillary acidic protein, showed different patterns in the two groups with a less demarcated tumour boundary in the CRCTU group. **Conclusion:** Walker 256 tumour cells obtained from two sources have different tumorigenicity, growth characteristics and interactions with the host brain. Such variability should be considered when comparing studies using cell lines obtained from different sources.

ORAL-23-03

IDENTIFICATION OF PHOSPHORYLATION SITES IN THE ARISTALESS-RELATED HOMEODOMAIN TRANSCRIPTION FACTOR NEAR THE OCTAPEPTIDE DOMAIN

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Purpose: The *Aristaless*-related homeobox gene (*ARX*) is one of the most frequently mutated genes in X-linked intellectual disability (XLID). Mutations in *ARX* give rise to a broad spectrum of phenotypes, including ID with epilepsy or infantile spasms. Despite the clinical significance, the exact role of the ARX homeodomain transcription factor, and the mechanisms by which ARX function is regulated remains unknown. Phosphorylation of ARX *in vitro* suggests post-translational processing may regulate aspects of ARX function. To examine this further, our study aimed to identify the actual residues modified by phosphorylation, the kinases responsible and investigate the effect of phosphorylation on ARX function. **Methods & Results:** Using *in vivo* labelling assays, we demonstrated at least four phosphorylation sites are present across the ARX protein. Using a combination of mass spectrometry and *in silico* prediction analysis, we identified serine 67 as a novel phosphorylation site. In addition, our data strongly suggests serine 37 is a likely phosphorylation site. **Conclusion:** We have shown that ARX is a phosphoprotein and contains multiple phosphorylation sites. We identified two serine residues at the N-terminus of ARX as novel phosphorylation sites. The conserved N-terminal octapeptide domain plays an important role in the transcription repression activity of ARX. The proximity of phosphorylated serine residues to the octapeptide domain suggests phosphorylation of these residues may regulate the activity of this domain and influence ARX function. We predict two naturally occurring missense mutations (L33P and P38S causing non-syndromic XLID) may affect the normal phosphorylation status of ARX and contribute to disease pathogenesis.

ORAL-23-04

INDUCING PLURIPOTENT STEM CELLS TO MODEL RETT SYNDROME

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Induced pluripotent stem (iPS) cells hold great promise for making patient-specific cell culture disease models for central nervous system disorders. Rett Syndrome (RTT) is an autism spectrum disorder caused by mutations in the methyl CpG-binding protein 2 (*MECP2*) gene. Due to the inaccessibility of patient neurons, it is difficult to study RTT *in vitro* or perform drug screens. As a consequence, underlying phenotypes have been primarily described using mouse models. iPS cells provide a potential solution, whereby neuronal differentiation of RTT-specific iPS cells creates a limitless supply of defective neurons for *in vitro* disease study. However, it remains unclear whether iPS cells accurately model autism spectrum disorders. Here we describe the characterization of mouse RTT *Mecp2*^{308} iPS cell lines to validate this technology for characterizing human neurons derived from RTT patient iPS cells. This mouse model expresses a truncated *Mecp2* allele and reproduces the defects in synaptic function, behaviour, and learning typical of RTT. These wild-type and heterozygous iPS cell lines express endogenous pluripotency markers, reactivate the X-chromosome, and differentiate into the three germ layers *in vitro* and *in vivo*. Via retinoic acid-mediated differentiation of embryoid bodies, the lines were differentiated into active glutamatergic neurons that form functional synapses and produce action potentials captured by whole-cell patch clamp recordings. Glutamatergic synapses were examined by immunofluorescence for the presynaptic marker vesicular glutamate transporter 1 (VGLUT1) and the post-synaptic density 95 (PSD95) marker. In these preliminary studies, iPS cell-derived neurons generate action potentials and miniature excitatory postsynaptic currents (EPSCs). We anticipate that electrophysiology will reveal RTT iPS cell-derived glutamatergic neurons recapitulate defects previously reported in RTT mouse brain samples. Detailed studies of synaptic function of iPS cell-derived neuronal cells are currently underway and will allow investigation of phenotypes in comparison to normal and RTT cortical neurons to validate the iPS cell system.

ORAL-23-05

PHENOTYPIC CHARACTERISATION OF CHMP2B KNOCKOUT MICE

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Purpose: C-terminal truncating mutations in the gene for Charged Multivesicular Body Protein 2B (CHMP2B) on Chromosome 3 have been described in a Danish family with hereditary Frontotemporal Dementia. Patient brains show gross atrophy of the frontal and temporal lobes accompanied by ubiquitin and p62 positive inclusions that are negative for TDP-43 and FUS. CHMP2B is part of the ESCRT-III complex, important for both trafficking of endocytosed proteins to the lysosome and autophagy. We generated homozygous CHMP2B knockout mice to determine whether loss of function of CHMP2B contributes to disease pathology. **Methods:** The modified SHIRPA protocol was used to systematically assess behaviour at 6 and 12 months old. Immunocytochemistry for GFAP, IBA-1 ubiquitin and p62 was performed on brain and spinal cord from 6 to 24 months old. **Results:** Kaplan-Meier analysis showed that homozygous Chmp2b knockout mice have a significantly reduced lifespan. SHIRPA analysis identified an impaired righting reflex, an abnormal splayed gait and a dystonic posture in either one of the front paws at 12 months old. We investigated the pathology of these mice using immunohistochemistry on spinal cords and brains. We found that, even at 24 months, the knockout mice had no obvious morphological changes in brain or spinal cord (n=3). Furthermore, normal levels of astrogliosis (GFAP) and normal microgliosis (IBA-1) were observed, whilst there was no abnormal accumulation of p62 or ubiquitin. **Conclusion:** Despite having a distinct phenotype, the knockout mice showed no gross neuropathology, suggesting further investigation is required to determine the pathological basis of the observed phenotype.

ORAL-23-06

EFFECT OF INFLAMMATORY ACTIVATION ON THE NEUROSUPPORTIVE FUNCTIONS OF ASTROGLIA

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Purpose A complex relationship exists between astrocytes and neurons involving astroglial uptake of glucose, glutamate and glutathione precursors and the release of lactate, glutamine and glutathione, which are in turn taken up by neurons. This metabolic exchange occurring between astrocytes and neurons is vital to normal neuronal functions such as energy metabolism, glutamatergic signalling and GSH-dependent cellular defence. We hypothesise that a breakdown in astroglial-neuronal interaction, due to inflammation-activated astrocytes altering their phenotype, could lead to neurodegeneration, as observed in Alzheimer's disease. **Methods** U373MG human astrocytes were activated up to 120 hours using various concentrations of IL-1β and TNF-α. Glucose, lactate, glutamate, glutathione and related thiols were measured in the conditioned media to determine how activation affects three important neuroprotective functions of astrocytes. Astroglial-neuronal co-cultures were then utilised to determine the affect of cytokine-activation of astroctyes on neuronal viability. **Results** Activated astrocytes show numerous time and cytokine concentration dependent changes in their neuroprotective functions. Most notably, chronically activated astrocytes significantly decrease glucose (40%) and glutamate (5%) uptake, decrease lactate (90%) and glutathione release (90%) and increase production of neurotoxic substances such as IL-6 (400%) and homocysteine (200%)(n=6-9). Immunohistochemical co-culture studies and astrocyte-targeted interventions demonstrate the detrimental affect that inflammation-induced modulation of astroglial phenotype has on neuronal viability. **Conclusions** Inflammation-induced changes in astroglial neuroprotective functions are believed to play a role in neurodegeneration observed in Alzheimer's disease. Therefore, our findings enable us to suggest evidence-based astrocyte-targeted therapeutic approaches for the treatment of Alzheimer's disease.

ORAL-23-07

ADULT HUMAN MICROGLIA RESPONSES TO M-CSF

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Purpose: Macrophage colony stimulating factor (M-CSF) is a cytokine found in the brain and its receptor is expressed by microglia, the primary immune cells in the brain. M-CSF has been shown to be involved in monocytic cell proliferation and function. While most of the research about M-CSF and microglia has been carried out using rodent models, it is becoming increasingly clear that there are important differences between rodent microglia and their human counterpart. This study investigates the effects of M-CSF on adult human microglia. **Results:** Following exposure to M-CSF *in vitro* for several days, M-CSF increased microglial proliferation as shown by increased BrdU and Ki67-positive microglia. PU.1 is a constitutive transcription factor expressed exclusively by cells of the monocytic lineage, including microglia. M-CSF increased PU.1-positive microglia and the level of PU.1 expression in microglia. M-CSF was also found to increase phagocytosis, an important microglial function in the brain, as well as induce a marked morphological change in microglia. Interestingly, there was a degree of variability in the response of adult human microglia to M-CSF, and not all donors microglia exhibited all of the above effects. **Methods:** For this study, microglia were isolated from biopsy (n=6) and post-mortem (n=2) adult human brain tissue using previously described methods. Immunocytochemical, phagocytic and morphological observations have been quantified using Discovery-1 automated fluorescence microscopy and MetaMorph image analysis software. **Conclusion:** M-CSF dramatically influences the phenotype of adult human microglia. However, microglia from different cases responded to M-CSF to varying extents. This observation may be related to the variable susceptibility and severity of a range of neurological diseases for which microglia are involved.

ORAL-23-08

THE ROLE OF CAMKII IN NEURONAL SENSITIVITY TO ISCHAEMIA

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Calcium/calmodulin stimulated protein kinase II (CaMKII) plays an important role in ischaemic neuronal cell death induced by transient middle cerebral artery occlusion (MCAo) (J Biol Chem 285:20675-82). Spontaneously hypertensive rats (SHR) are more sensitive to MCAo- and excitotoxicity-induced neuronal cell death compared to their parent Wistar Kyoto (WKY) strain. This enhanced sensitivity has been proposed to be due to increased expression of CaMKII leading to increased basal phosphorylation of AMPA receptors at S831-GluR1 (Stroke 38:3007-15). To test this proposal we have used western blotting to measure the expression and basal phosphorylation of CaMKII, the GluR1 subunit of the AMPA receptor and the NR2B subunit of the NMDA receptor in the striatum and cortex (relatively sensitive and resistant brain regions, respectively) from SHR (hypertensive, more sensitive) and WKY and Sprague Dawley (SD) (normotensive, relatively resistant) rats (N=6 per group). We have confirmed that the level of CaMKII expression and basal GluR1 phosphorylation at S831 is significantly higher in the striatum of SHR than WKY rats ($p<0.01$). However, there was no significant difference in these variables between cortex and striatum in the same animal, nor between SHR and SD rats despite significant differences in sensitivity to MCAo-induced injury. There was no correlation between sensitivity to MCAo and the level of expression or basal phosphorylation of CaMKII, GluR1 or NR2B. Therefore, if differences in CaMKII mediated events are responsible for the differences in sensitivity to ischaemia between brain regions, the mechanism must involve alterations in ischaemia-induced CaMKII activation, probably due to altered CaMKII targeting, rather than differences in basal levels of CaMKII expression or activity.

ORAL-24-01

BEHAVIOURAL AND NEUROCHEMICAL CONSEQUENCES OF MDMA SELF-ADMINISTRATION IN RATS

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Current estimates of drug abuse in NZ are that ~24% of those aged 15-34 years use amphetamines, including 3,4-methylenedioxymethamphetamine (MDMA; ecstasy). The Ministry of Health and 2006 National survey data point to an epidemic of drug abuse, with NZers being amongst the world's highest consumers of amphetamine-type stimulants. Collectively, in NZ, ~250,000 people are affected across all socioeconomic, ethnic and indigenous groups. The prevalence rates in NZ mirror those in other western countries and world-wide estimates are that 300 million people now suffer from the effects of drug abuse with prevalence rates increasing annually. We have developed a protocol whereby rats self-administer MDMA, and other drugs of abuse, during daily sessions. Because self-administration is considered the gold standard for modelling effects of human drug abuse, results of our studies have external and predictive validity. Our results indicate persistent deficits in brain serotonin, sensitization of brain dopamine and either tolerance or sensitization to some of the behavioural effects of MDMA, depending on the exposure regimen. The results are consistent with the idea that following repeated exposure to self-administered MDMA serotonergic effects become less pronounced whereas dopaminergic effects become more pronounced. We suggest that it is this shift that underlies the development of MDMA as an effective reinforcer and underlies its abuse liability and propensity to relapse following period of withdrawal.

ORAL-24-02

INTRACELLULAR TRAFFICKING OF CANNABINOID RECEPTOR 2, A POTENTIAL CNS DRUG TARGET

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Purpose: Cannabinoid Receptor 2 (CB2) is a well established modulator of peripheral immune function. Evidence is emerging to suggest that CB2 also plays important roles in the central nervous system and shows promise as a drug target, including for the treatment of neuroinflammation and glioma. Intracellular trafficking pathways determine cell surface receptor expression levels and thereby fundamentally influence drug responses. We sought to investigate the trafficking properties of CB2. **Methods:** Human CB2 was stably expressed in HEK-293 cells. Alterations of surface CB2 in response to CB2-selective ligands and/or co-expression of trafficking adaptor proteins were monitored immunocytochemically and quantified via an established high-throughput fluorescence microscopy technique (Grimsey et al. 2008) (n=3-5 per assay). **Results:** CB2 rapidly internalises upon agonist stimulation, however recycles back to the plasma membrane following agonist removal. Internalisation was Rab5-dependent, as demonstrated by the inhibitory effect of co-expressing a dominant-negative form of Rab5 (an internalisation adaptor protein). Meanwhile, CB2 recycling was influenced by expression of mutant forms of Rab11, but not Rab4, indicating that the recycling pathway taken by CB2 is likely the "long" route via the perinuclear recycling compartment. **Conclusion:** This study represents the first thorough characterisation of CB2 trafficking and suggests that CB2 has the potential to readily re-sensitise following agonist stimulation. Continued investigation into CB2 trafficking may provide insight into in vivo drug responses and may ultimately aid in therapeutic design. Reference: Grimsey et al. (2008) Clin. Exp. Pharmacol Physiol. 35:1377-82.

ORAL-24-03

5HTTLPR AND DRD2 TAQIA POLYMORPHISMS ARE ASSOCIATED WITH PERSONALITY TRAITS THAT CORRELATE WITH ALCOHOL CRAVING IN ALCOHOL-DEPENDENT SUBJECTS

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Purpose: Serotoninergic and dopaminergic neurotransmissions play indispensable roles in modulation of addictive behaviours through personality and stress response. This study aims at exploring the relationships among personality traits, salivary cortisol and alcohol craving in alcohol-dependent subjects during detoxification, and the associations between these parameters and two gene polymorphisms involve in these neurotransmission systems: 5HTTLPR and DRD2 TaqIA. Methods: Alcohol-dependent subjects (n=156) were recruited during a five-day detoxification treatment. Neuroticism (N), extraversion (E) and conscientiousness (C) were measured by NEO PI-R; harm avoidance (HA), reward dependence (RD), novelty seeking (NS), persistence (P) and self-directedness (SD) were measured by Tridimensional Character Inventory. Alcohol craving level was measured by Alcohol Urge Questionnaire in a morning of day3 – day5, and a saliva sample was collected subsequently for cortisol measurement. Genotyping was done on by PCR-RFLP. Results: Alcohol craving correlated with salivary cortisol level in female subjects (Spearman's rho=.430, p=0.022) but not in male subjects. Alcohol craving correlated significantly ($p<0.05$) with P in females and RD in males, in addition to its correlation with C and NS in all subjects. 5HTTLPR short allele carriers scored higher in C (t=-2.178, p=0.032) while DRD2 TaqIA non-A1 allele carriers scored higher in RD (t=-2.202, p=0.031). Conclusions: These preliminary results suggest that correlations between alcohol craving, salivary cortisol and certain addiction-related personality traits are gender specific. 5HTTLPR and DRD2 TaqIA polymorphisms may be associated with C and RD, which may involve in determining craving in alcoholic patients during detoxification.

ORAL-24-04

BEHAVIOURAL CORRELATES OF GESTATIONAL LOW DOSE ETHANOL EXPOSURE IN ADULT AND AGED OFFSPRING

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Purpose: Excessive alcohol consumption during pregnancy can lead to a wide spectrum of disorders and defects in offspring, which are collectively referred to as Foetal Alcohol Syndrome. However, recent evidence suggests that mild alcohol consumption during pregnancy may not have detrimental effects on cognition and behaviour of the offspring. The aim of this study was to examine the effect of exposure to a low dose ethanol during gestation on behavioural changes in aged and adult offspring. **Methods:** Female Sprague Dawley rats were fed a liquid diet containing a low dose of ethanol (6% v/v, Ethanol) or a calorie matched control diet for the duration of pregnancy (Control). Male (Control: Adult n=12, Aged n=17; Ethanol: Adult n=13, Aged n=12) and female (Control: Adult n=12, Aged n=14; Ethanol: Adult n=10, Aged n=13) offspring were tested at 6-9 months (Adult) and 15-18 months (Aged) of age to assess a number of behavioural domains including anxiety, exploration, sensorimotor gating and spatial memory, as well as ethanol preference. **Results:** Prenatal exposure to a low dose ethanol diet resulted in a subtle, approaching significant ($p=0.055$), behavioural phenotype affecting aspects of anxiety and neophobia at 6-9 months of age but not at 15-18 months. There was no effect of prenatal treatment on locomotion, sensorimotor gating, spatial memory or ethanol preference at either age ($p>0.05$). **Conclusions:** Exposure to low doses of ethanol during early neural development does not lead to long lasting behavioural changes in adult life.

ORAL-24-05

NALMEFENE, A MIXED OPIOID COMPOUND AS A POTENTIAL ANTI-ADDICTION PHARMACOTHERAPY

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Purpose Kappa opioid receptor (KOPr) activation attenuates drug seeking behaviours but produces severe adverse effects, which prevents its clinical use. Since Mu opioid receptor (MOPr) agonists produces rewarding behaviours and KOPr activation results into aversion, compounds with partial KOPr agonist/MOPr antagonists properties have potential anti-addiction properties. Nalmefene is currently approved by US-FDA for opioid overdose and is a MOPr antagonist. However, recent binding studies indicated that nalmefene is also a partial KOPr agonist/antagonist and binds to KOPr with more affinity than to MOPr. Also, nalmefene suppressed ethanol seeking in laboratory animals. **Methods** The aim of our current study was to determine anti-addiction properties of nalmefene by using cocaine induced drug seeking paradigm in laboratory animals. We further tested the effect of acute exposure to nalmefene on sucrose reinforcement and conditioned taste aversion in rats. Also, effect of a single injection of nalmefene was tested on cocaine induced hyperactivity and spontaneous locomotion in rats. **Results** Our results indicate that single injection of nalmefene dose dependently attenuated cocaine induced drug seeking ($p<0.05$), which was KOPr antagonist reversible ($p<0.05$). Also, nalmefene neither suppressed sucrose reinforcement ($p>0.05$) nor produced taste aversion to novel tasting saccharin solution ($p>0.05$). Nalmefene had no significant effect on cocaine induced hyperactivity in self-administering rats ($p>0.05$). However, a significant suppression in spontaneous locomotion was observed in drug naive rats ($p<0.05$) indicating motor suppression. **Conclusion** These findings support future research in developing mixed opioid compounds as potential anti-addiction pharmacotherapeutics with better side effect profiles.

ORAL-24-06

INHALATION OF TOLUENE DURING ADOLESCENCE: IMPLICATIONS FOR BRAIN FUNCTION

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Purpose: The abuse of volatile organic solvents via inhalation, such as toluene, is comparatively prevalent in adolescent populations, posing a significant risk to the developing brain. Despite this, our understanding of the behavioral and neuropathological implications of toluene abuse are relatively sparse. **Methods:** Adolescent (4-5 weeks at start) male Wistar rats were exposed to either air or chronic intermittent toluene (CIT, 3000ppm) for 3 x 1hr sessions per week, a paradigm relevant to human use patterns. After either 4 or 8 weeks exposure we investigated behavioural and neuropathological parameters. **Results:** Following 8 weeks CIT exposure rats displayed anxiety-like behaviour evident as an increased latency to enter (p=0.019), total entries (p=0.016) and decreased duration (p=0.009) in the light compartment of a light-dark box compared to air-exposed rats. Assessment of locomotion in the same cohort revealed decreased vertical plane entries ($F_{1,25}$=17.331, p=0.009) and time spent rearing ($F_{1,25}$=10.702, p=0.022) of CIT-exposed compared to air-exposed rats. This deficit was also present in a separate cohort of rats exposed to CIT for 4 weeks (n=12 CIT, n=12 air; $p<0.05$). However stereological investigation revealed no overt neuropathology following 4 weeks CIT exposure. **Conclusions:** Adolescent CIT exposure for up to 4 weeks is sufficient to induce specific behavioural deficits without the presence of any overt neuropathology. These behavioural deficits become more pronounced upon continued exposure. The results from this study increase our knowledge of the long-term behavioural consequences of inhalant abuse on the developing brain and highlight the need for future study of this issue.

ORAL-24-07

DIFFERENTIAL ROLE FOR MGLU5 AND ADENOSINE A_{2A} RECEPTOR INTERACTIONS IN REGULATING THE CONDITIONED REINFORCING VERSUS THE PSYCHOMOTOR EFFECTS OF COCAINE

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Purpose: The striatum is known to play a crucial, integrative role in processes such as reward, motivation and drug-seeking behaviour. Adenosine A_{2A} receptors and metabotropic glutamate type 5 (mGlu5) receptors are co-localised both presynaptically and postsynaptically in the striatum and have been shown to functionally interact in heteromeric complexes. In the present study this interaction was explored using antagonism of mGlu5 receptors with 3-[(2-methyl-1,3-thiazol-4-yl) ethynyl]-pyridine (MTEP) in combination with genetic deletion of A_{2A} receptors. **Methods:** The conditioned rewarding and locomotor activating properties of cocaine were evaluated using the conditioned place preference (CPP) paradigm. Adenosine A_{2A} receptor knockout (n=16) and wildtype (n=26) mice were subjected to alternating daily conditioning injections of cocaine (20mg/kg, *i.p.*) or saline. 20min prior to cocaine administration mice were pre-treated with either MTEP or vehicle. CPP was assessed following 8 days of conditioning. During each session the time spent in each compartment (sec) as well as locomotor activity (distance moved in cm) was measured. **Results:** Vehicle-treated mice of both genotypes achieved a CPP to cocaine ($p < 0.01$) while MTEP was found to abolish CPP in wildtype mice only. In contrast, MTEP attenuated the locomotor activating properties of cocaine in both genotypes ($p < 0.001$). **Conclusion:** These data provide evidence for a functional interaction between adenosine A_{2A} and mGlu5 receptors in mediating the conditioned rewarding effects of cocaine but not cocaine-induced hyperactivity.

ORAL-24-08

RELATING NEUROCHEMICAL CHANGES ASSOCIATED WITH CANNABIS USE TO JUVENILE LEARNING AND MEMORY

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Purpose: Cannabis, the most widely used drug amongst adolescents, impairs learning and memory via the action of Δ^9-tetrahydrocannabinol (THC) on the cannabinoid receptor 1 (CB1R) in the brain. The molecular events underlying learning impairment and not well understood and adolescents may be particularly vulnerable. We examined these molecular events in adolescent animals. **Methods:** THC (n=16) and control (n=16) adolescent male Sprague-Dawley rats were trained in a spatial learning and memory task using the radial maze; following the completion of training the hippocampus was assessed by western blot, qRT-PCR and immunohistochemistry. **Results:** THC inhibited the acquisition of spatial memory (learning) in the radial maze task by disrupting ordered search strategies (*p<0.05*), suggesting impairment of higher cognitive function. Western blotting and qRT-PCR revealed that pre- (synapsin-I and -II) and post-synaptic markers (PSD95), as well as well as CB1R were more abundant in vehicle-treated trained animals (*p<0.001*) and that their expression was enhanced by training in vehicle- (*p<0.001*), but not THC-treated animals. Training in the radial maze promoted the survival of labelled neurons in control animals (*p<0.001*, trained v untrained animals) which was not affected by THC-treatment. PSA-NCAM, a marker of maturing neuroblasts, was not affected by THC treatment although synapsin-III, predominantly associated with neurogenic brain regions and implicated in cognitive functioning, was elevated by training in vehicle-, but not THC-treated animals (*p<0.01*). **Conclusions:** These data suggest that THC exposure in adolescence is capable of disrupting learning through impairment of higher cognitive function and that this disruption appears to be mediated by the attenuation of specific neurochemical changes in response to learning.

ORAL-25-01

INFLUENCE OF HIPPOCAMPAL MICROGLIA ON NEURAL PRECURSOR CELL ACTIVATION

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Purpose: Microglia are capable of secreting factors that can either stimulate or inhibit proliferation of neural precursor cells. The activation status and secretory profile of microglia thus partly shapes the molecular microenvironment of the neurogenic niche, which in turn can influence neurogenesis under both normal and pathological conditions. Here, we investigated whether microglia contribute to hippocampal neural precursor activation induced by voluntary exercise. **Methods:** This study took advantage of MacGreen mice, which express green fluorescent protein (GFP) under the promoter of the macrophage colony-stimulating factor 1 receptor (M-CSF1R). Experimental mice were given unlimited access to a running wheel for voluntary exercise over a period of 2 weeks. Flow cytometry was used to deplete GFP-positive microglia from neurosphere cultures in order to assess the importance of their presence for neurosphere formation frequency. **Results:** As anticipated, in mice with access to running wheels, a 50% increase in neurosphere formation frequency was observed. In turn, when microglia were depleted from hippocampi of runner mice, a significant decrease in neurosphere formation was recorded. Depletion of microglia did not influence neurosphere formation in mice housed under sedentary conditions (4 experimental repeats; $p<0.05$). **Conclusion:** These results indicate that microglia exert instructive and stimulating effects on neural precursor cell activation within the hippocampus following running.

ORAL-25-02

STRUCTURAL AND BEHAVIOURAL CORRELATES OF DIMINISHED DOPAMINE SYNTHESIS IN THE DEVELOPING ZEBRAFISH

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Introduction: Schizophrenia is a neurodevelopmental disorder with underlying abnormalities in dopamine (DA) signaling in the brain. Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthetic pathway of the catecholamine neurotransmitter DA and is expressed in all DA neurons. To obtain a better understanding how alterations in DA signaling impact on DA ontogeny we have developed a model in which TH activity was suppressed during early zebrafish development. **Method:** We used morpholino (MO) injection to knock down TH function. Neuronal connectivity and DA content were analysed by immunochemistry and HPLC. We conducted open-field experiments as a first step to characterize the larval and adult behavioural phenotype. **Results:** Depending on morpholino design we achieved either partial or complete loss of TH positive neurons in the MO injected larval brain until at least 12 days post fertilization. The degree of TH knock-down also correlated with the levels of DA and its major metabolite DOPAC. Because of the transient nature of morpholino mRNA knock-down DA content returned to normal levels in adult fish. Although locomotion appeared to be grossly normal in the adult morphants, they appeared to show increased thigmotaxis as assessed by increased time spent in the corners of the tank and less time in the center compared with controls. **Conclusion:** Our findings suggest that transient morpholino-mediated knock down of TH function during development may be anxiogenic in the adult fish even though brain DA content was normal. Further analysis of anxiety-related behaviours as well as drug screens will be carried out to characterize the present phenotype in more detail. TH knock-down in zebrafish may represent a useful model for examining how abnormalities in DA ontogeny relate to mature DA signaling and may even help to explore the etiology of serious psychiatric disorders such as schizophrenia.

ORAL-25-03

MACROMOLECULAR TRANSFER ACROSS THE BLOOD-CSF INTERFACE

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Purpose: Early developmental cerebrospinal fluid (CSF) in fetal brain contains high concentrations of protein generated by transfer from plasma across choroid plexus epithelial cells. Previous studies demonstrated that about 10% of plexus cells in neonatal opossum (*Monodelphis domestica*) are involved in unidirectional transfer of plasma proteins from blood to CSF. We investigated physiological plasticity of this mechanism by altering levels of individual proteins in plasma. **Methods:** Protein transfer across plexus epithelial cells was investigated by intraperitoneal injection of endogenous (adult plasma) or exogenous (bovine fetuin) protein into opossum pups at different postnatal (P) ages. Plexus cell numbers, protein concentration and ventricular volumes were measured. **Results:** Injections of adult plasma resulted in increased protein concentration in CSF within 6 – 24 hours at P65 and P110, but not at P9 due to ventricular expansion at this age only. Protein content in CSF increased at all 3 ages. Albumin and hemopexin increased in CSF at P9 and P65, but not in adults. The number of choroid plexus cells immunopositive for individual proteins mirrored these changes. The concentration of α-fetoprotein did not change, but the number of immunopositive cells decreased. Injection of bovine fetuin resulted in increased CSF protein content and the exogenous protein was detected both in CSF and within protein-transferring plexus cells. Fetuin displaced some endogenous proteins - hijacking the native protein transport system of the choroid plexus. **Conclusions:** Results suggest that there may be specific, developmentally regulated, transporters responsible for protein transfer across the blood/CSF barrier. This raises the possibility of designing delivery systems from the blood to CSF and brain via the choroid plexus epithelial cells.

ORAL-25-04

PROTEIN PHOSPHATASE 2A METHYLATION PLAYS A CRITICAL ROLE IN NEURITE OUTGROWTH

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Neuritic alterations are a major feature of many neurodegenerative disorders. Methylation of protein phosphatase 2A (PP2A) catalytic C subunit by the leucine carboxyl methyltransferase LCMT1, and demethylation by the methylesterase PME-1, is a critical PP2A regulatory mechanism. It modulates the formation of PP2A holoenzymes containing the Bα subunit, which dephosphorylate key neuronal cytoskeletal proteins, including tau and amyloid precursor protein. Significantly, we have reported that LCMT1, methylated C and Bα expression levels are down-regulated in Alzheimer disease-affected brain regions. Here, we show that enhanced expression of LCMT1 in cultured N2a neuroblastoma cells, which increases endogenous methylated C and Bα levels, induces changes in F-actin organization. It promotes serum-independent neuritogenesis and development of extended tau-positive processes upon N2a cell differentiation. These stimulatory effects can be abrogated by LCMT1 knockdown and S-adenosylhomocysteine, an inhibitor of methylation reactions. Expression of C subunit mutant, which decrease PME-1 and the methylation-site L309Δ intracellular methylated C and Bα levels, block N2a cell differentiation and LCMT1-mediated neurite formation. Our results establish a novel mechanistic link between PP2A methylation and neuritogenesis, and suggest that LCMT1 dysfunction could contribute to axonal defects in Alzheimer disease.

ORAL-25-05

STIM1 IS NECESSARY FOR REGULATION OF GROWTH CONE MOTILITY

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The intricate neuronal networks of the nervous system are connected as navigating growth cones connect with target cells. Growth cones are extremely sensitive motile organs located at the distal tips of growing axons. Cytosolic calcium is a crucial mediator of growth cone navigation. Understanding the mechanisms that regulate cytosolic calcium is vital to understanding growth cone function. The work described here focuses on molecular mechanisms that regulate a crucial store of calcium, the endoplasmic reticulum (ER). Stromal Interacting Molecule 1 (STIM1) is a calcium sensing protein in the ER membrane, which interacts with Orai proteins in the plasma membrane, initiating calcium influx and repletion of depleted intracellular calcium stores. Our hypothesis is that STIM1 and Orai1/2 are necessary for growth cone chemotactic responses to extracellular guidance cues. STIM1 function in navigating growth cones was determined using an in vitro growth cone turning assay combined with STIM1 knockdown, immunofluorescence and western blot analyses. We demonstrate that STIM1 and Orai co-localise upon store depletion and during growth cone turning with expression biased towards the growth cones turning side. Furthermore, STIM1 knockdown perturbed growth cone turning responses to guidance cues Brain derived neurotrophic factor and Semaphorin-3a (Sema-3a). The normal chemorepulsive response to Sema-3a (-7.90 ± 2.94°) was abolished by STIM1 knockdown (0.95 ± 2.48°; $p<0.05$). Turning was rescued by activating cyclic AMP (cAMP), restoring the normal chemorepulsive response to Sema-3a (-12.18 ± 4.36°). Our data demonstrate the necessity of STIM1 for growth cone turning and importantly, that STIM1 likely regulates cAMP levels in addition to stored calcium in growth cones. This work has significant implications for neuronal development, and calcium dysregulation associated with neurodegenerative disease.

ORAL-25-06

SOCS2 OVEREXPRESSION INDUCES NEURITE OUTGROWTH IN DORSAL ROOT GANGLIA NEURONS

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Overexpression of Suppressor of Cytokine Signalling-2 (SOCS2) promotes increases in neurite length and neurite number in PC12 cells and cortical neurons. The mechanisms by which SOCS2 regulates the signals that control neurite outgrowth and neuronal differentiation are unresolved but appear to involve the Trk neurotrophin receptors. To examine the involvement of SOCS2 in Trk signalling in primary neurons, the morphology of TrkA expressing Dorsal Root Ganglion (DRG) neurons from SOCS2 overexpressing (SOCS2-Tg) mice was compared to wildtype neurons. DRG neurons were dissected from 1 day post-natal mice, dissociated, cultured for 4 hours with nerve growth factor (NGF) and neurite morphology then scored. DRG neurons from SOCS2-Tg mice demonstrated an increased proportion of neurons with complex neurite morphology. The inhibitor K252a was applied to cultures and significantly blocked neurite outgrowth, thus confirming the observed neurite outgrowth was dependent upon phosphorylation of the TrkA receptor. To examine if overexpression of SOCS2 could influence NGF-mediated survival, the survival profile of DRG neurons cultured with 50 ng/mL, 1 ng/mL or 0.1 ng/mL NGF or in the absence of NGF was examined and the number of viable DRG neurons (large, phase bright cell bodies) was recorded at 24 hour intervals for a period of 72 hours. The survival of SOCS2-Tg DRG neurons compared to wildtype controls was unaltered. In summary, whilst overexpression of SOCS2 has no impact on NGF-mediated survival of cultured DRG neurons, SOCS2 overexpression was able to promote the culture of a neuronal subpopulation with increased neurite complexity. These data highlight the independence of the molecular mechanisms governing survival and differentiation signalling downstream of the TrkA receptor in DRG neurons.

ORAL-25-07

CHARACTERISATION OF THE DISTRIBUTION OF CONNEXIN 30 IN THE ADULT SUBVENTRICULAR ZONE/ROSTRAL MIGRATORY STREAM

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Purpose: Connexin 30 (Cx30) is one isoform of a family of transmembrane proteins which have multiple established roles in perinatal neurogenesis. Early studies of the adult hippocampus indicate a role for Cx30 in modulating neuronal proliferation and differentiation, however the role of Cx30 in the adult subventricular zone (SVZ) - rostral migratory stream (RMS) is currently unknown. Determining the factors that facilitate neurogenesis in the adult brain could provide a novel means of enhancing neurogenesis after brain injury. **Aim:** To characterise the role of Cx30 in adult SVZ/RMS neurogenesis. **Methods:** Adult C57 mice (n=5) were killed and perfused transcardially with ice cold 0.9% saline followed by 4% paraformaldehyde. Coronal sections (30 µm) were cut from frozen brains using a sliding microtome. Co-localisation of Cx30 with specific cell markers was carried out using immunohistochemistry. **Results:** Experiments indicated that Cx30 is present in the adult mouse SVZ/RMS. Cx30 is expressed by both neuroblasts (Dcx+) and astrocytes (GFAP+) throughout the SVZ/RMS. The number of Cx30 puncta on Dcx+ cells is 200% higher in the RMS than in the SVZ ($p=0.0004$). Similarly, the number of Cx30 puncta on GFAP+ cells is 44% greater in the RMS compared to the SVZ ($p=0.00005$). Interestingly, Cx30 puncta on GFAP+ cells of the SVZ/RMS that are in contact with Dcx+ cells are 3 times larger than those not in contact with Dcx+ cells ($p=0.001$). **Conclusion:** Cx30 may facilitate interaction between neuroblasts and astrocytes. Cx30 expression is most intense in the RMS, a specialised pathway of neuroblast migration, suggesting that Cx30 may be involved in facilitating this process.

ORAL-25-08

THE DOWN SYNDROME-RELATED GENE EURL IS IMPORTANT FOR CORTICAL NEURON MIGRATION AND TERMINAL MORPHOGENESIS

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Aneuploidy disorders such as Down's Syndrome (DS) and tetrasomies of human Chromosome 21 can predispose to abnormal brain development and mental retardation. The etiology of mental retardation endophenotypes within these aneuploid diseases is, in part, attributable to altered expression of genes within regions of the affected chromosome(s) which have been multiplicated. In pursuit of this "gene dosage hypothesis" to explain how excessive expression of key genes may affect normal brain development, we have identified *eurl* as a novel Down syndrome-related gene that is important for controlling neuronal morphology and migration within the developing mouse cerebral cortex. During mouse brain development, eurl protein is detected in immature neurons within the cortex and hippocampus, and forced overexpression of *eurl* perturbs cortical neuron migration in vivo in a dose-dependent manner. Furthermore, neurons which overexpress eurl also adopt changes to their morphology. Together, these findings identify *eurl* as a novel gene important for the maturation and migration of immature cerebral cortical neurons, and provides novel insight into the contribution of this previously uncharacterised gene within DS-implicated regions of chromosome 21 correlated with mental retardation.

ORAL-26-01

NOVEL ROLE FOR CAMKII IN REGULATING GROUP I MGLUR-MEDIATED LTD IN RAT HIPPOCAMPUS

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Previously, we have reported that group I mGluR-dependent LTD (mGluR-LTD) induced by the group I specific agonist DHPG is partially dependent on alpha calcium/calmodulin-dependent protein kinase II (CaMKII)-mediated protein synthesis through regulation of translation. Other reports suggest that synaptically- and chemically–mediated mGluR-LTD may involve different molecular mechanisms. **Purpose**: The present study tested whether CaMKII mediates both forms of mGluR-LTD, as well as phosphorylation of translation factors. **Methods**: Hippocampal slices with CA3 removed were prepared from 6-7 week old male Sprague-Dawley rats. Synaptically-mediated mGluR-LTD was induced by electrical stimulation in stratum radiatum (1200 pulses, 1 Hz) in the presence of the NMDAR blocker APV (50 µM) and the CaMKII inhibitor KN62 (10 µM), and the initial slope of the field EPSP measured. Chemically-mediated mGluR-LTD was induced by bath application of DHPG (100 µM, 10 min) following intracellular infusion of a second CaMKII blocker (AIP, 50 µM, 40 min) via a patch electrode attached to CA1 neurons, and EPSC amplitudes measured. Phosphorylation of translation factors was determined by Western blot following incubation of hippocampus-derived synaptoneurosomes with DHPG (10 µM, 5 min). **Results**: KN62 significantly reduced synaptically-mediated mGluR-LTD 75 min post-stimulation (control, -23±5%, n=7; KN62, -9±3%, n=8; p=0.028). Similarly, DHPG-induced mGluR-LTD was significantly reduced by AIP 30 min post-treatment (DHPG, -50±5%, n=10; DHPG+AIP, -26±5%, n=10; p=0.003). A 1.84 fold DHPG-induced increase in phosphorylation of the translation factor eIF4 (n=4, p=0.018) was blocked by KN62. **Conclusion**: These findings suggest that CaMKII mediates both forms of group I mGluR-dependent LTD and that this involves eIF4-dependent translation initiation.

ORAL-26-02

HETEROSYNAPTIC METAPLASTICITY IN AREA CA1 OF THE HIPPOCAMPUS

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It has been suggested that for long-term potentiation (LTP) and long-term depression (LTD) to underlie information storage, overall synaptic strengths need to be kept within a dynamic range by metaplasticity mechanisms. **Purpose:** Here we tested key predictions of one prominent computational model of synaptic plasticity, the Bienenstock, Cooper and Munro model (BCM, 1982): 1) that the history of cell firing regulates the ability to produce LTP and LTD, and 2) this occurs in a cell-wide manner. **Methods:** To test these predictions, field excitatory postsynaptic potentials (fEPSPs) or intracellular EPSPs were recorded in response to stimulation of the Schaffer collaterals in area CA1 of acute hippocampal slices from 6-7 week male Sprague-Dawley rats (group n's 4-7). **Results:** Strong, high-frequency priming stimulation delivered to afferents in either stratum oriens or stratum radiatum significantly inhibited LTP and facilitated LTD induced later by tetanisation of an independent stratum radiatum pathway. This confirms the BCM prediction that activity can induce a heterosynaptic metaplastic state that spreads widely across the dendritic arbour, including from basilar to apical dendrites. In contrast with the model, however, postsynaptic cell firing was neither necessary nor sufficient to induce the metaplastic state. Instead, the induction of the metaplastic state was mediated by the release of calcium from intracellular stores during the priming activity, as it was significantly blocked by store depletory CPA. **Conclusions:** These results indicate that synaptic plasticity in CA1 is homeostatically regulated by the cell-wide history of synaptic activity through a calcium signal generated from intracellular stores.

ORAL-26-03

HUNTINGTIN-ASSOCIATED PROTEIN 1 (HAP-1) REGULATES EXOCYTOSIS VIA MULTIPLE MECHANISMS

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Subcellular localisation and protein interaction data indicate that HAP-1 maybe important in vesicle trafficking and microtubule transport. However, no physiological evidence exists to verify this possibility. Our study reports a novel role of HAP-1 as a regulator of exocytosis by influencing the rate of exocytosis, fusion pore dynamics and the size of the readily releasable pool (RRP) which consists of vesicles released immediately upon stimulation. This role was identified using carbon-fibre amperometry on adrenal chromaffin cells, a classic model of neuronal exocytosis, cultured from HAP-$1^{-/-}$ (KO), HAP-$1^{+/-}$ (Het) and HAP-$1^{+/+}$ (WT) mice. Similar levels of exocytosis was found in WT (102.2 ± 10.2 exocytotic events, n= 29) and Het (90.8 ± 11.5, n=20) cells while exocytosis in KO cells was significantly reduced (60.4 ± 7.1, n=35) compared to WT ($p<0.01$) or Het ($p<0.05$) cells. The duration of the pre-spike "foot signal", an indicator of fusion pore opening, was found to be prolonged in KO cells (3.0 ± 0.1 ms) compared to WT (2.3 ± 0.1 ms, $p<0.05$) and Het (2.9 ± 0.1 ms, $p<0.05$) cells indicating that HAP-1 may function in stabilizing the formation of the fusion pore. The size of the RRP is also regulated by HAP-1 as the number of vesicles undergoing exocytosis following treatment with a hyperosmotic solution in KO cells (19 ± 5.3, n=7) is less than in WT (54.4 ± 8.9, n=7, $p<0.01$) or Het (46 ± 9.2, n=8, $p<0.05$) cells. Real-time PCR also indicates the downregulation of exocytosis-related genes in KO cells. Our findings implicate, for the first time, the involvement of HAP1 in the regulation of exocytosis at multiple levels including vesicle localization, membrane fusion and gene transcription.

ORAL-26-04

RELIABLE ESTIMATION OF PASSIVE MEMBRANE PROPERTIES OF LAYER IV EXCITATORY NEURONES

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Passive membrane properties of various neurons have been estimated but the errors inherent in estimation itself or caused by recording noise, have not been quantified. **Purpose**: To reliably estimate passive membrane properties with associted errors and apply it to excitatory neurons in layer IV. **Methods**: Experiments were done in 300 µm thick parasagittal slices of 17±2 day-old rats at 36±1°C. Whole-cell recordings were obtained from layer IV excitatory neurons which were filled with biocytin to allow for subsequent reconstruction. Standard patch solution was used with the following additions (mM): 10 TEA, 10 BAPTA and 5 QX-314. ACSF included (µM): 3 SR95531, 50 ZD7288 and 10 DNQX. Membrane responses to small current or voltage steps were obtained and checked for linearity by scaled superimposition and semi-log plotting to detect sag. In order to improve estimation accuracy and reliability, all recordings from a single neuron served to estimate specific membrane (r_m), axial resistivity (r_a) and membrane capacitance (c_m) using a chi-squared based minimization algorithm. Reliability of estimates was obtained using chi-squared and Monte Carlo techniques. **Results**: For spiny stellate, star pyramidal and pyramidal neurons, respectively, average neuron surface areas (µm^2) were 1326±185, 1745±68 and 2016±208. For spiny stellate c_m=1.02±0.03 µF/cm^2, r_m=30.0±1.2 kOhm.cm^2 and r_a=85±30 Ohm.cm; for star pyramid c_m=1.10±0.03 µF/cm^2, r_m=54.99±0.95 kOhm.cm^2 and r_a=109±30 Ohm.cm; and for pyramidal neurons c_m=1.25±0.08 µF/cm^2, r_m=50.74±1.50 kOhm.cm^2 and r_a=75±25 Ohm.cm. **Conclusion**: Our method provides robust estimates of membrane parameters of excitatory neurons together with associated errors. Initial data suggests that, despite similar membrane time constant, pyramidal and star pyramidal neurons have larger r_m than spiny stellate neurons potentially pointing to a homeostatic principle.

ORAL-26-05

α_1-ADRENERGIC RECEPTOR ACTIVATION CAUSES INCREASE IN MINIATURE EPSC FREQUENCY BUT DEPRESSION OF EVOKED EPSCS

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Presynaptic α_1-ARs activation by noradrenaline (NA) increases mEPSC frequency due to Ca^{2+} release from stores in rat barrel cortex. The result of this activation on evoked release is unknown. **Purpose**: To obtain the dose-response relationship, check if there is crosstalk between α_1-ARs with other IP_3-producing receptors and evaluate how α_1-ARs activation affects EPSCs. **Methods**: 300 µm thick parasagittal slices from rats served to record mEPSCs from pyramidal cells in layer II/III in the presence of tetrodotoxin (1µM) and gabazine (3µM) at 36±1°C and a superfusion rate of 4mL/min. Typically, 10µM NA was used. **Results**: Specificity of receptor activation was assessed by blocking α_2-ARs (yohimbine, 1µM) and β-ARs (propranolol, 1µM) in the presence of NA (*n*=8), which produced a significant mEPSC frequency change, consistent with applying cirazoline (5µM), an α_1-AR agonist alone. The dose-response curve of NA was obtained using concentrations from 0.1 to 100µM. It showed a full response at 1µM. Testing receptor cross-talk with (s)-3,5-dihydroxyphenylglycine (30µM; *n*=8) increased mEPSC rate by 24±2% but when NA was co-applied no further increase was seen. For evoked EPSCs, application of NA (*n*=4) decreased EPSC amplitude by 60±9%. In some cells, paired-pulse ratio did not change, whereas in others, a facilitatory component was uncovered. Recovery from depression at 500 ms was typically much larger than the initial response (22±5%; *n*=5). **Conclusions**: In rat barrel cortex, NA activates predominantly presynaptic α_1-ARs. Different IP_3 producing receptors may not operate independently of each other. In evoked transmitter release, NA dramatically alters synaptic dynamics. mEPSCs may be a poor predictor of evoked EPSCs.

ORAL-26-06

GABAERGIC INHIBITION IS DEVELOPMENTALLY REGULATED IN CORTICAL LAYER 5 PYRAMIDAL NEURONS

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Purpose: GABAergic inhibition in the neocortex is mediated by either GABA-A (ionotropic) or GABA-B (metabotropic) receptors. Activation of GABA-B receptors typically is thought to open G protein-coupled inwardly rectifying potassium (GIRK) channels and can also down-regulate voltage-activated calcium channels. Postsynaptic GABA-B receptors play a role in setting the resting membrane excitability, whereas presynaptic GABA-B receptors can regulate transmitter release. **Methods**: Here we investigated the impact of GABA-B receptor activation on the excitability of layer 5 pyramidal neurons in brain slices of barrel cortex from 4-5 and 8-9 week old rats. **Results**: At the soma, GABA-B receptor activation via bath application of baclofen (20 µM) was associated with hyperpolarization of the resting membrane potential and a decrease in input resistance, leading to a strong and reversible decrease in the number of action potentials evoked by somatic current injections in young (n=41) and adult (n=23) rats. Similarly, GABA-B receptor activation affects the dendritic resting membrane potential, input resistance in adult rats (n=10), but not in young rats (n=10). To confirm a differential contribution of GABA-B receptor activation on somatic and dendritic resting membrane excitability in young rats, we locally applied baclofen (50 µM) to the soma and distal dendrites. A transient hyperpolarisation of the dendritic membrane potential was observed in neurons from adult rats (n=7), where no effect was observed in young rats (n=7). In both groups, bath application of baclofen blocked dendritic calcium electrogenesis evoked by high frequency action potential trains (young: n=17; adult: n=10). **Conclusion**: These data suggest that GABA-B receptors regulate neuronal excitability via location and development-dependent mechanisms in the cortex.

ORAL-26-07

2'-METHOXY-6-METHYLFLAVONE: A NOVEL DIRECT ACTIVATOR OF α2β2/3γ2L AND α4β1/2/3δ $GABA_A$ RECEPTORS

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Introduction: Ionotropic $GABA_A$ receptors located on post- and extrasynaptic locations of neurons modulate 'phasic' and 'tonic' inhibition, respectively. Flavonoids are polyphenolic agents found in the foods we eat and drink. Recently more attention has been given to the central actions of flavonoids particularly as they exert anxiolytic effects through $GABA_A$ receptors. **Methods**: We have evaluated the effects of 2'-methoxy-6-methylflavone (2'MeO6MF) on recombinant $\alpha_2\beta_x\gamma_{2L}$, $\alpha_4\beta_x\delta$ and $\alpha_4\beta_x\gamma_{2L}$ (x=1-3) $GABA_A$ receptors expressed in Xenopus oocytes using two-electrode voltage clamp methods. **Results**: 2'MeO6MF had no effect on its own but enhanced the action of GABA (EC_{10}) at $\alpha_4\beta_{2/3}\gamma_{2L}$ (EC_{50} (α**4**β**2**γ**2L**) 37 [3.3, 413] µM; n=5; EC_{50} ($\alpha_4\beta_3\gamma_{2L}$) = 98 [48, 200] µM; n=4) by 400-450% at 300 µM. In contrast, 2'MeO6MF had no effect alone or as a potentiator of GABA on $\alpha_4\beta_1\gamma_{2L}$ $GABA_A$ receptors. In addition, 2'MeO6MF directly activated $\alpha_2\beta_2\gamma_{2L}$ (EC_{50} 54.52 (46.15 to 65.24) $\alpha_2\beta_3\gamma_{2L}$ (56.33 (38.49 to 71.40) $\alpha_4\beta_1\delta$ (EC_{50} 1.1 [0.8671, 1.432] µM; n=4), $\alpha_4\beta_2\delta$ (EC_{50} 1.6[1.3-1.9] µm; n=4) and $\alpha_4\beta_3\delta$ in a biphasic manner (EC_{50}(1) 0.011 [0.0043, 0.08] µM; EC_{50}(2) 11.5 [6.3, 21] µM; n=4; Comparison of fits F (DFn, DFd) 5.198 (3, 32)) indicating high and low affinity binding sites within this receptor. In mice 2'MeO6MF displayed sedative and anxiolytic effect without causing any myorelaxation.**Discussion and Conclusion**: The pharmacological effects of activation versus potentiation exerted by 2'MeO6MF on α_4-containing receptors appear to be mediated via the β-δ and β-γ interfaces, respectively. As 2'MeO6MF can differentially activate or potentiate certain $GABA_A$ receptor combinations, it serves as a useful pharmacological tool to characterize $GABA_A$ receptors in vitro and in vivo.

ORAL-26-08

TONIC MODULATION OF GABAERGIC SYNAPTIC INPUT TO NIGRAL DOPAMINERGIC NEURONS BY ENDOCANNABINOIDS

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Whilst the actions of endogenous cannabinoids (eCB) have been studied intensely in the hippocampus and the cerebellum, little is known of eCB function in dopaminergic neurons of the Substantia Nigra pars compacta (SNc) – the cells which die in Parkinson's disease (PD). **Purpose:** The aim of this study was to determine the mechanisms by which eCB control inhibitory synaptic transmission to SNc neurons. **Methods:** Whole-cell patch-clamp recordings were made from SNc neurons in midbrain slices obtained from juvenile rats. Synaptic GABA release was evoked by electrical stimulation within the Substantia Nigra pars reticulata. The evoked inhibitory post-synaptic currents (eIPSCs) were recorded in the presence of NMDA, AMPA and dopamine receptor blockers. **Results:** Bath application of a CB1 antagonist, rimonabant (SR141716A; 3 µM), caused a significant increase of eIPSC amplitude (to 127%; n=14). The effect was prevented by inclusion of high calcium buffering solution in the patch pipette (n=5). Rimonabant also reduced the ratio of amplitudes during paired-pulse stimulation. Blockade of metabotropic glutamate receptors (mGluR1) by CPCCOet (100 µM) also prevented the rimonabant-induced increase in eIPSCs. Inhibition of dopamine synthesis by carbidopa (300 µM) also prevented the increase of eIPSC amplitude caused by rimonabant. Non-dopaminergic neurons did not respond to rimonabant (n=5). **Conclusion:** Our results demonstrate that GABAergic inhibition of SNc neurons in brain slices is tonically suppressed by eCBs released from these neurons, and that eCB production depends on activation of metabotropic glutamate receptors. This new regulatory mechanism may be important for the function of SNc neurons both in health and disease, including PD.

ORAL-27-01

ABNORMAL EPIGENETIC MODIFICATION OF THE MICROTUBULE ASSOCIATED PROTEIN TAU GENE IN NEURODEGENERATIVE DISEASES

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Purpose: Alzheimer's disease (AD), and Parkinson disease (PD) are the two common neurodegenerative disorders. Epistatic interaction between the microtubule associated protein Tau (*MAPT*) and the glycogen synthase kinase-3β (*GSK3B*) genes occur to determine risk of idiopathic AD and PD [Kwok et al. Annals Neurol; 58: 829-39 (2005); Kwok et al. Annals Neurol; 64: 446-54 (2008)]. We have previously shown that increased *MAPT* expression, associated with the inheritance of the common *MAPT* H1 haplotype, was a pathogenic mechanism in idiopathic neurodegenerative diseases [Kwok et al. Annals Neurol; 55: 329-34 (2004)]. Environmental factors have important roles in disease aetiology, and may exert their influence via epigenetic modifications that result in altered expression of neurodegenerative genes. We aim to identify whether the *MAPT* gene was epigenetically modified in a disease-specific manner. **Methods:** We assayed for epigenetic modification of the *MAPT* promoter using PCR-sequencing of bisulfite-treated genomic DNAs to detect methylated cytosines. **Results:** We demonstrated that the level of methylation was significantly correlated with *MAPT* transcript levels in brain tissue (r^2 = 0.62, p =0.025), thus providing a novel mechanism for the regulation of *MAPT* expression. We further demonstrated that DNAs from frontal cortices of AD patients (n = 7) had a significant 4-fold (p = 0.002) lower methylation of *MAPT* compared with DNAs of neuropathologically normal controls (n= 10). We showed in a case-control cohort of AD patients (n = 124 cases), that the pattern of *MAPT* methylation was not uniform, but is significantly correlated (r^2 = 0.56, p = 0.032) with cigarette smoking in an haplotype-specific manner. Finally, the methylation of *MAPT* was 2-fold lower (p = 0.034) in a subset of patients who have inherited a specific *GSK3B* haplotype, consistent with the epistatic interaction between the two genes. **Conclusion:** Our evidence supports the hypothesis that epigenetic modification of *MAPT* plays an important role in neurodegenerative diseases.

ORAL-27-02

ISOFLURANE ANESTHESIA OF YOUNG TAU-P301L MICE PRECIPITATES UPPER AIRWAY DYSFUNCTION

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Introduction: Isoflurane, a commonly used volatile anesthetic, is suspected to hasten tauopathy and post-operative cognitive decline in Alzheimer's Disease (AD) (Baranov et al., 2009). Tau-P301L mice, a possible mouse model for AD, develop cognitive deficits, motor dysfunction, brain tauopathy (7-8 months) and die prematurely (8-12 months) following progressive upper airway dysfunction (Terwel et al., 2005 ; Dutschmann et al., 2010). **Aim:** As isoflurane anesthesia precipitates tauopathy in young Tau-P301L mice (Planel et al., 2009), we examined the effect of isoflurane on upper airway function and dysfunction in young Tau-P301L mice. **Methods:** Double-chamber plethysmography of wild-type and Tau-P301L mice (4 months, n=6 and 13 respectively) for upper airway function prior to and after anesthesia (1.3% isoflurane/ 30% O2, 4 hours)(Planel et al., 2009). **Results:** The 4-months old Tau-P301L mice showed normal upper airway function prior to anesthesia but significant upper airway dysfunction after isoflurane anesthesia, with reduced airflow despite marked increase in respiratory chest movements. The isoflurane deleterious effects on upper airway function were totally prevented by artificial ventilation of Tau-P301L mice during anesthesia, suggesting that the anesthesia-induced hypoventilation contributed to induce upper airway dysfunction in Tau-P301L mice. **Conclusion:** Isoflurane anesthesia precipitates upper airway dysfunction in spontaneously breathing, but not artificially ventilated, young Tau-P301L mice. These data suggest the use of artificial ventilation during volatile anesthesia of patients suspected to suffer a primary or secondary tauopathy.

ORAL-27-03

GENETIC CONTROL OF NEURODEGENERATION BY A CANONICAL HETEROTRIMETIC G PROTEIN SIGNALING PATHWAY IN A C.ELEGANS MODEL OF PARKINSON'S DISEASE

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PURPOSE: Accumulation of the protein alpha-synuclein (α-syn) in dopaminergic neurons is a pathological hallmark of Parkinson's Disease. Signal transduction mechanisms that mediate α-syn induced neurodegeneration are less known. Here we showed that a canonical Heterotrimeric G Protein Signaling Pathway plays a fundamental role in controlling neurodegeneration in an in vivo model of Parkinson's Disease. **METHODS**: Transgenic animals carrying over-expressed human α-syn in dopaminergic neurons were crossed with respective mutants of the Heterotrimeric G Protein Signaling Pathway. Respective mutants (n=90) were analyzed post-embryonically at days 4 and 7 for neurodegeneration in dopaminergic neurons. Treatment of animals (n=90) with phorbol ester (PMA) was used to activate a downstream component of the pathway. **RESULTS**- Overexpression of α-syn in dopaminergic neurons of wild-type animals (n=180) caused age-dependent neurodegeneration (p<0.001). Inactivation of Gqα(EGL-30) at days 4 (n=90; p=0.04) and 7 (n=90; p=0.027) enhanced neurodegeneration. Elimination of Goα (GOA-1) at days 4 (n=90;p=0.019) and 7(n=90, p=0.004) conferred neuroprotection. Inactivation of a negative regulator of Goα increased neurodegeneration (n=90; p=0.003) while inactivation of genes acting downstream of Gqα, *egl-8* (phospholipase Cβ) (n=90; p=0.0009) and *pkc-1* (Protein Kinase C) (n=90; p=0.003) all exacerbated neurodegeneration. Activation of PKC by PMA ameliorated neurodegeneration (n=90; p<0.05) **CONCLUSION**: Our genetic analysis indicates that the Heterotrimeric G Protein Signaling modulates neurodegeneration in vivo. Activation of Gqα is required for neuroprotection against α-syn toxicity. This neuroprotective mechanism partially requires activation of downstream factors such as EGL-8 (phospholipase Cβ) and PKC-1, and is negatively controlled by Goα. Moreover,a negative regulator of Goα positively controls this pathway.

ORAL-27-04

RECIPROCAL INDUCTION OF α-SYNUCLEIN AND AMYLOID-β IN ADULT PRIMARY NEURONS VIA PHOSPHATIDYLINOSITOL 3-KINASE PATHWAY

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Purpose: α-Synclein (αSyn) and amyloid beta peptide (Aβ) are pathogenic proteins responsible for Parkinson's and Alzheimer's neurodegenerations, respectively. Clinical, pathological and genetic observations point to robust interactions between the two proteopathogenic pathways presumably most active in middle aged humans. Yet there is dearth of knowledge of the interactions in relevant cell models. We examined interactions between αSyn and Aβ proteins in primary hippocampal neuron cultures derived from middle-aged rats. **Methods:** Hippocampal neurons were isolated using density gradient centrifugation from Sprague Dawley rats (12 month old, n=8), cultured in Neurobasal A plus fibroblast growth factor for 4 days. Recombinant αSyn or Aβ42 were applied to the cultures for 24 hours. Endogenous αSyn or Aβ40 levels were monitored by ELSA, cytotoxicity by TUNEL, and cell cycle re-entry by cyclin D1 immunostaining. **Results:** Following Aβ42 treatment, both intracellular and secreted αSyn levels were significantly increased. Conversely, αSyn treatment also increased intracellular and secreted Aβ40 levels. Neurons treated with high concentration of Aβ42 (2.5 μM) or αSyn (10 μM) showed significant apoptosis, whereas treatment with lower concentrations of Aβ42 (0.01 to 1.0 μM) or αSyn (0.1 to 2.0 μM) induced the neurons entering into cell cycle indicated by increased cyclin D1 staining. The phosphatidylinositol 3-kinase inhibitor, LY294002, which blocks cell cycle, suppressed αSyn-induced Aβ40 elevation, as well as Aβ42-induced αSyn elevation. **Conclusion:** Aβ42 and αSyn can induce the production of each other, and enhanced cell cycle re-entering through the phosphatidylinositol 3-kinase pathway in primary hippocampal neurons of middle-aged rats.

ORAL-27-05

THE EFFECT OF AGE ON α-SYNUCLEIN PATHOLOGY IN PARKINSON'S DISEASE

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Purpose: Age is the greatest risk factor for Parkinson's disease (PD) and an older age of onset significantly accelerates disease progression so that patients reach similar endpoints and pathological severities at the same age. This suggests that at any time in the course of their disease, PD patients of older onset should have greater α-synuclein pathology because of their more rapid disease progression. To test this we compared the quantity of α-synuclein pathology in the brains of younger versus older onset PD patients. **Methods:** Following study approvals, fixed brain samples were obtained through the Sydney Brain Bank from younger (N=14, average onset 61 years) and older (N=14, >70 years) onset PD cases matched for disease duration (8-16 years). Immunohistochemistry for α-synuclein was performed and the areal fraction of α-synuclein deposition determined in regions stereotypically affected in PD. Stepwise linear regression analysis was performed to determine whether the severity of regional α-synuclein pathologies predicted the onset or duration of PD. **Results:** In contrast to the prediction, increasing severity of α-synuclein deposition in the medial but not lateral regions of the midbrain predicted an earlier rather than later onset of PD (p=0.032), while greater α-synuclein pathology in the amygdala occurred in PD cases with shorter disease durations (p=0.009). **Conclusion:** This data suggests that the severity of α-synuclein pathology in many brain regions is not linearly related to the age of PD onset or disease duration. Further modelling of the influence of age and time on the tissue pathology of PD is required.

ORAL-27-06

SUPPRESSION OF β-AMYLOID PRECURSOR PROTEIN BY NITRIC OXIDE MAY LEAD TO TOXIC NIGRAL IRON ACCUMULATION IN PARKINSONS DISEASE

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Purpose: Parkinsons disease (PD) is typified by toxic iron elevation in the effected Substantia Nigra (SN). We recently demonstrated that the Alzheimers disease (AD) implicated β-Amyloid precursor protein (APP) is a neuronal ferroxidase that facilitates iron export. We revealed a failure of APP in AD contributing to iron accumulation, which prompted investigation of APP in related PD. **Methods:** Human SN from control and PD post mortem brains (n=10 each) were measured for iron content and levels and activity of APP. APP and iron levels were measured in a timecourse after administration of the toxin, MPTP to mice (5 groups, n=10 each). To determine if restoration of APP protected against MPTP toxicity, (1) mice over-expressing APP (Tg2576) (6 groups, n=8 each) and (2) mice treated with neuronal nitric oxide synthase (nNOS) inhibitor, 7-nitroindazole, (4 groups, n=8 each) were intoxicated with MPTP. Nigral iron content was measured to determine if iron accumulation was opposed and nigral cell counts were performed to measure if this protected against toxicity. **Results:** Iron was elevated in PD SN with a coincidental decrease in APP levels and activity. MPTP intoxication caused iron accumulation correlating with APP depression over the timecourse. APP overexpression (Tg2576) protected against iron elevation and toxicity. Elevation in Nitric oxide, which perturbs iron responsive elements (possessed by APP on 5 prime untranslated mRNA), was investigated for the mechanism of APP failure. nNOS elevation resultant from MPTP was inhibited by 7-nitroindazole. This restored APP levels, prevented iron accumulation and protected against toxicity. **Conclusions:** Nitric Oxide elevation in PD is likely upstream in a pathway that causes APP failure, iron elevation and eventual degeneration.

ORAL-27-07

LRRK2 AND α-SYNUCLEIN PROTEIN LEVELS IN PARKINSON'S DISEASE

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Purpose: Mutations in two genes, leucine-rich repeate kinase (Lrrk2) and α-synuclein, cause autosomal-dominant Parkinson's disease (PD) via Lrrk2 dysfunction and increased amounts of α-synuclein, respectively. The purpose of this study was to determine if the levels of these proteins are related in sporadic PD. **Methods:** Frozen brain samples from 10 PD patients and 10 controls were obtained from the Sydney Brain Bank following study approvals. Crude soluble brain proteins were extracted from regions with (amygdala, anterior cingulate) and without (occipital) α-synuclein deposition, and full-length Lrrk2, total and phosphorylated α-synuclein measured by western blotting. Multivariate linear regressions were used to test for PD-related protein associations. **Results:** Multivariate analysis revealed significant regional increases ($p<0.001$) in total (3.2x in amygdala, 1.5x in cingulate) and phosphorylated (>30x in amygdala and cingulate) α-synuclein levels in PD, but no significant change in the levels of full-length Lrrk2 over controls. Posthoc univariate analyses showed that the most substantial increase in PD was in α-synuclein phosphorylation, with the amygdala demonstrating the largest protein changes. Linear regression modelling using values from regions with PD changes revealed that the levels of both full-length Lrrk2 and total α-synuclein could predict PD (β coefficients=0.44 and 0.55 respectively, $p\leq0.001$). The non-significant average 30% increase in Lrrk2 levels in PD were negatively related to α-synuclein levels ($R=-0.49$, $p=0.03$), with PD cases with high α-synuclein levels having control levels of Lrrk2 and vice versa. **Conclusions:** The inverse relationship between increased Lrrk2 and α-synuclein protein levels in PD are consistent with Lrrk2 negatively regulating protein translation (that potentially includes increased α-synuclein expression) in PD[1]. [1]Gehrke et al. Nature 2010;466:637-43.

ORAL-27-08

DISEASE-SPECIFIC DEFICIENCIES IN METABOLIC FUNCTIONS AND STRESS RESPONSES IN OLFACTORY NEUROSPHERE-DERIVED CELLS FROM PARKINSONS DISEASE PATIENTS

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Purpose: To develop an in vitro system to identify molecular and/or environmental mechanisms underlying idiopathic Parkinsons Disease. Ideally the model should reflect functional differences implicated in vivo. **Methods**: We have developed an in vitro cell system derived from human olfactory mucosa biopsies. Following biopsy cells undergo a neurosphering stage, before expansion as adherent cell lines maintained in DMEM/F12 and 10%FCS. Metabolic functions, and stress responses were assayed in 96-well format on a plate reader.**Results**: There was a 16% decrease in glutathione (p=0.016) and 18% decrease in MTS metabolism (p=0.019) in PD-derived hONS cells (n=26 Controls, n=28 PD). There was no difference in other metabolic functions. There was no correlation with age, gender or passage number in culture. The only correlation was with disease status of the donors. Transcriptomic analysis identified the Nrf2 oxidative stress response pathway as the most dysregulated between PD and control hONS. Activation of Nrf2 produced a 10%-20% increase in MTS (p=0.09) and glutathione (p=0.002) levels in the 12 of 14 PD hONS lines. PD hONS cells (n=8) more readily undergo apoptosis than controls (n=8) following exposure to Rotenone (p=0.001), H2O2 (p=0.014) and epoxomicin (p=0.009) but not other stresses. Results repeated in another 8 PD and 8 control hONS lines. **Conclusions**: 1. hONS cells replicate some molecular mechanisms implicated in PD. 2. hONS cells are a valuable in vitro model of PD as they reflect normal human genetic diversity. 3. Nrf2 was identified as a potential therapeutic target for PD. 4. PD hONS cells are more sensitive to stress.

ORAL-28-01

Aβ42-P75 NEUROTROPHIN RECEPTOR DEATH SIGNALLING THROUGH G-PROTEIN ACTIVATED INWARDLY RECTIFYING POTASSIUM (GIRK) CHANNELS: A NEW MECHANISM OF EXCITOTOXIC NEURONAL DEATH?

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The Alzheimer's disease peptide amyloid β (Aβ42) has been shown to induce neuronal death through the p75 neurotrophin receptor (p75NTR). We have previously identified a novel p75NTR-initiated death pathway involving pathological potassium efflux through activation of G-protein activated inwardly rectifying potassium (GIRK) channels. In the present study we investigated the link between these pathways. Using cultured hippocampal neurons we demonstrated that Aβ42 triggered the p75NTR-GIRK channel death pathway. Aβ42 treatment also increased intracellular calcium in synaptically active neurons which resulted in an increase in GIRK channel expression; this correlated with initiation of p75NTR-mediated neuronal death signals. Both calcium influx and GIRK channel upregulation was prevented by blocking NMDA receptors or by activation of GABAb receptors, both of which promoted neuronal survival. We demonstrated in vitro that glutamate also upregulated GIRK channel expression and potentiated p75NTR-induced death in hippocampal neurons. Importantly, GIRK channels were also upregulated in vivo in a seizure model, where p75NTR is known to cause neuronal death, and around amyloid plaques in APP/PS transgenic mice, as assessed via immunohistochemistry and electron microscopy. These data provide evidence that Aβ42 toxicity and p75NTR death signalling intersect at the point of GIRK channels. Our results demonstrate a mechanism of neuronal death where Aβ42 activates p75NTR while simultaneously increasing GIRK channel expression following calcium dysregulation. This upregulation increases the efficiency of the p75NTR-mediated pathological potassium efflux resulting in neuronal death. We propose that this mechanism serves as a general model for the neuronal death pathway triggered by other forms of excitotoxicity.

ORAL-28-02

PLATELET-RELEASED FACTORS (PRFs) ATTENUATE NEURONAL APOPTOSIS

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The exchange of material between blood and the brain parenchyma is tightly regulated by the blood-brain barrier (BBB). In several neuropathologies, such as in stroke and traumatic brain injury (TBI), the BBB becomes compromised allowing extravasation of blood components, such as platelets, into the brain. Platelets are pivotal in processes of inflammation, thrombosis and in wound repair. In these processes, platelets become activated and release a plethora of bioactive platelet-released factors (PRFs). **Purpose:** To investigate the effect of PRFs on neuronal viability which currently, is poorly understood. **Method:** Primary neurons were challenged with injury-mimicking agonists including etoposide, SIN-1, glutamate and OGD, either alone or in the presence of PRFs. The ability of PRFs to modulate the extent of neurotoxicity elicited by each injury agonist was assessed using biochemical assays that measure: 1) cell death, 2) cell viability and 3) caspase 3/7 activity. Western blot analysis for PARP cleavage (an indicator of apoptosis) and evaluation of culture viability *via* fluorescence microscopy were performed accordingly. **Results:** PRFs significantly protected neuronal cultures *in vitro* against pro-apoptotic substances etoposide (n=4, $p<0.05$) and SIN-1 (n=4, $p<0.05$). Conversely, PRFs did not elicit any obvious protective effect on neuronal cultures subjected to glutamate (n=4) or OGD conditions (n=5). **Conclusion:** PRFs provide substantial protection to neuronal cultures challenged with pro-apoptotic reagents etoposide and SIN-1, but not to cultures exposed to glutamate or OGD conditions. Our data suggest that the neuroprotection offered by PRFs is injury-specific and may involve a selective protection against neuronal apoptosis. These novel findings have implications for stroke, TBI and other neurodegenerative conditions where platelet activation, BBB breakdown and neuronal injury coincide.

ORAL-28-03

PIKFYVE INHIBITION AFFECTS AUTOPHAGY IN NEURONS AND PROMOTES NEURONAL CELL DEATH

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Purpose: Phosphoinositides are a family of lipid molecules with diverse roles in cellular processes including cytoskeletal remodelling and membrane trafficking. The phosphoinositide PtdIns(3,5)P_2 is synthesised by the kinase PIKfyve. PIKfyve forms a complex with Fig4 and Vac14. Disruption of this complex reduces PtdIns(3,5)P_2 levels and leads to strong neurodegenerative phenotypes. In Fig4 and Vac14 mutant mice, PtdIns(3,5)P_2 levels are significantly reduced and swollen vacuole-like structures are observed in neurons, the appearance of which is proposed to precede the onset of neurodegeneration. Here we investigate the effect of directly reducing PIKfyve activity on neuronal cell death and autophagic status using the specific PIKfyve inhibitor YM-201636. **Methods:** E18 hippocampal neurons were cultured for 48h prior to incubation with 1 μM YM-201636 or DMSO alone for 22-24h. Neurons were used for survival assays, for immunocytochemistry using sub-cellular markers, or for Western blotting to detect changes in the level of LC3-II, a marker for autophagic activity. **Results:** We demonstrate that YM-201636 treatment causes significant neuronal death that cannot be rescued by the pan-caspase inhibitor Z-VAD-fmk (n=4). YM-201636 treatment promotes the appearance of swollen vacuole-like structures in neuronal cell bodies that can be visualized by light microscopy and are LAMP1 positive (a lysosomal marker). Time-lapse imaging demonstrates that the formation of vacuoles precedes cell death (n=3). By Western blotting, YM-201636 treatment increases LC3-II levels (n=4) in neurons and PC12 cells, a sign of increased autophagic activity. **Conclusion:** Direct inhibition of PIKfyve activity causes death of cultured E18 hippocampal neurons through a caspase-independent mechanism. Since PIKfyve inhibition increases autophagic activity, we are investigating the possibility that cell death occurs through an autophagy-related process.

ORAL-28-04

REMOVING THE DEAD: OLFACTORY ENSHEATHING CELLS PHAGOCYTOSE AXONAL DEBRIS

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Olfactory ensheathing cells (OECs) are the glial cells of the olfactory system. Their primary role is thought to be to provide support and guidance for primary olfactory axons. However, OECs are known to phagocytose bacteria and express immune markers and thus they may help to maintain a healthy environment. Interestingly, following widespread death of primary olfactory axons, there is minimal mobilisation of macrophages but yet the axonal debris is rapidly cleared. **Purpose**: We have therefore investigated whether OECs are the cells that are primarily responsible for removal of axonal debris. **Methods**: We cultured red fluorescent OECs from S100beta-DsRed mice and green fluorescent primary olfactory neurons from OMP-ZsGreen mice. **Results**: In explant cultures of DsRed-OECs and ZsGreen-neurons, OECs clearly contained green fluorescent axonal debris. When cellular debris from green fluorescent neurons was added to cultured OECs, the OECs extended pseudopodia and rapidly phagocytosed the axonal debris (n=5). We examined sections through the olfactory system in healthy animals throughout development (E15 to adult, n=5 animals at each time point) and found that with increasing age OECs contained increasing levels of axonal debris. Following degeneration of olfactory neurons by injection of methimazole, OECs had significantly more axonal debris (30-50% more, n=5, $p<0.005$) compared to controls. In comparison, macrophages within the olfactory system did not display increased levels of axonal debris, indicating that OECs, rather than macrophages, are the cells that are primarily responsible for removal of axonal debris. **Conclusion**: These results clearly demonstrate that OECs actively phagocytose cellular debris and thus is another mechanism by which they maintain the health of the olfactory system.

ORAL-28-05

TRAUMATIC BRAIN INJURY (TBI) STIMULATES PRODUCTION OF NEW IMMATURE NEURONS, BUT THEIR SURVIVAL MAY REQUIRE EXOGENOUS NEUROTROPHIC SUPPORT

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Purpose: Neurogenesis is stimulated by experimental TBI, and provides a compelling target for novel therapies to improve recovery following trauma. In this study, we characterised the neurogenic response in a closed head injury (CHI) model of focal TBI, then attempted to augment specific regulatory stages with growth-factor treatment. **Methods:** Adult C57BL/6 mice were subjected to CHI or sham-operation and BrdU was administered (200mg/kg 2xdaily, d1-4 post-CHI) to label proliferating cells. For treatment studies, mice were administered EPO (d0-11 post-CHI) and/or BDNF (d7-11 post-CHI). Following twice-weekly functional assessment, brains were collected at 1,2,4 and 8w post-CHI (n=4-5), or at 6w for treatment studies (n=4). **Results:** In the dentate gyrus (DG) and subventricular zone (SVZ), the number of BrdU+cells increased after CHI by 3-fold ($P<0.001$) and 2-fold ($P<0.05$), respectively. Immature neurons detected by DCX-immunolabelling increased by 8-fold at 1w post-CHI in the DG ($P<0.001$), and an abundance of DCX+cells was observed in the pericontusional-cortex at 1 and 2w. However, assessment of neuronal maturation/survival by BrdU/NeuN immunofluorescence at 4 and 8w, revealed no difference in the number of new neurons in the DG after trauma, and no new neurons were detected in the pericontusional-cortex. Interestingly, treatment with EPO+BDNF improved behavioral ($P<0.001$) and motor ($P=0.002$) outcomes of CHI mice compared to vehicle controls. Ongoing analysis will determine whether this increased neurological recovery is related to augmented neuronal production. **Conclusion:** These data suggest that while early stages of neurogenesis are stimulated by TBI, therapies to enhance the maturation and survival of new neurons may be an effective strategy to increase ultimate neuronal production.

ORAL-28-06

METHIONINE-93 IS REQUIRED FOR LIPID ANTIOXIDANT FUNCTION OF APOLIPOPROTEIN-D

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Purpose: Apolipoprotein-D (apoD) expression is upregulated with age, in Alzheimer's disease and upon oxidative stress. Recent studies indicate apoD inhibits lipid peroxidation in the brain. We aimed to elucidate the basis of this lipid antioxidant function. Previous studies indicate specific Met residues of plasma apoA-I inhibit lipid oxidation via direct reduction of radical-propagating lipid hydroperoxides (LOOH) to non-reactive hydroxides (LOH); with concomitant formation of Met sulphoxide (MetSO). Since apoD has three conserved Met residues (amino acids 49, 93, 157), we hypothesised that they may similarly prevent lipid peroxidation. **Methods:** We generated recombinant apoD and mutants in which either one ($apoD_{M49\text{-}A}$, $apoD_{M93\text{-}A}$, $apoD_{M157\text{-}A}$) or all ($apoD_{M\text{-}A}$) of the Met residues were replaced by Ala. The recombinant proteins were incubated with eicosatetraenoic acid-derived lipid hydroperoxides (5sHpETE, 12sHpETE, 15sHpETE) or cholesteryl linoleate hydroperoxide (CLOOH). The conversion of apoD Met to Met-SO and LOOH to LOH was investigated using HPLC. **Results:** Incubation of apoD and $apoD_{M49\text{-}A}$, $apoD_{M157\text{-}A}$ mutants with HpETEs induced a shift in apoD retention time (RT) consistent with apoD MetSO formation. HPLC analysis also revealed the conversion of all HpETEs to their corresponding hydroxy (H)ETEs. Importantly, there was no shift in RT for $apoD_{M\text{-}A}$ or $apoD_{M93\text{-}A}$, a result associated with insignificant conversion of HpETE to HETE. There was no change in RT for either apoD or any of the mutants with CLOOH treatment and also no conversion of CLOOH to CLOH. **Conclusions:** Met-93 is essential for L-OOH reduction by apoD. ApoD converts HpETE to HETE but has no effect on bulkier lipid hydroperoxides such as CLOOH.

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OXIDATIVE STRESS AND NEURODEGENERATIVE CELL DEATH: PRIMARY CORTICAL NEURONS UNDERGO DIVERSE CASPASE-INDEPENDENT CELL DEATH PATHWAYS

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Neuronal cells can undergo a diverse range of death responses. These death outcomes were previously thought to be limited to either apoptosis (involving caspases and energy-dependent) or unregulated necrosis (independent of both caspases and the need for ATP). Here, we show that primary cortical neurons from C57 Black 6J mice exposed to oxidative stress are capable of undergoing diverse forms of cell death, highly regulated yet caspase-independent. Acute insult of hydrogen peroxide (H_2O_2) was shown to initiate cell death with hallmarks of necrosis, such as rapid loss of plasma membrane integrity, changes in nuclear morphology (but with little fragmentation characteristic of apoptosis) as well as rapid mitochondrial depolarization and redistribution of intermembrane space proteins (cytochrome c, Smac, AIF and endonuclease G). Through siRNA experiments, we demonstrated that the cell death triggered by H_2O_2 was programmed necrosis that is directly dependent on Endo G. Further investigations also showed that autophagic cell death involving Beclin-1 and Atg7 is also activated under acute H_2O_2 conditions (specific siRNA knockdown blocked cell death). Under a more chronic oxidative stressor, superoxide ($O_2^{\cdot-}$; provided by exogenous xanthine oxidase in the presence of catalase, acting as a sink for H_2O_2), knockdown of either Endo G (programmed necrosis) or Atg7 (autophagic cell death) using siRNA was much less potent in blocking cell death induced by chronic $O_2^{\cdot-}$. Therefore, oxidative stress invokes a diverse death response that involves some autophagic cell death and programmed necrosis involving mitochondria, but under chronic oxidative conditions unregulated necrosis predominantly results.

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DEFICIENCY IN COMPLEMENT ANAPHYLATOXIN RECEPTORS C3AR AND CD88 WORSENS THE OUTCOME FROM SPINAL CORD INJURY IN MICE

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Purpose: Neuronal death and axonal degeneration at sites of spinal cord injury (SCI) are inevitably linked to neuroinflammation. The complement system is thought to be a major contributor to the innate immune response following SCI but the role of its individual components in the developing neuropathology remains ambiguous. Here, we explored the role of the complement anaphylatoxin receptors, C3aR and CD88, on neuropathology and functional recovery following contusive SCI in mice. **Methods:** Wild-type (n=13), $CD88^{-/-}$ (n=12) and $C3aR^{-/-}$ (n=13) mice on a C57BL6/J background were subjected to moderate-severe contusive SCI using the Infinite Horizon impactor device. Open-field locomotor scoring was used to monitor functional recovery up to 35 days post-injury. Magnetic resonance imaging (MRI), together with standard histological and immunofluorescent staining procedures, was used to compare histopathology between genotypes. **Results:** $C3aR^{-/-}$ mice showed significantly worsened recovery from SCI compared to their wild-type counterparts as evidenced by consistently poorer locomotor performance. These findings were paralleled by significantly greater lesion volumes as determined by MRI, reduced white matter sparing, and increased presence of $Ly6B.2^{+}$ neutrophils / inflammatory monocytes. In contrast, $CD88^{-/-}$ mice displayed a more intermediate and delayed phenotype that only became functionally apparent at 35 days post-injury. **Conclusion:** Deficiency in complement receptors, particularly C3aR, worsened the outcome from SCI. These findings suggest that signalling through these receptors via their anaphylatoxin ligands, C3a and C5a, has modulating rather than detrimental roles on the inflammatory processes that take place in the acute and/or sub-chronic stages following SCI.