ORAL SESSIONS

ORAL-01-01

NOVEL ASPECTS OF *PRESENILIN1* BIOLOGY REVEALED BY ANALYSIS IN ZEBRAFISH EMBRYOS

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The *PRESENILIN1 (PSEN1)* gene is the major locus for dominant mutations causing inherited early onset Alzheimer's disease (AD). Mutations in this gene can also cause frontotemporal dementia (FTD). Fertilized zebrafish eggs represent a macroscopic cell that can be subtly manipulated to increase or decrease the expression of multiple genes simultaneously in a physiologically-relevant manner and to alter transcript splicing etc. The results of manipulation can be observed by quantifying changes in embryo development and/or by molecular biological analysis. **Purpose**: We aim to understand the central role of *PSEN1* in AD pathology by investigating the molecular biology of normal and mutant forms of psen1 (the zebrafish orthologue of human *PSEN1*) in zebrafish embryos. **Results**: 1) In recently published work we showed that disruption of *PSEN1* transcript splicing can have potent dominant negative effects on the function of *PSEN1* and the related gene *PSEN2*. We hypothesise that aberrant splicing of *PSEN1* in ageing neural cells may contribute to sporadic AD. 2) In unpublished work we have evidence that partial disruption of *PSEN1* splicing can result in compensatory increases in the stability of *PSEN1* protein to maintain normal *PSEN1* levels. 3) We have disrupted zebrafish psen1 splicing in a similar manner to that seen for a Picks disease (FTD) mutation of *PSEN1*. Contrary to expectations we see an apparent increase in Notch signalling. This raises the possibility that the AD and FTD mutations in *PSEN1* may be distinguished by decreased and increased γ-secretase activity respectively.

ORAL-01-02

DECREASED EXPRESSION OF GOLGI-ASSOCIATED GGA1 PROTEIN IN ALZHEIMER'S DISEASE

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Alzheimer's disease Aβ amyloid peptide is derived from the amyloid precursor protein by sequential cleavages by BACE and gamma-secretase. Our previous work has shown that BACE protein is increased in frontal cortex of patients with sporadic AD compared to age-matched healthy controls and neurological controls. No change in mRNA expression was observed, suggesting the cellular trafficking of BACE or/and its metabolism may be altered in AD. Among the proteins known to interact with BACE, the Golgi-associated GGA were shown to control the trafficking of BACE and its recycling from endosomes to Trans-Golgi network. Their expression may thus affect BACE cellular levels. **Purpose:** To study the expression of GGA proteins in AD and control brain, and investigate a possible correlation between GGA and BACE expression. **Methods:** Frontal cortex samples from AD patients (N=20) and from age-matched controls (N=20) were homogenized with Trizol and the protein analysed by western blotting for BACE, GGA1 and GGA3. Signals were captured and quantified with a Syngene DCC instrument. The data were normalized to the neuronal marker β-tubulin III. **Results:** An overall 1.2 fold increase in BACE 70 kDa protein was observed in AD compared to controls. AD cases could be subdivided in two subpopulations that may correspond to different stages of the disease, 30 % showing decreased BACE levels compared to average controls, and 70% having high to very high BACE levels. GGA1 was decreased in the AD group whereas GGA3 was unchanged. Statistical analysis of the results is in progress to determine if there exists an inverse correlation between GGA1 and BACE expression. **Conclusion:** This is the first report of a decrease in GGA1 expression in AD brain. This finding suggests that membrane protein trafficking is impaired in AD and may result in mis-targeting of BACE, leading to increased Aβ production.

ORAL-01-03

AMYLOID-β AND HUMAN AMYLIN SHARE COMMON NEUROTOXIC MECHANISMS

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Purpose: Type 2 Diabetes Mellitus (DM) and Alzheimer's disease (AD) share epidemiological and biochemical features. Specifically, both diseases are amyloidoses or conformational diseases as they are characterized by insoluble protein aggregates with a fibrillar conformation - amylin in Type 2 diabetes pancreatic islets, and Aβ amyloid and tau-containing neurofibrillary tangles in Alzheimer's brains. Amylin aggregation is associated with pancreatic β-cell loss, whereas Aβ and tangle formation is associated with neuronal cell loss. Epidemiological studies establish a link between the two diseases, and more importantly, they share clinical and biochemical features suggesting common pathogenic mechanisms. **Methods:** To investigate the pathomechanisms between DM and AD, primary murine cultures (n=5) and SH-SY5Y human neuroblastomas (n=4) were incubated with Aβ42, human amylin, and the non-amyloidogenic rat amylin. **Results:** Aβ42 and human amylin were found to cause a dose-, time- and cell type specific neurotoxicity, which was not observed with incubations with rat amylin. Co-incubation of Aβ42 and human amylin did not produce an additive neurotoxic effect, suggesting common pathogenic pathways. Aβ42 and human amylin were found to exert similar mitochondrial dysfunction, which was not observed with treatments with rat amylin. Proteomics approaches revealed several interesting proteins that are deregulated by both human amylin and Aβ42. **Conclusion:** Collectively, the results suggest a possible common patho-mechanism in the pathogenesis of DM2 and AD.

ORAL-01-04

REGIONAL POST-SYNAPTIC SCAFFOLD PROTEIN LOSS IN AD

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Glutamate-evoked excitotoxicity, possibly from the over-stimulation of NMDA receptors, may contribute to the regional loss of glutametergic synapses observed in Alzheimer's disease (AD). NMDA receptor expression and activity at the synapse is highly regulated through binding to the post-synaptic scaffold proteins PSD-95 and SAP-102. This study looked for changes in PSD-95 and SAP-102 mRNA and protein levels in human autopsy brain tissue that could contribute to NMDA receptor subunit loss in AD. Using absolute quantification Real-Time PCR, we detected reduced expression, albiet not significant, in PSD-95 and SAP-102 mRNA transcripts in the pathologically susceptible inferior temporal cortex (ITC), but not the hippocampus (HIP), of AD cases (n=13) compared with controls (n=12). In contrast, the pre-synaptic protein synaptophysin was markedly less abundant in both ITC (P=0.019) and HIP (P=0.10), consistent with previous reports. No difference was observed for any protein in the occipital cortex, a region spared from marked cell loss, between AD cases and controls. Proteins were precisely quantified against recombinant truncated protein standards. PSD-95 and SAP-102 protein expression showed similar trends to the mRNA data but were now significantly lower in the ITC (P=0.028) and (P=0.0017) respectively, between AD cases (n=15) and controls (n=15). Our data suggest a possible mechanism for the reduction in NMDA receptor subunit expression in AD ITC. The lack of reduction in scaffolding-component mRNA and protein in HIP, an area affected early in the disease, may reflect a compensatory mechanism driving post-synaptic neurones to search for new synaptic contacts following extensive neuronal death. This research provides further understanding of the excitotoxic pathology of AD at the molecular level.

ORAL-01-05

ALPHA-SYNUCLEIN AND BETA-AMYLOID INTERACTION IN ADULT NEURONS: ROLE OF CELL CYCLE IN PRODUCING AMYLOID AND NON-AMYLOID PEPTIDES IN ALZHEIMER'S DISEASE

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Introduction: In spite of familial Alzheimer's Disease (AD), the causes of Aβ (amyloid plaque component) and α-synuclein (non-amyloid plaque component) elevation in sporadic AD are unknown. Increasing those proteins perform through enhancement of Amyloid Precursor Protein (APP) or its processing and α-synuclein expression. Also Aβ induces its own production in smooth muscle and neurons. Because APP and α-synuclein undergo cell cycle-dependent phosphorylation, so if Aβ and α-synuclein trigger cell cycle re-entry, APP cleaving and α-synuclein expression will be elevated. **Purpose:** The objective was to determine if Aβ42 and α-synuclein increase α-synuclein and Aβ via cell cycle re-activation. **Method:** We used hippocampal neuronal culture of 10-12 months rats. To find the toxic concentrations by TUNEL, we used 2×10□6, 2×10□5, 2×10□4, 0.01, 0.1, 1 and 2.5 μM of Aβ42 and 0.1, 1, 5 and 10 μM of α-synuclein. To determine Aβ and α-synuclein effect on each other's production by ELISA and on cell cycle re-entry by immunocytochemistry for Cyclin D1, we used 0.01, 0.1, 1 μM of Aβ42 and 0.1, 1, 2, 4 μM of α-synuclein. **Results:** The toxic concentrations for Aβ and α-synuclein were 2.5 and 10μM. 1 μM Aβ increased α-synuclein and 2 and 4 μM α-synuclein enhanced Aβ. 0.01, 0.1, 1 μM Aβ and 0.1, 1 and 2 μM of α-synuclein induced cell cycle re-entry. **Conclusion:** Aβ and α-synuclein toxic concentrations in adult neurons were lower than in embryonic neurons. Aβ and α-synuclein elevate each others and induced cell cycle re-entry which could be one of the mechanisms in increasing amyloid and non-amyloid proteins in AD.

ORAL-01-06

MITOCHONDRIAL DYSFUNCTION TRIGGERS NEURONAL ALZHEIMER-LIKE CYTOSKELETAL STRIATIONS CONTAINING PHOSPHORYLATED MICROTUBULE-ASSOCIATED PROTEIN TAU AND ACTIN

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Hyperphosphorylation and accumulation of the microtubule-associated protein tau into striated neuropil threads is an early neuropathological feature of Alzheimer's disease (AD). Similarly, intraneuronal aggregates of actin are also commonly identified in neurites and cell soma of AD brains. Although the mechanisms involved in formation of these structures remains poorly understood, they are directly correlated to cognitive decline and disease progression. Moreover, as with many other age-related diseases, AD is associated with mitochondrial dysfunction and cellular energy deprivation, the understanding of which may give clues to the disease pathogenesis. **Results:** Here we demonstrate that inducing mitochondrial dysfunction and energy deprivation in primary chicken neuron cell cultures causes the rapid accumulation of phosphorylated tau into striated rod-like structures along neurites. These rods bear a striking resemblance to the tau-positive neuropil threads observed in postmortem AD brains. Upon further investigation of our primary cultures, we found the striated inclusions to also contain actin. **Conclusion:** Taken together, these observations show that rod-like phosphorylated tau and actin accumulations are linked and suggest that classic tau- and actin-neuropathologies could be directly caused by mitochondrial dysfunction as an early triggering event in AD neurodegeneration.

ORAL-01-07

AGE-RELATED REGULATION OF APOLIPOPROTEIN-D EXPRESSION IN HUMAN PREFRONTAL CORTEX

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Purpose: Apolipoprotein D (apoD) is a lipid binding protein that is highly expressed in the central nervous system, however, its function in the brain remains largely unknown. **Methods**: Based on changes in lipid metabolism and deposition that occur in the human brain during postnatal development, we investigated potential changes in apoD expression in the prefrontal cortex in a cohort of 69 normal cases ranging in age from 40 days to 49 years utilising gene microarray, quantitative PCR and western blotting methods. **Results**: In contrast to the expression of APOE, LRP8 and HMGCR (genes that are thought to play a role in lipid related pathways in human brain development) apoD expression was very low in neonates and infants and increased in expression throughout life resulting in 6- to 8-fold higher levels at the mRNA and protein levels in adults compared to neonates. Recent studies suggest that apoD may have a novel antioxidant function in the brain and we found that the increased apoD expression throughout development and into adulthood was significantly correlated with the expression of antioxidant genes SOD1 and GPX3 as well as proteins that were modified by the aldehydic lipid peroxidation end-product 4-hydroxynonenal. **Conclusions**: These studies show for the first time that apoD expression is increased age-dependently in the human prefrontal cortex and that this is correlated with genetic and biochemical markers of oxidative stress.

ORAL-01-08

NEUROPROTECTION INDUCED BY COPPER-COMPLEXES

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Purpose: Neurodegenerative illnesses such as Alzheimer's disease (AD), Parkinson's disease (PD), familial amyotrophic lateral sclerosis (ALS) and prion diseases are characterized by altered biometal metabolism. This has led to the development of novel therapeutic strategies designed to modulate metal metabolism in the brain and restore biometal homeostasis. However, little is known about the consequent effects associated with altering metal metabolism in the brain. We have investigated the effect of lipid soluble copper-complexes on neuronal survival and function in cell culture and animal models of neurodegeneration. **Methods:** Neuronal cell cultures and murine models of AD, PD, ALS and prion disease have been treated with copper-bis(thiosemicarbazone) metal complexes (Cu-GTSM and Cu-ATSM) (n = 4-10 per treatment group). **Results:** The copper complexes increase neuronal metal uptake and stimulate activation of cell signaling involving phosphoinositol-3-kinase (PI3K) and down-regulation of the critical regulatory kinase, glycogen synthase kinase 3β (GSK3β). Modulation of this pathway by copper complexes resulted in remarkable neuroprotective effects. The copper complexes inhibited amyloid beta levels and tau phosphorylation in cell culture and an animal model of AD (APP/PS1). The complexes also inhibited dopamine neurotoxicity both in vitro and in vivo and have ameliorated disease symptoms and extended lifespan in a murine model of ALS. Furthermore, the complexes have reduced prion protein expression in neurons and are being tested in an animal model of human prion disease. The neuroprotective processes are currently under investigation but our initial studies have identified robust positive effects of copper-complexes on synaptic-associated functions including increased expression of synaptic proteins and improvements in long-term potentiation in situ and memory function in vivo. We are continuing to map the neuroprotective activity of copper-complexes in a range of cell and animal models of neurodegeneration. **Conclusions:** Our studies provide the first evidence that low doses of copper delivered to neurons can stimulate neuroprotective pathways that may provide the basis for therapeutic treatments for a number of neurodegenerative illnesses.

ORAL-02-01

ACTIVATION OF BRAIN REGIONS IN RESPONSE TO AIRPUFF

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Purpose: Acute psychological stress, such as airpuff stress, increases blood pressure and heart rate. This study used Fos expression to investigate which autonomic brain regions may mediate this response, with particular reference to monoamine neurons in the medulla. **Method:** A series of airpuffs was applied to male Sprague-Dawley rats for 30 min (~ 60 puffs total, n=5). Rats in the control group did not receive airpuffs (n=4). Two hours later, the rats were deeply anaesthetised and perfused, and the brains were immuno-processed for Fos [or Fos double labelled with PNMT (adrenaline), TH (noradrenaline) and 5-HT (serotonin)]. **Results:** There were significant increases ($p<0.05$) in the number of Fos-positive neurons in the caudal ventrolateral medulla and nucleus of the solitary tract in rats subjected to airpuff. In particular, approximately 15 and 20%, respectively, of TH-positive neurons in these regions (A1 and A2 neurons) were also Fos-positive. There was also a significant ($p<0.05$) increase in Fos expression in the ventral part of the rostral medulla, but the Fos-positive neurons were located mainly medially in the parapyramidal region and were not double labelled with PNMT, TH or 5-HT. Fos expression also increased significantly ($P<0.05$) in the defence-related regions of the midbrain periaqueductal grey, the paraventricular, dorsomedial, perifornical and lateral hypothalamus, and the locus coeruleus. **Conclusions:** In contrast to sympathoexcitatory responses to challenges such as hypotension or haemorrhage, the pressor response to airpuff stress does not appear to be associated with activation of adrenaline neurons (C1 neurons) or serotonin neurons in the rostral ventrolateral medulla. The stress-evoked pressor response could be mediated by other sympathetic premotor neurons within the medullary parapyramidal region or in the hypothalamus.

ORAL-02-02

CUTANEOUS VASOMOTOR ALERTING RESPONSES (SCVARS) ARE ASSOCIATED WITH HIPPOCAMPAL THETA RHYTHM IN NON-MOVING CONSCIOUS RATS

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Purpose: In rabbits, alerting-related reduction of thermoregulatory cutaneous blood flow (i.e. SCVARs) is accompanied by theta rhythm (5-8Hz) in the hippocampus, an EEG marker of alerting reactions. The present study determined the relation between SCVARs and hippocampal theta in male Sprague Dawley rats. **Methods**: SCVARs were measured by a Doppler ultrasonic flow probe chronically implanted subcutaneously around the base of the tail artery[1]. Unipolar electrodes were implanted in the hippocampus (CA1 region) to measure EEG. Rat activity was monitored with a webcam. Six standard alerting stimuli were administered[1] during continuous recording of tail blood flow and EEG. Stimuli were given only when the rats were motionless and theta was absent. The SCVAR index was calculated as a percentage fall from pre-alerting blood flow values using both mean blood flow and mean pulse amplitude as parameters[1]. **Results**: Alerting stimuli produced SCVARs as previously reported[1]. The proportion of theta power in the total frequency range (0-20 Hz) increased significantly after alerting stimuli (46 ± 1 % vs. 29 ± 1% before the stimuli, $p< 0.0001$, 34 stimuli), while the rats remained immobile. Theta proportion began to increase approximately 0.5 s after the stimuli, and preceded SCVARs by approximately 1 s. The SCVAR index was correlated with the magnitude of the increment in theta proportion (significant linear regression, $p= 0.02$, $r^2=0.163$, n=7 rats). **Conclusion**: Our study demonstrates that alerting responses resulting in selective vasoconstriction of the tail vascular bed are associated with hippocampal theta rhythm in conscious rats. (1) Blessing, WW, Psychopharmacology (Berl) 181(3):518-28, 2005).

ORAL-02-03

ACTIVATION OF 5-HT1A RECEPTORS PREVENTS CARDIAC ARRHYTHMIAS AND ATTENUATES TACHYCARDIA DURING SOCIAL STRESS IN RATS

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Purpose: To apply a behavioral stress paradigm for studying the neural mechanisms underlying stress-induced arrhythmias, and to test whether such arrhythmias could be suppressed by systemic administration of 8-OH-DPAT, a 5-HT1A agonist possessing central sympatholytic properties. Methods: The study was conducted on adult male rats (n=19) instrumented for telemetric recordings of ECG, body temperature and locomotor activity. In the first experiment, rats were subjected to social defeat after either 8-OH-DPAT (100 μg/kg s.c.) or vehicle. In the second experiment, prior to vehicle/8-OH-DPAT administration, animals were pre-treated with zatebradine, a blocker of the pacemaker current. Results: 8-OH-DPAT caused prolongation of basal RR interval, increase in locomotion and hypothermia. Subjecting vehicle-treated animals to social defeat caused shortening in RR interval, increase in locomotor activity and hyperthermia, and provoked the occurrence of premature ventricular and supraventricular beats; all these effects were substantially attenuated by 8-OH-DPAT. Zatebradine caused prolongation of RR interval. In zatebradine/vehicle-treated rats, the incidence of ventricular and supraventricular premature beats during defeat increased 2.5-fold and 3.5-fold, respectively. 8-OH-DPAT administered after zatebradine significantly reduced these stress-induced arrhythmias. Conclusions: i) Pharmacologically induced prolongation of RR interval may contribute to an increased susceptibility to stress-induced cardiac arrhythmias, possibly due to the prolongation of the ventricular diastolic period with restored excitability; and ii) Systemic administration of 8-OH-DPAT abolishes these arrhythmic events, likely by suppressing stress-induced cardiac sympathetic outflow.

ORAL-02-04

ANGIOTENSIN TYPE 1A RECEPTOR KNOCKOUT MICE SHOW NO BLOOD PRESSURE RESPONSE TO MICROINJECTION OF ANGIOTENSIN II IN THE ROSTRAL VENTROLATERAL MEDULLA

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Brain angiotensin (AngII) regulates several functions, including cardiovascular function and fluid homeostasis. The rostral ventrolateral medulla (RVLM) expresses AngII receptors and is one site where AngII acts to regulate blood pressure (BP). Studies in neonatal in vitro preparations indicate that AngII-mediated excitation of RVLM neurons is dependent upon the angiotensin type 1A (AT_{1A}) receptor. In the absence of AT_{1A} receptors, AngII hyperpolarizes RVLM neurons via the AT_2 receptor. Purpose: This present study aim to examine the cardiovascular responses to AngII in the RVLM of adult mice in vivo with targeted deletion of AT_{1A} receptors. Methods: AT_{1A} receptor knockout ($AT_{1A}^{-/-}$, n=6) and wild-type ($AT_{1A}^{+/+}$, n=6) mice were anesthetized with urethane (0.75mg/g, IP) and chloralose (0.05mg/g, IP). Mice were tracheotomized, BP and heart rate (HR) were monitored. Glutamate (10mM, 10nl) and AngII (1mM, 50nl) were microinjected unilaterally into the RVLM and cardiovascular responses recorded. Results: Resting BP in $AT_{1A}^{-/-}$ mice (43±3mmHg,) was lower than in $AT_{1A}^{+/+}$ mice (55±5mmHg) and HR levels were similar. Microinjection of glutamate produced a similar pressor response in both groups. Microinjection of AngII into the RVLM increased BP by 10±3 mmHg in $AT_{1A}^{+/+}$ mice and had no effect in $AT_{1A}^{-/-}$ mice (+0±1mmHg). AngII microinjections caused no change in HR in either $AT_{1A}^{-/-}$ (-1±5bpm) or $AT_{1A}^{+/+}$ (+0±1bpm) mice. Conclusion: These results indicate that the AT_{1A} receptor is the main AngII receptor subtype responsible for the AngII induced pressor response in the RVLM and provide no support for a role of the AT_2 receptor in the adult mouse.

ORAL-02-05

ROLE OF CAMP IN CARDIOVASCULAR REGULATION OF THE RAT BRAIN STEM

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Purpose: The role ionotropic receptors play in regulating the cardiovascular regions of the rat brainstem is well understood. In contrast, the role GPCRs and, in particular, their signalling transduction proteins play in regulating neuronal activity in the rostral and caudal ventrolateral medulla is poorly understood. The aim of this study was to determine the importance of cAMP/PKA in the tonic and reflex regulation of blood pressure. **Methods**: Adult male Sprague Dawley rats (n=4) anaesthetized with urethane (1.3g/kg; ip) were ventilated, vagotomised and paralyzed. Splanchnic sympathetic nerve activity (sSNA) was recorded. Heart rate (HR) was derived from ECG and the femoral artery was cannulated for arterial pressure (AP) measurement. Drugs were microinjected into the ventrolateral medulla. Baroreceptor and somatosympathetic reflex function was tested. **Results**: Sp-cAMPS (Sp-Adenosine-3'5'-cyclic monophosphorothioate) a cAMP analog and activator of protein kinase A (0.5, 1.5, 5nmol) evoked a dose dependent increase in the sSNA, HR, BP n the RVLM. The highest dose elicited an increase of 55±13 mmHg in MAP, 115±19 % sSNA, and 31±2 beats per min. The response returned to baseline within 60-70 min. Subsequent similar doses evoked responses of equal magnitude indicating tachyphylaxis does not occur. **Conclusion**: Activation of PKA in the rostral ventrolateral medulla elicits a profound sympathoexcitation and pressor response. These findings indicate that cAMP/PKA pathways play an important role in regulating the activity of neurons in this area. Whether or not the cAMP/PKA pathway is tonically active in the RVLM is currently being explored.

ORAL-02-06

THE ROLE OF Gαi/O PROTEINS IN THE ROSTRAL VENTROLATERAL MEDULLA IN NORMOTENSION AND HYPERTENSION

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Purpose: The rostral ventrolateral medulla (RVLM) generates vasomotor tone and sets the level of blood pressure (BP). There is some evidence to suggest that the elevated sympathetic tone characteristic in all forms of hypertension is due increased activity of presympathetic neurons in the RVLM. Methods and Results: We tested the hypothesis that increased sympathetic tone in hypertension was due to differences in the activation Gαi/o proteins, within the RVLM. We compared the autonomic responses (MAP, HR and temperature) evoked by 8-OHDPAT or oxotremorine in conscious telemetered SHR (n=9) and WKY (n=10). The cardiovascular responses to oxotremorine and 8-OHDPAT are sympathetically mediated and evoked predominantly in the RVLM. SHR exhibited a dose related increase in the duration and degree of depressor ($p \leq <0.01$) and bradycardic ($p \leq <0.01$) response to 8-OHDPAT compared with WKY. The tachycardic ($p \leq <0.001$) response to oxotremorine was also enhanced in SHR. In light of this, we tested the effect of inhibiting Gi/o proteins, using pertussis toxin, in the RVLM on resting BP. In SHR (n=3), microinjection of pertussis toxin into the RVLM had no effect on resting level of BP at any time point following pertussis toxin. The cardiovascular responses to 8-OHDPAT (1mg/kg) were normal at day 2 following pertussis toxin but diminished over the following 4 day period with no cardiovascular response to oxotremorine (0.2mg/kg) or 8-OHDPAT able to evoked by day 6-8. Conclusion: Activation of 5HT1A and muscarinic receptors linked to Gi proteins evoked enhanced responses in SHR versus WKY. These effects were blocked by inhibition of Gi proteins in the RVLM. Blockade of Gi proteins in the RVLM does not alter BP in SHR.

ORAL-02-07

CELLULAR SOURCES OF ANGIOTENSIN II – INDUCED SUPEROXIDE PRODUCTION IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM)

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Purpose: Brain angiotensin II (ANGII)–induced superoxide production is implicated in the central regulation of cardiovascular function. However, cellular sources of superoxide released in response to increased ANGII concentration in the brain remain elusive. Using living rat brain slices, we examined the role of neuronal, glial and perivascular cells in superoxide generation evoked by ANGII in the RVLM, an area critical for cardiovascular regulation. **Methods:** Slices containing the RVLM were pre-incubated with a lectin-conjugated fluorescent marker to visualize blood vessels and perivascular cells. Real-time superoxide production within individual cells was detected using a superoxide-sensitive fluorescent dye dihydroethidium (DHE) and time-lapse confocal microscopy. **Results:** Following DHE (10 μM, n=7) superfusion, strong fluorescence was observed in microvascular pericytes and some but not all neurons within the RVLM. By contrast, little fluorescence was detected in cells with astroglia-like morphology. Application of ANGII (1 μM, n=7) rapidly increased superoxide levels by ~40% in neurons and pericytes, and also initiated robust superoxide production in astroglia (that reached the level observed in neurons). Application of a superoxide dismutase (SOD) mimetic tempol (100 μM, n=5) attenuated ANGII-induced increase in DHE oxidation in all cell types. Application of a SOD inhibitor (DETCA; 1-10 mM) dose-dependently increased DHE oxidation in the RVLM by up to 3-fold. This effect was mostly mediated by increases in superoxide production in astroglia. **Conclusion:** Pericytes and neurons are major sources of superoxide in RVLM slices under basal conditions, whereas glial contribution is less essential. However, all these cell types, and particularly astroglia, may contribute to pro-oxidative effect of ANGII in the RVLM.

ORAL-02-08

LACK OF DETECTABLE CGMP IN THE RAT ROSTRAL VENTROLATERAL MEDULLA

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It is well documented that the premotor sympathetic neurons in the RVLM are critical to the maintenance and control of blood pressure and that endogenous NO is an important mediator in this regulatory process. Functional studies provide strong evidence that NO acts through the soluble guanylate cyclase (sGC)-cGMP signalling pathway in the RVLM to drive sympathoexcitatory responses. Furthermore, there is evidence to support perturbations in NO and cGMP signalling in association with hypertension. We examined cGMP expression as a marker of active NO signalling in the C1 region of the RVLM, comparing Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Double label immunohistochemistry for cGMP-immunoreactivity (IR) and NO synthase (NOS) or C1 neurons (as identified by phenylethanolamine N-methyltransferase (PNMT-IR) or tyrosine hydroxylase TH-IR) failed to reveal cGMP-IR neurons in the RVLM of either strain, despite consistent detection of cGMP-IR in the nucleus ambiguous and vasculature (n = 8). This was unchanged in the presence of the phosphodiesterase (PDE) inhibitor isobutylmethylxanthine (IBMX, n=6) or the use of specific stimulators of cGMP synthesis in slice preparations (DETA-NO, NMDA and sodium nitroprusside (SNP) (n=10). sGC-IR was found throughout neurones of the RVLM, but did not co-localise with PNMT, TH or NOS-IR neurons (n=6). Overall, results indicate that within the RVLM, cGMP is not detectable using immunohistochemistry in the basal state and cannot be elicited by phosphodiesterase inhibition, NMDA receptor stimulation or NO donor application. The sGC-IR results indicate the capacity for cGMP synthesis and other inputs may be required to drive a sGC/cGMP cascade in the RVLM.

ORAL SESSION 3 – ARTHROPOD VISION

Sponsored by ARC Centre of Excellence in Vision Science

ORAL-03-01

THE VISUAL DETERMINANTS OF TEMPORAL CODING IN THE CENTRAL BEE BRAIN

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Purpose: Photoreceptors in the eye provide information about light intensities from which visual interneurons extract different kinds of visual cues (e.g. color, movement, pattern). How do the properties and response characteristic of visual interneurons differ from the periphery to the central brain? We used the bee visual system to answer this question since bees rely on such stimuli during flight and foraging and show sophisticated visual learning abilities. **Methods:** We intracellularly recorded from and subsequently filled individual neurons with fluorescent dye in the second and third optic ganglia (medulla and lobula) and the central brain (protocerebrum) of bumblebees (total number of neurons= 162; total number of bees=145; Apidae: *Bombus impatiens*) while presenting color and motion stimuli. **Results:** We found that neurons at the three stages of the visual system exhibited differences in color and motion sensitivity. The distal and the proximal medulla regions were distinguished by differences in their responses to color. In the next visual stage, neurons in the distal lobula (layers 1-4) mainly process motion information while the proximal lobula (layers 5 and 6) seems to combine color and motion responses. Anterior parts of the central brain receive complex input representing combinations of motion and color information characterized by specific temporal properties (e.g. temporal precision, 'novelty' information). In contrast, posterior parts of the central brain receive mainly motion information with more reliable responses yet significantly less precise spike timing (Mann-Whitney U test; $p<0.05$). **Conclusion:** While temporally precise or novelty information in anterior pathways is suited to form stimulus associations relevant during foraging, the latter, more reliable information is thought to support fast optomotor flight control maneuvers. Therefore, the central bee brain appears to be segregated along functional lines, possibly allowing the bee to process visual cues along parallel pathways to better deal with the complex natural scene.

ORAL-03-02

FLYING LIKE A DRAGONFLY: THE SECRETS OF THE OCELLI

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Anisoptera (true dragonflies) are one of the phylogentically oldest arthropod taxons in existence, and this may be reflected in their relatively simple brain architecture and suite of sensory systems. In comparison to other insects, dragonflies have poorly developed antennal lobes and mushroom bodies. They also lack specific gravity receptors and gyroscopes such as halteres or mechanosensory antennae like those found in flies and moths (Sane et al. 2007). Despite these limitations, dragonflies are one of the most accomplished fliers known. What underlies their exceptional flight ability? Dragonflies are endowed with excellent vision; the compound eyes may contain more than 28 000 ommatidia, and interommatidial angles may be as little as 0.24° (Sherk 1978). However, dragonflies, like many other flying insects, also possess a triplet of eyes found on the front and top of the head, known as the ocelli. The ocelli have long been postulated to have a role in controlling flight stability. Could it be that these eyes are the key, for the designers of micro-aerial vehicles, to learning how to fly like a dragonfly? Here we present an overview of the results from several comprehensive studies and show that the ocelli of dragonflies are exceptionally well tuned to provide fast, sensitive and directionally selective information about the world along one dimension. We hypothesise that the peripheral processing strategies employed by this system forms a 'matched filter' (Wehner 1987) for detecting the horizon, and that this signal is subsequently passed on to efferent motor centres driving wing movement.

ORAL-03-03

EFFECTS OF ADAPTATION AND SATURATION ON NATURAL IMAGE CODING

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Purpose: Based on visual cues alone, animals can accurately perceive self-motion, compute speeds, and calculate distances travelled. Each of these tasks requires, at least, the accurate coding of image velocities across the retina. In the fly visual system the Lobula-Plate Tangential Cells code for directional image motion and influence flight responses. When presented with natural images, these neurons respond in a fashion that is dependent upon image velocity, yet independent of other image parameters (e.g. contrast). These neurons are amenable to long duration stable intracellular recordings, and thus represent an ideal model to probe the neural strategies of image speed estimation. **Methods:** Natural images (26) were displayed at a range of velocities and directions whilst recording in-vivo from identified neurons (n=15) in hoverflies. In addition to displaying the images at their normal contrast we artificially manipulated image contrasts to investigate further the effects of response saturation and motion adaptation. **Results:** We characterized 3 different motion-dependent effects that have important consequences for velocity coding. 1. A rapid adaptive component (200-300 ms) increases the response only when the initial response is weak (7/26 images). 2. Slower onset adaptive components (300-3000 ms) decrease the response to higher contrast images when the initial response is strong (14/26). 3. Local response saturation set limits to the responses of all the images. **Conclusion:** The 3 components characterized here lead to a strong normalization of the responses to images that would otherwise be highly variable. The inherently high contrast of natural images combined with the high contrast sensitivity of insect motion-detectors mean such non-linearities would be regularly recruited in naturalistic conditions.

ORAL-03-04

SYNCHRONIZATION OF THE WING BEAT CYCLE OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA* BY PERIODIC LIGHT FLASHES

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Purpose: The desert locust *Schistocerca gregaria* can adopt its wing beat frequency to the frequency of periodic light flashes (Waldron 1968, Z vgl Physiol 57:331-347). We examined (1) differences in the effect of UV and green light, (2) whether the ocelli or the compound eyes mediate this effect, and (3) the speed and time course of entrainment. **Methods**: Tethered flying locusts were presented with light flashes in the UV (n = 20) and in the green (n = 5) spectrum. To identify the visual system mediating entrainment we tested for synchronization after cutting the optic nerves of the compound eyes, the two lateral ocellar nerves, or the median ocellar nerve (n = 19). In these experiments only UV light was used. **Results**: Both stimuli caused synchronization and the flight cycle had the same phase to both light stimuli. This means that the relationship of a specific wing position during a wing beat and the time of a light flash was always the same. UV light had a stronger effect, and still caused synchronization at 5-80 times lower intensities than green light. All of the three ocelli mediate light entrainment of the wing beat. The compound eyes might play an additional minor role. Latencies of the response varied greatly and ranged from the duration of a single wing beat cycle to several seconds. **Conclusion**: We suggest that this phenomenon is part of the flight stabilization system with respect to the horizon.

ORAL SESSION 3 – ARTHROPOD VISION *(contin.)*

Sponsored by ARC Centre of Excellence in Vision Science

ORAL-03-05

THE INFLUENCE OF NOISE ON RELIABLE VELOCITY CODING IN A COMPUTATIONAL MODEL OF FLY VISUAL PROCESSING

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Purpose: The neuronal pathway for biological motion vision highly complex and non-linear. Despite considerable research effort it has defied accurate modelling for over 50 years. We have proposed a computational model for the calculation of ergo-motion that explains a number of outstanding issues, such as reliable coding in different environments and responses to images that ware artificially contrast rescaled. Here we have used the computational motion model and varied the amount of signal (i.e. photon) noise and internal (i.e. synaptic and stochastic) neuronal noise to determine the robustness of the model under more biological conditions. Methods: High-dynamic range panoramic images (n=15) taken from various environments were used as inputs to a computational motion model of biological motion vision. Noise was approximated as Gaussian, white and additive. Signal noise was added at the level of early visual processing. Internal noise (n=3) was inserted at key stages of the model corresponding to the assumed locations of synapses in the biological pathway. Result: The addition of biologically relevant levels of noise resulted in a significant ($p<0.05$) 18% increase in the reliability of velocity coding. Furthermore, the model was robust, as velocity coding not significantly degraded under low signal to noise conditions. Conclusion: While the phenomenon of stochastic resonance has been observed previously in biological systems it is most common in systems that perform non-linear thresholding, such as spike generation. Our findings are unusual as they show noise being beneficial in a model of a graded system. We also highlight the robustness of the biological visual system to large levels of noise, both internal and external.

ORAL-03-06

THE TOPOGRAPHY OF FIDDLER CRAB VISION

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Purpose: Many animals rely on vision to collect information about their environment. Given the great range of visual systems, tasks and habitats, there is surprisingly little experimental evidence of how the structure of the visual sampling array affects behavioural strategies and vice versa. **Methods:** We measured size and (utilising the corneal pseudopupil) viewing directions of most of the 9000 ommatidia in a *Uca vomeris'* compound eye. We then calculated the distribution of contrast sensitivity and spatial resolution over the whole visual field. To confirm differences between frontal and lateral acute zones we measured vertical strips of ommatidia in additional animals (n=7). Spectral and polarisation sensitivity were determined by intracellular recordings from photoreceptors (n>20) in live animals. **Results:** We found three zones characterised by distinct combinations of contrast sensitivity and resolution: High acuity (1.4 cyc/deg) and maximum contrast sensitivity laterally; maximum acuity (1.6 cyc/deg) frontally; and very low resolution and narrow acceptance angles, leading to drastic undersampling, but reasonable contrast sensitivity, in the dorsal visual field. We have identified two spectral sensitivities (λ_{max}=400 and λ_{max}=460) which are co-expressed in most photoreceptors. **Conclusion:** The measured parameters relate directly to observed behavioural strategies. Fiddler crabs always face their burrow sideways, suggesting that the lateral visual field (highest contrast sensitivity) is involved in detecting conspecifics approaching their burrow. The high resolution in the front helps recognise individual carapace patterns of females. Increased contrast sensitivity at the cost of undersampling in the dorsal visual field can be seen as an adaptation to early detection of predatory birds.

ORAL-03-07

ASTAXANTHIN BASED LINEAR POLARIZED LIGHT SIGNALS IN STOMATOPODS

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It has been shown that many stomatopod crustaceans can perceive the polarization of light. It is also known that various species of stomatopods contain polarized light reflecting body parts. However, here we find on the antennal scale of the *Odontodactylus scyllarus* a polarizer which reflects a steady e-vector angle of light that is not highly affected by orientation. The optical active structure lies in the cuticle of the antennal scale. By measuring the polarization while tilting or rotating the antennal scale, it was found that the e-vector angles of the resulting polarized light is always parallel to the rotation axis. We failed to find any photonic structures that could be responsible for this type of polarization. Based on the spectral properties of the polarized light, however, we hypothesized that the polarizer is based on dichroic molecules. By submersing the cuticle in acetone, we successfully extracted the pigment from the antennal scale and abolished the polarization properties of the antennal scale. The absorbance spectrum of the extract was nearly identical to that of astaxanthin, a typical crustacean carotenoid. Based on the polarization properties, we predicted that all the astaxanthin in the cuticle were aligned such that only e-vector angles parallel to the surface normal will be absorbed. As a result, rotation of the polarizer along the axis perpendicular to the surface should have minimal affect on the e-vector of the reflected light.

ORAL-03-08

A NEW CATEGORY OF VISUAL SYSTEM: CIRCULAR POLARISATION VISION

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Purpose. As well as seeing differences in intensity, many animals also discriminate colour, and some discriminate or at least use plane of polarization, the e-vector direction of light. Up until now, intensity, colour and plane of polarization have been the three visual modalities known in the animal kingdom. The discovery of a fourth modality, the perception of circular polarized light (CPL), is described here in a marine crustacean, the stomatopods (mantis shrimps). **Methods.** Four lines of evidence demonstrating this astonishing feat of physics in a biological system are described: the optical mechanism by which discrimination is achieved, the anatomy of the photoreceptors, physiological evidence from individual photoreceptors and behavioural proof of circular polarization vision **Results.** The optical mechanism that allows this form of vision is a ¼ wave retarder followed by linear polarization detectors, a specialty of arthropod microvillar photoreceptors. The elegant structural basis for this form of vision in a compartmentalized part of the eye is described. Electrophysiological evidence demonstrates circular polarization sensitivity to both left and right handed CPL. Behavioural training showed that, remarkably, these animals were both sensitive to CPL and could discriminate its handedness (left or right). The final bit of evidence, that stomatopods possess gender-specific circular polarized reflections, reveals the likely biological significance for this new form of vision; sex. **Conclusion.** This is the first time circular polarization vision has been demonstrated in any animal and prompts the re-evaluation of this possibility in both arthropods and vertebrates. As well as sexual communication, circular polarization vision may be useful for object detection and increased contrast. Human camera systems have re-invented this ability for such purposes.

ORAL-04-01

LATENT STEM AND PROGENITOR CELLS IN THE HIPPOCAMPUS ARE ACTIVATED BY NEURAL EXCITATION

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The regulated production of neurons in the hippocampus throughout life underpins important brain functions such as learning and memory. Surprisingly, however, studies have so far failed to identify a resident hippocampal stem cell capable of providing the renewable source of these neurons. Here we report that depolarizing levels of KCl produce a 3-fold increase in the number of neurospheres generated from the adult mouse hippocampus. Most interestingly, however, depolarizing levels of KCl led to the emergence of a small subpopulation of precursors (approximately 8 per hippocampus) with the capacity to generate very large neurospheres (>250 µm in diameter). Many of these contained cells which displayed the cardinal properties of stem cells: multipotentiality and self-renewal. In contrast, the same conditions led to the opposite effect in the other main neurogenic region of the brain, the subventricular zone, where neurosphere numbers decreased by approximately 40% in response to depolarizing levels of KCl. Most importantly, we also show that the latent hippocampal progenitor population can be activated in vivo in response to prolonged neural activity found in status epilepticus. This work provides the first direct evidence of a latent precursor and stem cell population in the adult hippocampus, which is able to be activated by neural activity. Since the latent population is also demonstrated to reside in the aged animal, defining the precise mechanisms that underlie its activation may provide a means to combat the cognitive deficits associated with a decline in neurogenesis.

ORAL-04-02

INDUCTION OF NEURAL CREST STEM CELLS FROM HUMAN NEURAL PROGENITORS DERIVED FROM EMBRYONIC STEM CELLS

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Purpose: Neural crest (NC) cells are multipotent stem cells that are specified within the embryonic neural tube, delaminate and migrate to stereotyped sites for differentiation into multiple lineages. A number of neurocristopathies involve a deficit of NC-derived cells, raising the possibility of stem cell therapy. To investigate this possibility it would be ideal to have an unlimited source of human NC that can be differentiated to functional lineages. One of the most appropriate sources that may serve this purpose is NC derived from human embryonic stem cell (hESC) lines. Thus, our aim was to develop a method to derive NC from hESC and test their functional capacity. **Methods:** We have a robust system to generate neural progenitors from hESC using noggin treatment. We found that co-culturing neural progenitors onto mouse fibroblasts promoted their differentiation towards migrating NC. To examine their functional potential, hESC-derived NC were harvested and co-cultured with explants of E11.5 aneural murine colon. **Results:** Neurospheres cultured onto mEFs resulted in over 90% of migrating cells that expressed the NC marker, p75. hESC-derived NC were able to migrate within aneural explants of colon in vitro (>80%, n=25). **Conclusions:** These data show promise for using hESC as a source of human NC to further study NC migratory behaviour, differentiation and molecular traits, as well as their therapeutic potential for treating neurocristopathies.

ORAL-04-03

ISOLATION OF HUMAN BMPRII+ NEURONAL-RESTRICTED PRECURSORS FROM SPINAL CORD DERIVED NEURAL EMBRYOID BODIES

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Purpose: In this study we examined organogenesis in primary aggregate cultures derived from the developing human spinal cord. Our research is the first to demonstrate histogenic recapitulation of human neural tissue - this is where dispersed cells self-organize into spheroid structures with histotypic characteristics of the tissue from which they were derived. In developing this technique for human tissue, we are now able to recapitulate aspects of early human neural development by means of controlled culture conditions and can study the de novo formation of tissue-like cell arrangements, including the isolation of neural phenotypes, in culture. **Methods:** Data were collected from human spinal cord specimens aged 7.5-19.5 weeks gestation. Samples were dissociated and expanded in enriched neural basal media. Resultant aggregates were dissociated at low density or reaggregatated at high cellular density with or without leukaemia inhibitory factor (LIF). The spheres generated were either equilibrated in sucrose, fixed, cryosectioned and examined for cytoarchitecture; plated and their progeny examined or sorted by fluorescent-activated cell sorting (FACS) and then cultured. **Results:** The principal findings from this study are that: (*i*) histotypical spheres can be formed from human neuroepithelial stem cells (NSC) which we named neural embryoid bodies (NEB) (*ii*) sequential development is observed for human NSCs in culture; (*iii*) passage via reaggregation decreases cellular senescence from occurring; (*iv*) tissue culture technique (reaggregation), media condition (addition of LIF) and sphere size (200-500µm diameter) all significantly affect histotypical sphere formation; (*v*) NEB cytoarchitecture is characterized by a surface layer of neuron restricted precursor cells; and (*vi*) BMPRII^{+}NRP can be isolated by single-label FACS. **Conclusion:** Taken together, our study provides novel and compelling evidence for the in vitro modeling of human neural development in culture. We show how NEB cytoarchitecture reflects neural development and take advantage of this to isolate immature precursor cells of the neuronal lineage. Through the use of NEB assays it is possible to better model neural development in culture and therefore more accurately reflect true biological function in vitro, which has application for cell-based pharmaceutical assays as well as regenerative medical technologies.

ORAL-04-04

THE ROLE OF NEOGENIN AND ITS LIGANDS IN DIFFERENTIATION AND MIGRATION IN THE ADULT BRAIN

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Purpose: The ability of the adult brain to produce neurons is well established. The molecular mechanisms controlling differentiation to a neural fate and those guiding migration of neurons from a proliferative zone to their final destination are still poorly understood. The largest proliferative region in the adult brain is the subventricular zone (SVZ) where cells are initially quiescent and then differentiate into neuronal precursors. These new neurons migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB) where they integrate into the neuronal circuitry and replace older neurons. The multi-functional receptor, Neogenin, is expressed in the SVZ and RMS, and its ligands, Netrin-1 and RGMa are expressed in complementary patterns. Our studies indicate these molecules have a role in differentiation and migration leading to neuronal replacement. **Method:** Expression studies were conducted on frozen sagittal sections of mice (8–12 weeks). The expression pattern of Neogenin was analysed with immunofluorescence (n=5). Netrin expression was inferred from LacZ staining in the Netrin-1 mutant forebrain (n=4). Functional studies were conducted on neurospheres generated from Neogenin mutant and wild-type mice (n=5). **Results:** Our experiments show Neogenin is expressed in the SVZ and on migrating neuroblasts throughout the RMS. Netrin-1 is expressed ventral to the RMS. Functionally, differentiated neurospheres from Neogenin mutant mice have significantly fewer neurons than wild-type mice; and RGMa also appears to affect differentiation. **Conclusion:** Together, these data suggest Neogenin and its ligands have a role in differentiation and are potential guidance molecules for RMS migration. Our preliminary human studies show the expression of these molecules is conserved, suggesting their function is the same in our own brains.

ORAL-04-05

GLYCOPROTEIN 130 SIGNALING IS NOT REQUIRED FOR MAINTENANCE OF ADULT NEURAL PRECURSOR CELLS IN VITRO

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Purpose: We have previously demonstrated that exogenous leukemia inhibitory factor (LIF) potentiates the clonogenicity of embryonic neural precursor cells (NPCs). LIF signals via a heterodimeric receptor complex comprising LIFRβ and gp130, the latter being the common receptor component for all IL6-family cytokines. To assess the role of gp130 signaling upon the clonogenicity of adult NPCs, we have characterised NPCs derived from the subventricular zone of adult mice in which gp130 is conditionally deleted. **Methods:** Adult NPCs were isolated from the subventricular zone of GFAP-Cre^{+} gp130$^{fl/fl}$ (n=8) and GFAP-Cre^{-} gp130$^{fl/fl}$ (n=8) mice and cultured as neurospheres in the presence of EGF and FGF-2 to assess their clonogenicity and growth kinetics in vitro. In addition, the clonogenic response of embryonic (n=8) and adult (n=4) wild-type NPCs to exogenous LIF pre-treatment was assessed. **Results:** Deletion of gp130 from adult NPCs resulted in loss of responsiveness to exogenous LIF, as demonstrated by the absence of phosphorylated STAT3, GFAP immunoreactivity and adherent morphology. In the absence of gp130 signaling, NPCs formed neurospheres that could be maintained and expanded over serial passages with clonogenicity and growth kinetics that did not differ from GFAP-Cre^{-} gp130$^{fl/fl}$ NPCs. Furthermore, we found that pre-treatment of wild-type NPCs with LIF did not modulate the clonal potential of adult, E10.5 or E16.5 NPCs regardless of growth factor conditions. **Conclusions:** We have demonstrated that deletion of the gp130 cytokine receptor has no effect on the clonal potential or self-renewal capacity of adult NPCs in vitro. We are currently investigating whether the effects of LIF that we previously observed in E14.5 NPCs reflect a role for LIF at a specific developmental epoch and whether conditional deletion of gp130 modulates adult neurogenesis in vivo.

ORAL-04-06

A NOVEL CRIPTO ANTAGONIZING PEPTIDE IMPROVES EMBRYONIC STEM CELL BASED THERAPY IN PARKINSON'S DISEASE

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Purpose: Embryonic Stem Cells (ESCs) hold considerable promise as a potential regenerative therapy for neurological conditions, including Parkinson's disease (PD). Two major hurdles exist for ESC in this context; adequately restricting cell fate and preventing tumor formation. In this regard Cripto, a GPI-anchored protein and key player in the Activin type IB receptor (ALK-4)/Nodal/Smad2 signaling pathway, holds particular interest as it has been shown to control cell fate in ESCs and has been implicated in tumor biology. **Methods:** By combinatorial approach, we identified a tetrameric tripeptide made of non-natural amino acids, which prevents Cripto/ALK-4 receptor interaction and Cripto-dependent Smad2 phosphorylation in ESCs. We examined the ability of this peptide to prevent cardiomyogenesis and promote neuronal induction of ESCs, thereby increasing the pool for dopaminergic (DA) differentiation. Subsequently, we examined the ability of this peptide to improve ESC grafting in a rodent model of Parkinson's disease. **Results:** ELISA and western blotting confirmed the ability of the Cripto blocking peptide (CriptoBP) to antagonise Smad2 phosphorylation. Application in vitro significantly enhanced neural induction (n=3, $p<0.005$) and the proportion of DA-enriched colonies (386% greater than control peptide). In vivo, ESC-grafts+CriptoBP infusion significantly reduced the incidence of tumor formation (42% vs 75%) and tumor size compared to ESC-grafts+Control peptide. Furthermore, these grafts significantly enhanced functional recovery in PD rats. Behavioural recovery was supported by the elevated DA neurones within the graft. **Conclusion:** These findings imply that this novel peptide may represent a unique, safe, and effective molecule for cell-based transplantation therapies in neurodegenerative disorders such as PD.

ORAL-04-07

PEPTIDERGIC INFLUENCES ON NEUROGENESIS IN THE ADULT RODENT BRAIN

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Purpose: Neurogenesis is an ongoing process in the subventricular (SVZ) and hippocampal subgranular zone (SGZ) of the adult brain, and evidence is emerging that peptidergic systems, including cholecystokinin and somatostatin, may be involved in its regulation. **Methods:** We used a histochemical approach and mice lacking CCK1 or CCK2 receptors, to examine the influence of these receptors on proliferation, migration and differentiation of neural progenitors in the adult SVZ/rostral migratory stream (RMS) and SGZ, and generation of neurons in the olfactory bulb (OB) and hippocampal formation (HF). We also examined the distribution of the ciliary somatostatin receptor 3 (SSTR3) protein in the developing and adult HF. **Results:** Mice lacking CCK1- or CCK2 receptors had 35% and 38%, respectively, fewer Ki67-ir proliferating cells and 50% and 41% less doublecortin-ir neuroblasts in the SVZ/RMS than wildtype mice. Similar trends were observed in the SGZ. Fewer calbindin- (CCK1-/- 14%; CCK2-/- 16%), calretinin- (CCK1-/- 29%; CCK2-/- 26%) and tyrosine hydroxylase-ir (CCK1-/- 35%; CCK2-/- 15%) interneurons were observed in the OB of these mice, with no changes in GFAP-, calbindin- and NeuN-ir cell numbers in the dentate gyrus (DG). SSTR3-ir cilia first appeared in the DG on P2, increased to high levels by P20, and their density remained high in adults. SSTR3-ir cilia did not colocalize with neuroblasts in the DG (or OB), but localized to mature GABA- and calbindin-ir cells in these regions. **Conclusion:** Cholecystokinin, through CCK1 and CCK2 receptors, and somatostatin, through SSTR3 signalling, may regulate neurogenesis and cell cycle/apoptotic processes in the adult forebrain.

ORAL-04-08

ROLE OF LYSOPHOSPHATIDIC ACID IN THE NEURAL DIFFERENTIATION OF NEURAL STEM/PROGENITOR CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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Purpose: lysophosphatidic acid (LPA) is a signalling lysophospholipid that plays broad and major roles within the nervous system both during early development and neural injury, with its concentration increasing in the nervous system following the impairment of the blood brain barrier. Neural stem cells (NSC) have been extensively studied with the aim of using endogenous and/or donor NSC to replace neurons and restore circuitry in a neurodegenerative microenvironment. One of the most challenging aspects of NSC biology is that despite showing efficient neural differentiation in vitro, their differentiation potential in vivo tends to be biased towards glial lineages particularly during degeneration/injury when there is extensive and ongoing inflammation. It is therefore pertinent to study how NSC differentiation may be influenced by factors released during injury. In addition, the understanding of the downstream signalling pathways involved in this process provides an important step towards refining potential therapeutic strategies. **Results**: we describe the potent effect of LPA as an inhibitor of neuronal differentiation of human embryonic stem cell (hESC)-derived NSC. Moreover, we identified the intracellular signalling pathways involved and demonstrated that both the PI3K/Akt pathway and the Rho/ROCK pathway are involved in the anti-differentiation effect of LPA on hESC. Altogether, these original data are the first demonstration of a role for LPA signalling in neuronal differentiation of hESC. **Conclusion**: this work suggests that, as LPA concentrations increase in the nervous system during inflammation, LPA's inhibition of neuronal differentiation might contribute to the non-regeneration of neurons observed following neurotrauma. Thus, modulation of LPA signalling may have a significant impact in injury within the nervous system, allowing new avenues for potential therapeutic approaches.

ORAL-05-01

COMPARATIVE NEUROANATOMY ONLINE - RESEARCHING THE MAMMALIAN HIPPOCAMPUS VIA THE INTERNET

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Purpose: Studies of comparative mammalian neuroanatomy are severely restricted by barriers to obtaining histological sections from many different animals. Geographical, financial, and ethical factors are responsible for this. One solution is to make use of images of existing collections that are available on the Internet. The best of these are 'www.brainmuseum.org' and 'www.brainmaps.org'. The former offers great variety with low to medium resolution images; the latter offers less variety but with very high resolution images. **Methods**: This study used these two sites to study hippocampal variation in relation to navigation behaviour in the major groups of mammals. Serial sections of the brains of 2 monotremes, 3 marsupials, and 55 from the 4 superorders of placentals (Afrotherians - 4, Xenarthria - 3, Laurasiatheria - 26, and Euarchontoglires - 22) were examined. **Results**: The CA fields and the dentate gyrus vary widely in relative size and complexity among these mammals. Most striking is the relatively huge and complex hippocampus in the elephant shrew and in the platypus, both of which repeatedly patrol a home territory. In many nomadic animals, the hippocampus is relatively modest in size. **Conclusion**: These findings are consistent with the role of the hippocampus in memorising features in the immediate environment. These are only preliminary results that now need to be subjected to quantitative analysis, but they show that valuable comparative anatomical data can be gleaned cheaply and easily from the Internet.

ORAL-05-02

DURATION BUT NOT ONSET OF WORKING MEMORY PROCESSES DIFFERS FOR INEFFICIENT VERSUS EFFICIENT VISUAL SEARCH: AN EEG/ERP STUDY

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Purpose Visual search involves perception, attention, memory and response selection. We investigate how these component processes differ during efficient (possibly parallel) and inefficient (possible serial) visual search. **Methods** Participants (n=11) identified the location of a target item (coloured, oriented rectangular bar) in search displays also containing non-target distractors. In the condition yielding efficient search, where response time was independent of set size (two or four items), distractors had no feature in common with the target. In the condition yielding inefficient search, where response time increased with set size, distractors shared one feature (colour, or orientation) with the target. Stimulus- and response-locked event-related potentials (P1, N1, P2, N2, P3, N2pc, SPCN, and rLRP) were analyzed. **Results** P3, an indicator of working memory engagement, showed no significant difference in onset latency between efficient and inefficient conditions, but P3 activity over time was greater for inefficient search (p=.0001). Sustained posterior contralateral negativity (SPCN), which has been linked to working memory load, was greater for the inefficient condition (p=.037). The duration of response-locked lateralized readiness potential (rLRP), indicating response selection, was also longer in the inefficient condition (p=.047). By contrast, indicators of selective attention, P1 peak amplitude (p=.001) and N1 peak latency (p=.016), were sensitive to number of items; and P2 peak amplitude (p=.008) and N2pc mean amplitude (p=.001) to number of non-target features. (N2 results were inconclusive.) Analysis of phase-locking values, indicating synchronization between brain regions, revealed greater frontoparietal synchronization in the lower gamma band (20-40 Hz) around 300-500 ms post-stimulus for inefficient search (p<.01). **Conclusion** These results suggest that efficient and inefficient search differ not in the time required to select items as potential targets of search (selective attention), but in the time required to confirm which of those items is indeed the target (target confirmation).

ORAL-05-03

A SHORT ATTENTION SPAN IN THE MEMORY CONSOLIDATION MUTANT RADISH

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Purpose: Although the fly *Drosophila melanogaster* has been used for over thirty years as a model system to study learning and memory, investigations relating memory defects to attention-like processes have only recently been initiated. We investigated short-term visual processes in the memory consolidation mutant *radish*. **Methods**: Two behavioural paradigms were used to address short-term processes in flies, an optomotor maze and the tethered flight arena. Local Field Potentials (LFPs) were recorded from the brains of tethered flies in the arena to examine responsiveness to competing visual stimuli. **Results**: *radish* mutants displayed defective short-term processes in all three paradigms (maze, tethered flight, and electrophysiology). Poor responsiveness in the optomotor maze was found to result from random turning behaviour, (n=40 flies). In closed-loop tethered flight, *radish* attended to visual objects for less time than wild-type flies (1.3 s vs 3.8 s). Closer examination of torque behaviour during flight revealed a ~1.7 Hz oscillation in *radish* mutants (n=24 flies). Electrophysiological analysis revealed that *radish* responsiveness to visual novelty was significantly briefer compared to wild type (n=14 flies). A measure of alternation dynamics for LFP activity showed that *radish* brain activity alternated randomly between stimuli whereas wild type displayed a sustained bias for one or the other object across successive image exposures. **Conclusion**: Our behavioural and electrophysiological paradigms all suggest that *radish* is defective in short-term processes relevant to selective attention. We propose that *radish* is defective in memory consolidation because of periodic interruptions in behaviour, or a shortened attention span. As such, *radish* may provide a Drosophila model for attention deficit disorders.

ORAL-05-04

RYANODINE RECEPTOR-DEPENDENT INTRACELLULAR CALCIUM STORES: RELATIONSHIP TO NITRIC OXIDE AND NORADRENALINE IN CONSOLIDATING LONG-TERM MEMORY IN THE YOUNG CHICK

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Purpose: Nitric oxide (NO) and noradrenaline (NA) are vitally important molecules in memory processing. In young chicks trained on a single-trial discrimination avoidance task, these two molecules are required for memory processing at a time consistent with a role in priming the long-term memory stage. The literature suggests that both NO and NA can be linked to ryanodine receptor (RyR) calcium channels. In the chick, RyRs are also involved in memory processing at the same time as NO and NA. The aim of the current study was to explore whether these three molecules interacted to prime long-term memory. **Methods:** Chicks (*n*=15-18 per data-point) were trained on a single-trial discrimination avoidance task. Three challenge studies were performed. In experiments 1 and 2, concentrations of the RyR antagonist dantrolene were challenged with NA or the spontaneous NO donor sodium nitroprusside (SNP). In experiment 3, the amnestic action of the NO synthase inhibitor L-NAME was challenged with the RyR agonist 4-CMC. **Results:** Experiment 1: The amnestic action of dantrolene (5 mM, ic) was overcome by either NA (100 µM; ic) or SNP (150 µM; ic). Experiment 2: NA (100 µM; ic) effectively challenged higher doses of dantrolene (7.5 and 10 mM). Experiment 3: 4-CMC (500 µM; ic) overcame the amnestic action of L-NAME (500 µM; ic). **Conclusion:** These findings appear to suggest that: (1) the roles of RyRs, NO and NA in this model are functionally related; and (2) NO action is likely to be up-stream of RyRs while NA down-stream of RyRs. These findings give important insights into the mechanisms underlying the priming of long-term memory.

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ORAL-05-05

A CYTOKINE MODEL OF COGNITIVE FUNCTION

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Purpose: A formulation of a cytokine model of cognitive function under immunologically unchallenged physiological conditions. Methods: The proposed cytokine model of cognitive function under unchallenged conditions is based on empirical work by this group investigating the effects of cytokines at the protein and genetic level on cognitive function in humans as well as in animal models investigating the effects of TNF in transgenic mice. Results: In a study among 369 healthy elderly from the general population, we found the chemokine IL-8 to be significantly associated with poor cognitive performance in the memory, attention and motor domains (1). In a similar study among 369 healthy individuals, genetic variants of IL-1beta were related to poor memory, whereas TNF-alpha was related to better cognitive speed performance. In contrast, genetic variants of IL-6 showed no association with cognitive performance in humans (2). Animal studies of our group show that the absence of TNF (B.6TNF-/-; N=10) is detrimental of cognitive function during neurodevelopment (3), while during aging the absence of TNF (B.6TNF-/-; N=10) is related to improved cognitive performance as opposed to wt mice (C57BL/6; N=10). The cytokines IL-1β, IL-6 and TNF-α have effects on complex cognitive processes at the molecular level, such as synaptic plasticity, neurogenesis, as well as neuromodulation (4). Conclusions: The findings provide evidence for a cytokine model of cognitive function, which shows that cytokines play an intimate role in the molecular and cellular mechanisms subserving learning, memory and cognition under physiological conditions (4). References: (1) Baune, B.T., et al.,Neurobiol Aging, 2008. 29(6): p. 937-44; (2) Baune, B.T., et al.,Psychoneuroendocrinology, 2008. 33(1): p. 68-76. (3) Baune, B.T., et al.,Am J Med Genet B Neuropsychiatr Genet, 2008. 147B: p. 1056–1064; (4) McAfoose, J., Baune BT.,Neuroscience and BioBehavioral Reviews (in press).

ORAL-05-06

INCREASING CU BIO-AVAILABILITY INHIBITS Aβ OLIGOMERS AND TAU PHOSPHORYLATION, AND IMPROVES COGNITION IN ALZHEIMER'S DISEASE MODEL MICE

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Cognitive decline in Alzheimer's disease (AD) involves pathological accumulation of synapto-toxic amyloid-β (Aβ) oligomers and hyper-phosphorylated tau. As recent evidence indicates glycogen synthase kinase 3β (GSK3β) activity regulates these neurotoxic pathways we developed an AD therapeutic strategy to target GSK3β. The strategy involves using copper-*bis*(thiosemicarbazonoto) complexes to increase intracellular copper bio-availability and inhibit GSK3β through activation of ERK/Akt pathways. We treated cognitively impaired AD model mice with our lead compound Cu^{II}gtsm by oral gavage for 11 weeks. Cu^{II}gtsm crosses the blood-brain barrier and is cell permeable, and once inside the cell the bound Cu^{II} is reduced to Cu^{I} by cellular reductants, causing intracellular release of the copper. Sham treated AD mice (n=8) showed a typical decline in cognitive function as determined using the Y-maze test for memory and learning, but treatment with Cu^{II}gtsm (n=7) restored cognitive function to levels expected for normal, healthy mice. Biochemical analysis of the brains revealed treatment with Cu^{II}gtsm activated ERK1/2 and Akt, and increased phosphorylation of (i.e. inhibited) GSK3β. Further to this, inhibition of GSK3β correlated with decreased levels of phosphorylated tau. We also used a novel mass-spectrometry technique to show Cu^{II}gtsm decreased the abundance of Aβ trimers in the brain. This study demonstrates that increasing intracellular copper bio-availability can restore cognitive function by inhibiting the accumulation of neurotoxic Aβ trimers and phosphorylated tau.

ORAL-05-07

BEHAVIOURAL ANALYSIS OF CONGENIC MOUSE STRAINS CONFIRMS STRESS-RESPONSIVE LOCI ON CHROMOSOME 1 AND 12

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PURPOSE: Chronic stress is directly related to anxiety disorders. To identify the genetic factors that influence anxiety, we have studied the stress-responsiveness of inbred mouse strains using the elevated open field activity test (eOFA). Two strains show high (DBA/2J) or low (C57BL/6J) stress-responsiveness in the eOFA. Genetic studies of an F_2 intercross between these two strains identified two regions, on chromosomes (Chr) 1 and 12, linked to anxiety-related behaviour. Our aim was to confirm the phenotypes linked to these regions and further fine map the loci to facilitate gene identification. **METHODS:** We established separate congenic mouse strains containing the linked Chr1 and Chr12 regions from the DBA/2J strain on a C57BL/6J genetic background. Cohorts of parental and congenic mice were analysed for a series of stress-responsive phenotypes using the eOFA test. **RESULTS:** Both congenic strains had significantly different (*p<0.0001*) stress-responsive phenotypes compared to C57BL/6J parental strain. The DBA/2J-derived Chr12 interval had a greater genetic effect than the Chr1 interval for changing the behavioural phenotype of the parental C57BL/6J mouse strain. We also identified new stress-related phenotypes, which aided in comparing and differentiating the parental and congenic strains. We further mapped the Chr12 interval to ~20Mbp using 4 different sub-strains that dissect the original interval. **CONCLUSION:** We confirmed the presence of stress-responsive loci on Chr1 and Chr12 congenic strains. We have also mapped the Chr12 interval to ~20Mbp, which if further mapped to <5Mbp should help identify the stress-responsive genes.

ORAL-05-08

POST-PARTUM DEPRESSION IN MOTHER RATS INDUCED BY PROLONGED SEPARATION FROM PUPS IS REVERSED BY CONSUMPTION OF PALATABLE HIGH FAT DIET

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Purpose: The effects of maternal separation (MS) in pups are well known, unlike those in the mother rats. This study investigated the influence of palatable high fat diet (HFD) on the behavioural responses in mothers, after repeated, prolonged MS. Methods: After giving birth, female Sprague-Dawley rats (n=17) were assigned to either brief separation from their pups of 15 minutes (S15) or 180 minutes (S180)/day from postnatal day (PND) 2-14. These mother rats were assessed for anhedonic behaviour using sucrose preference test at PND 25; forced swim test (FST) was carried out at PND 27/28. Rats were assigned to either chow or HFD at PN week 4 for 16 weeks. The anxiety level was assessed with elevated plus maze (EPM) at PN week 17 and re-assessed with FST at PN week 19. Results: S180 mother rats showed anhedonic behaviour, with decreased sucrose preference compared to S15 mothers (0.65±0.08 vs 0.93±0.02 sugar/water intake, t=3.34, p<0.01). S180 mothers spent more time being immobile (3.37±0.36 vs 1.85±0.25 minutes, t=3.52, p<0.01) and less time swimming (0.29±0.08 vs 0.95±0.29 minutes, t=3.28, p<0.01) in FST compared to S15 mothers, indicative of depression-like behaviour. Interestingly, at PN week 17 onwards, HFD fed S180 rats had increased frequency of head dip postures in the open arm of EPM compared to chow fed rats (12.8±0.96 vs 4.0±2.9, t=4.78, p<0.01) which suggests decreased anxiety, and in the FST they spent more time swimming (0.83±0.17 vs 0.03±0.01 minutes t=7.61, p<0.001). Conclusion: Anxiety and depression-like behaviours induced in mothers by prolonged separation from pups were reversed with voluntary consumption of HFD diet.

ORAL-06-01

BALANCE: A SPECIAL TYPE OF MOTOR CONTROL

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Purpose: To understand how the brain controls muscles. Methods: (I) Subjects (n=10) were asked to report the force applied by the leg muscles to stand by producing a voluntary contraction of the same muscles. Force output, muscle EMG and cortical EEG were measured. (II) Subjects (n=10) were supported upright with inactive muscles and then released so that they had to actively stand or asked to voluntarily produce the muscle contraction used to stand. Blood pressure was measured. Results: (I) Subjects greatly underestimated this force, reporting about one-fifth of the active force actually used to stand. When balancing an inverted pendulum that had the same properties as their own body, they accurately reported this force. There was a high coherence between EEG and EMG (20-30Hz bandwidth) during the voluntary contraction but not during standing. (II) When released to stand, there was no change in blood pressure. When they made the same voluntary contraction, mean blood pressure rose by 8%. Conclusions: A central signal that is a corollary of the motor command is used to estimate muscle force. We conclude that most of the drive to motoneurones during standing arises from a balance system independently of cortical voluntary muscle activation and is not accessible to perceptual processes. When a muscle is activated voluntarily, a central pressor response acts to increase systemic blood pressure. This response arises through a corollary signal of the motor command, sent to cardiovascular centres. We conclude that the balance drive used to stand does not evoke a pressor response, again showing an independence of the balance system from cortical motor processes.

ORAL-06-02

UNILATERAL STRENGTH TRAINING INCREASES VOLUNTARY ACTIVATION OF THE OPPOSITE UNTRAINED LIMB

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Purpose: We investigated whether an increase in neural drive from the motor cortex contributes to the cross-limb transfer of strength that can occur after unilateral strength training. **Methods:** Twitch interpolation was performed with transcranial magnetic stimulation to assess changes in strength and cortical voluntary activation in the "untrained" left wrist, before and after 4-weeks of unilateral strength-training involving maximal voluntary isometric wrist extension contractions (MVC) for the right wrist (n = 10, control group = 10). **Results:** Wrist extension MVC force increased in both the trained (31.5 ± 18%, mean ± SD, $p < 0.001$) and untrained wrist (8.2 ± 9.7%, $p = 0.02$), whereas wrist abduction MVC did not change significantly. The amplitude of the superimposed twitches evoked during extension MVCs decreased significantly by 35% (± 20%, $p < 0.01$), which contributed to a significant increase in voluntary activation (2.9 ± 3.5%, $p < 0.01$). Electromyographic responses to cortical and peripheral stimulation were unchanged by training. There were no significant changes for the control group which did not train. **Conclusion:** Unilateral strength training increased the capacity of the motor cortex to drive the homogolous untrained muscles. The data show for the first time that an increase in cortical drive contributes to the contralateral strength training effect.

ORAL-06-03

REORGANISATION OF THE MOTOR CORTEX IN CHRONIC LOW BACK PAIN

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Purpose: Changes in trunk muscle control are common in low back pain (LBP). Although control can be improved with training, it is unclear how this is mediated in the nervous system. As deficits in anticipatory postural activation of transversus abdominis (TrA) are common, and this involves motor cortical input, we investigated the representation of TrA at the motor cortex and whether this changes with training. **Methods:** Eleven healthy volunteers and 20 with chronic LBP participated. Patients were randomly allocated into two groups: trunk muscle control training or walking exercise for two weeks. Electromyographic activity (EMG) of TrA was recorded with intramuscular electrodes. TrA control was assessed as the postural response with arm movements. Transcranial magnetic stimulation (TMS) was delivered over a scalp grid to examine the cortical representation of TrA. A map was generated from the EMG amplitude of the responses to TMS. Training groups were reassessed at 4 weeks. TrA EMG onset relative to deltoid, and TMS map centre of gravity (CoG) were compared between groups, and between pre- and post-training in the LBP group. **Results:** TrA CoG was 2 cm anterior and lateral to the vertex in controls. However, LBP subjects had a significant posterior and lateral shift of the CoG ($P<0.05$). The shift of TrA CoG was correlated with the TrA timing during arm movements. After two weeks of trunk control training, TrA CoG had moved towards that observed in controls. The TrA CoG did not change after walking exercise. **Conclusion:** These findings show reorganisation of the trunk muscle representation at the motor cortex in chronic LBP. Reorganisation can be restored with training and is associated with improved motor behaviour.

ORAL-06-04

MOTOR CORTEX PLASTICITY INDUCED BY A BALLISTIC MOTOR LEARNING TASK IS DIMINISHED IN OLDER ADULTS

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Purpose: Numerous studies indicate that the ageing process is accompanied by deficits in neural plasticity, particularly in relation to age-related impairments in cognition and memory. In contrast, the ability of the ageing brain to adapt to a period of motor training is less well established. The aim of this study was to examine changes in motor cortex (M1) plasticity and motor performance after a ballistic thumb abduction task in young and old adults. **Methods:** Electromyographic (EMG) recordings were obtained from the right abductor pollicis brevis (APB) muscle in 10 young (18-24 years) and 10 old (61-81 years) adults. The training task consisted of 300 ballistic abductions of the right thumb with the aim of maximising peak abduction acceleration. Transcranial magnetic stimulation of the left M1 was used to assess changes in APB motor-evoked potentials (MEPs) and short-interval-intracortical inhibition (SICI) before, immediately after, and 30 minutes after motor training. **Results:** Motor training resulted in improvements in peak thumb acceleration in the young (189% improvement, $P<0.0001$) and old (149% improvement, $P=0.003$) subjects, with greater improvements in the young subjects ($P=0.003$). The improvement in motor performance was accompanied by a significant increase in APB MEPs after training in young (67% greater than before, $P<0.0001$) but not old subjects (9% reduction, $P=0.49$). SICI remained unchanged following training in both groups. **Conclusion:** Use-dependent M1 plasticity and improvements in motor performance were diminished in older adults for the ballistic motor learning task. We suggest that the mechanisms responsible for reduced M1 plasticity may contribute to the impaired motor learning ability in older adults.

ORAL-06-05

REFLEX AND BIOMECHANICAL PROPERTIES WITH AGEING

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Aim: This study investigated the influence of ageing on the tonic stretch reflex (TSR) and mechanical joint properties in the human hand. **Methods:** Data were collected from 20 adult volunteers and analysed in two age groups (below and above 53 years). The metacarpal joint of the index finger was sinusoidally perturbed at sixteen frequencies (2.5 to 40 Hz) while the subjects maintained a range of contraction levels (0-50%MVC). Joint angle, surface electromyogram (EMG) from the first dorsal interosseous muscle and angle-torque data were recorded. Cross correlation analysis between joint angle and the EMG provided TSR gain estimates. Inertial, viscous and elastic properties of thh joint were derived by modeling the joint as a second order system and averaged across all frequencies. **Results:** TSR gain increased with contraction level in both groups but was significantly decreased in the old group (mean gain 9.96 %MVC/deg) compared to the young group (mean gain 16.15 %MVC/deg). In both groups, the viscous and elastic torque components increased with contraction level but the inertia remained unchanged. Mean elasticity estimates were similar in both groups, however viscosity was significantly reduced in the old group (5.92×10^{-6} Nm.s.rad^{-1}) compared with the young group (1.00×10^{-5} Nm.s.rad^{-1}). **Conclusions:** There were marked differences in TSR gain and joint mechanics in the two age groups. The decrease in TSR and viscous resistance with ageing suggests that the TSR may contribute positively to joint stiffness through the viscous component. The current results emphasize the importance of using age-matched controls in TSR studies particularly those involving disorders in elderly subjects.

ORAL-06-06

THREAT OF PAIN ALTERS MOTONEURONE DISCHARGE

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Purpose: Motoneurone discharge properties are altered during matched-force contractions when pain is induced in muscle or non-muscle tissue. This change involves reduced rate or cessation of discharge of one population, and recruitment of new motoneurons. This cannot be explained by uniform inhibition of the agonist motoneurone pool as suggested by the pain adaptation theory and provides evidence for alternative mechanisms for motor control changes during pain. Animal data suggest direct effects of nociceptive afferents on motoneurones. However, it's unclear whether changes in motoneurone discharge result from nociceptive synaptic inputs or whether similar changes can be induced by descending inputs. One way to test whether motoneurone changes are dependent on nociceptor activity is to induce threat-of-pain, in the absence of nociceptor discharge. We tested whether threat-of-pain changes motoneuron discharge. Method: Motoneurone discharge was recorded from 4 fine-wire electrodes in quadriceps of 7 volunteers. Subjects matched isometric knee extension force (~21.6(13.8)N) for 3x ~20s before and during trials of unexpected painful electrical shocks (~0.5s duration; 0.5-8s apart; pain intensity ~5/10 on visual analogue scale) to the infrapatellar fat pad. Discharge properties were compared between conditions. Results: Fifty-six motor units were discriminated, of these, 29 discharged in both control and trials with threat-of-pain. Twenty-one units discharged during the control trial only, while 6 units discharged only during threat-of-pain. The discharge rate of units recruited in both conditions decreased significantly during threat-of-pain (9.2(2.0) to 8.2(1.9)Hz; $P<0.0001$). Conclusion: Motoneuron discharge pattern changes similarly when there is nociceptive afferent discharge or simply threat-of-pain. Changes in motoneuron discharge pattern during pain may be due to changes in motor command from higher centres rather than simple nociceptor barrage at the spinal cord.

ORAL-06-07

MODELLING THE RECRUITMENT ORDER OF ELECTRICALLY ACTIVATED MOTOR UNITS

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Purpose: During voluntary contractions, single motor units are generally activated with a stereotypical progressive recruitment order from the slowest, weakest units to stronger, larger motor units. While this principle is generally accepted for voluntary contractions it is often assumed that recruitment order is reversed by electrical stimulation. We used a combination of experimental data and mathematical modelling to investigate the recruitment order of electrically stimulated motor units. **Methods:** Detailed stimulus-response curves were recorded from tibialis anterior muscle, electrically stimulating the motor point using 0.1 ms pulses with 0.1 mA increments, and recording isometric dorsiflexion force of the ankle which was flexed to approximately 90° (n=20). We implemented a modified Fuglevand model with 445 motor units using an iterative approach until the simulated data matched experimental data. **Results:** The experimental and modelled data were closely matched with correlation coefficients ranging from 95.3-99.8. The first positive inflection of the curve was only matched by recruiting the smallest motor units in a Henneman-type size order. The steepest portion of the curve was only achieved by next recruiting the largest, fastest units. The remaining motor units were then recruited in random order. **Conclusions:** Motor units are recruited during electrical stimulation in an orderly manner for the first type I units according to the Henneman size principle. The recruitment order amongst the remaining units is relatively random but the largest units (presumably type IIb) are recruited before the remaining type I and IIa units. The last group of motor units to be recruited is the least homogenous and may reflect the absence of a distinct tripartite distribution for human motor units.

ORAL-06-08

STEREOTYPED SYNERGY OF HUMAN THUMB AND FINGERS DURING GRASPING

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Purpose: To determine invariant aspects of how the central nervous system controls grasping. **Methods:** In eight subjects using the right dominant hand, we studied the static phase of grasping with all or just three fingers, and the dynamic force changes when individual fingers were transiently removed from the grasp. To simplify the mechanical constraints, the grasped object was immobilised. For static grasps we recorded force under each digit for each of the 5 tasks (grasping with all 5 digits or without one of the fingers) to compute coefficient of variation (CV) and force coherences. For "dynamic" grasping, we recorded transient forces under each digit for repetitive lifting and replacing each finger. **Results:** During grasping, the CVs (a measure of force steadiness) were lowest for the thumb and index finger. The coherence between forces produced by the digits was generally high (>0.4) but the coherence levels between digit pairs did not change when a finger was removed from the grasp. Similar results were obtained when the thumb did not contribute to the task. During the 300ms encompassing the lift off and replacement of the test finger on the object, there were repeatable transient forces in the non-test fingers. They did not adapt on repeated trials. Similar behaviour occurred when the protocol was repeated 2 months later, and when performed without the thumb contacting the object. **Conclusions:** There is a stereotyped neural synergy which co-ordinates the digits during grasping.

ORAL-07-01

INVOLVEMENT OF MESOLIMBIC DOPAMINE IN DISRUPTIONS OF PREPULSE INHIBITION INDUCED BY SEROTONIN-1A RECEPTOR ACTIVATION

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Purpose: Prepulse inhibition (PPI) is a measure of sensorimotor gating, which is deficient in schizophrenia. In rats, administration of the serotonin-1A (5-HT_{1A}) receptor agonist, 8-hydroxy-di-propyl-aminotetralin (8-OH-DPAT), causes a disruption of PPI. This study aimed to explore whether this effect is due to 5-HT_{1A} receptor-mediated modulation of mesolimbic dopamine activity. Methods: Ovariectomised female Sprague-Dawley rats were subcutaneously treated with saline or 0.5 mg/kg 8-OH-DPAT alone and in combination with the 5-HT_{1A} receptor antagonist, WAY 100,635, or the dopamine D_2 receptor antagonist, haloperidol. Another group of rats was instrumented with an indwelling cannula into the ventral tegmental area and micro-injected with 1 or 3 µg/0.5 µl of 8-OH-DPAT before being tested for PPI. Results: Systemic injection of 8-OH-DPAT caused a significant disruption of PPI (from 59% to 40%), which was blocked by pretreatment with both WAY 100,635 and haloperidol. Direct injections of 8-OH-DPAT into the ventral tegmental area also caused a disruption of PPI (from 46% to 35%). Conclusion: These data suggest that the disruption of PPI observed in rats with systemic 8-OH-DPAT treatment involves the activation of 5-HT_{1A} receptors, as this effect can be blocked by a 5 HT_{1A} receptor antagonist. Subsequently, this causes activation of dopamine D_2 receptors, as the disruption of PPI can also be blocked by a dopamine D_2 receptor antagonist. The mesolimbic dopamine system originating in the ventral tegmental area may be the site of action of these effects. These findings are important for our understanding of serotonin/dopamine interactions in schizophrenia and the action of antipsychotic medications.

ORAL-07-02

RELATED PATHOLOGICAL CHANGES OF SEROTONIN 2A, MUSCARINIC M1 AND GABA A RECEPTORS IN THE SUPERIOR TEMPORAL GYRUS IN SCHIZOPHRENIA

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The superior temporal gyrus (STG) is strongly implicated in the pathophysiology of schizophrenia, particularly with regards to auditory hallucinations. **Purpose**: The objective of this study was to investigate the relationships of pathological changes in 5-HT_{2A}, muscarinic M1 and $GABA_A$ receptors in the STG of schizophrenia. **Methods**: Using *in situ* quantitative autoradiography, the bindings of 5-HT_{2A}, muscarinic M1 and $GABA_A$ receptors in the STG were examined. **Results**: Significant decreases in binding density of 5-HT_{2A} and M1 receptors were observed in schizophrenia patients in comparison with control subjects (n=8; $p<0.05$). However there was an increased density of $GABA_A$ receptor in the STG of schizophrenia ($p<0.05$). A clear positive correlation between 5-HT_{2A} and muscarinic M1 receptor bindings ($r=0.44$, $p=0.087$) and negative correlations between muscarinic M1 and $GABA_A$ receptor bindings ($r=-0.49$, $p=0.057$) have been revealed. **Conclusions**: These results suggest that the related pathological alterations of the 5-HT_{2A}, muscarinic M1 and $GABA_A$ receptors contribute to pathophysiology of the STG in schizophrenia. There is a possible mechanism of auditory hallucinations through interactions between 5-HT_{2A}, muscarinic and GABA transmissions in the STG in schizophrenia.

ORAL-07-03

DIFFERENTIAL NEUROBEHAVIOURAL EFFECTS OF ACUTE AND CHRONIC CANNABINOID TREATMENT ON HETEROZYGOUS NEUREGULIN 1 MUTANT MICE

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Purpose: A mouse model for the schizophrenia candidate gene neuregulin 1 (*NRG1*) [i.e. transmembrane domain Nrg1 mutant mice (*Nrg1* HET)] exhibit a marked schizophrenia-related behavioural phenotype. Schizophrenia is a multi-factorial mental disorder and cannabis exposure may increase the risk of developing schizophrenia by unmasking the disorder in genetically vulnerable individuals. **Methods:** We investigated the neurobehavioural phenotype of male heterozygous *Nrg1* HET and their wild type-like (WT) littermates ($n \geq 10$), which were treated acutely/chronically with either vehicle or cannabinoids [acute: 5 and 10 mg/kg Δ-9-tetrahydrocannabinol (THC) / chronic: 0.4 mg/kg CP 55,490 (CP)]. All mice were tested in a variety of tasks for locomotion, exploration, anxiety and prepulse inhibition (PPI). Fos B/ΔFos B as well as c-Fos expression analyses determined neuronal correlates for the behavioural effects of cannabinoids. *Results:* Acutely, *Nrg1* HETs were more sensitive to the locomotor suppressant actions of THC and expressed a greater THC-induced enhancement in %PPI. Mutants were also more susceptible to the anxiogenic effects of THC. THC selectively increased c-Fos expression in the ventrolateral septum (LSV), the central nucleus of the amygdala and the paraventricular nucleus of *Nrg1* HETs. Chronically, *Nrg1* HET mice developed more rapidly tolerance to CP-induced hypothermia and locomotor suppression. Conversely, tolerance to the anxiogenic effect of CP was only observed in WT mice. A selective increase in Fos B/ΔFos B expression was detected in the LSV of *Nrg1* mutants following CP exposure. CP treatment on day 1 facilitated PPI in *Nrg1* HET mice and decreased it in WT mice. **Conclusion:** These data suggest that a variation in the *Nrg1* gene alters the sensitivity to the neurobehavioural effects of cannabinoids and results in differential adaptive processes to repeated cannabinoid exposure. Overall, our findings support the idea of an interactive relationship between neuregulin and the cannabinoid system.

ORAL-07-04

APOE EXPRESSION IS INCREASED AND LRP12 EXPRESSION IS DECREASED IN THE PREFRONTAL CORTEX OF SUBJECTS WITH SCHIZOPHRENIA

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Purpose: Altered apoE and reelin expression has been reported in subjects with schizophrenia. Both apoE and reelin mediate their activity through various members of the low density lipoprotein receptor-related protein (LRP) family. Thus, signalling through LRP molecules may represent sites of molecular dysfunction in the pathology of schizophrenia. We examined the expression of LRP2, LRP4, LRP6, LRP8, LRP10 and LRP12, representing different structural subtypes within the LRP family, as well as apoE and reelin expression in the post-mortem prefrontal cortex of subjects with schizophrenia. Methods: Western blotting was used to examine apoE and reelin protein expression in Brodmann's Area 46 from 15 subjects with schizophrenia and 15 pair matched control subjects. Real-time PCR was used to analyse LRP2, LRP4, LRP6, LRP8, LRP10 and LRP12 mRNA expression in Brodmann's Area 46 from subjects within the same cohort. Results: There was a significant increase in apoE protein expression in subjects with schizophrenia ($P<0.05$). LRP12 mRNA expression was significantly decreased in subjects with schizophrenia ($P<0.05$). No significant difference was observed in reelin protein levels nor in the mRNA levels of the other LRP molecules examined between control subjects and subjects with schizophrenia ($P>0.05$). Conclusion: Our data supports a role for ApoE and for LRP12 in the pathology of schizophrenia. Our data does not support the involvement of reelin in the pathology of schizophrenia.

ORAL-07-05

REGULATION OF TYROSINE HYDROXYLASE BY THE MALE-SPECIFIC GENE SRY: MECHANISM FOR SEXUAL DIMORPHISM IN DOPAMINE DISORDERS

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SRY (Sex-determining Region on the Y chromosome) is the key male sex-determination gene that directs embryonic gonads to develop as testes. Remarkably, our group has shown that *SRY* is also expressed in the brain, which includes areas such as the substantia nigra (SN) and ventral tegmental area (VTA) in males. We have shown that inhibition of *SRY* function in the rat SN led to a reduction in tyrosine hydroxylase (TH) expression and deficits in motor function. Thus, *SRY* plays an important role in positively regulating the biochemical and motor functions of dopamine neurons in the male brain. In the present study, a human dopaminergic neuronal cell line, NT2N, was used as an *in vitro* model to investigate putative *SRY*-mediated TH regulation in the brain. NT2 is a pluripotent embryonic teratocarcinomal cell line that differentiates into NT2N 'neurons' following retinoic acid (RA) treatment. Here, NT2 precursor cells were differentiated over a 28 day period into NT2N neurons and characterized for TH and *SRY* mRNA expression. Quantitative RT-PCR revealed a positive correlation between time of RA treatment and *SRY*and TH expression, with a 2.7-fold increase in *SRY* mRNA and 8.2-fold increase in TH mRNA expression (n=3, one-way ANOVA, $p<0.05$). Over-expression of *SRY* in NT2N cells by transfection induced a 2.8-fold increase in TH mRNA expression with respect to control cells transfected with empty vector (n=3, one-way ANOVA, $p<0.05$). Thus, *SRY* positively regulates TH expression in vitro, suggesting a mechanism by which *SRY* mediates dopamine levels and consequently motor function in males. In conclusion, the current study provides a molecular basis for gender differences in dopamine disorders such as Parkinson's Disease and Schizophrenia.

ORAL-07-06

THE DIFFERENTIAL EFFECT OF CLOZAPINE COMPARED TO OTHER ANTIPSYCHOTIC DRUGS ON CORTICAL AND STRIATAL CELL SIGNALLING: A NOVEL ANTIPSYCHOTIC DRUG MECHANISM

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Purpose: Antipsychotic drugs (APD) are the principal treatment for schizophrenia however, for many patients they are of limited benefit. For these treatment-resistant cases, the atypical APD clozapine is superior to other agents. The mechanism by which clozapine exerts this antipsychotic effect is unknown but may involve alternate cell signalling systems. A potential candidate is the mitogen activated protein kinase-extracellular signal regulated kinase (MAPK-ERK) cascade that links GPCR and ErbB growth factor signalling systems. We previously reported *in-vitro* that clozapine and other APD acutely inhibited ERK activation but only clozapine stimulated ERK with sustained treatment. This stimulation was mediated by the epidermal growth factor (EGF) receptor (ErbB1). Here we extend our findings *in-vivo* to determine if clozapine, haloperidol, quetiapine and aripiprazole differentially modulate the EGF-ERK1/2 pathway in prefrontal cortex (PFC) and striatum of C57Bl/6 mice (n=4) following acute treatment. **Methods:** Phosphorylation of the predominant neuronal ERK isoforms, ERK1/2 was measured by immunoelectrophoresis. **Results:** ERK1/2 phosphorylation was inhibited by clozapine at 20 and 60 min followed by subsequent activation at 8 hrs and normalization of the pERK1 response at 24 hrs. This *in-vivo* clozapine-induced ERK activation was significantly reduced by the EGF receptor inhibitor, AG1478, in both brain regions (PFC clozapine 8 hrs: 144.7±4% *vs* clozapine+AG1478 8 hrs: 46.7±10.7%, $p<0.001$). In contrast, aripiprazole triggered biphasic ERK phosphorylation in PFC, whilst haloperidol significantly stimulated pERK1 in striatum for up to 8 hrs. ERK activation seen with aripiprazole and haloperidol was not EGF receptor mediated. **Conclusion:** Clozapine recruitment of ErbB1 signalling to activate ERK1/2 may warrant investigation as a novel antipsychotic drug target for treatment-resistant patients.

ORAL-07-07

ANTIPSYCHOTICS REVERSE NMDA RECEPTOR ANTAGONIST-INDUCED ABERRANT CORTICAL γ OSCILLATIONS - A NOVEL MODEL OF ACUTE PSYCHOSIS?

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Purpose: A single non-anaesthetic injection of ketamine, a non-competitive NMDA receptor (NMDAr) antagonist with hallucinogenic properties, induces cognitive impairment and psychosis and aggravates schizophrenia symptoms. In conscious rats, an equivalent dose of ketamine or MK-801 increases the power of ongoing γ oscillations in the neocortex and concomitantly induces abnormal behaviour, including ataxia and hyperlocomotion, a key feature of animal models of acute psychosis. This study investigated whether NMDAr antagonist-induced aberrant γ oscillations are: 1) correlated with locomotion and 2) reversible with antipsychotic treatment. **Methods**: The relationship between quantitative measures of γ power and locomotion was assessed in 8 freely moving rats following a single injection of ketamine (<5 mg/kg) or MK-801 (<0.16 mg/kg). In subsequent experiments (n=8 rats), the effects of pretreatment with haloperidol or clozapine on NMDAr-induced increases in γ power and locomotion was assessed. **Results**: Ketamine and MK-801 induced correlated hyperlocomotion and aberrant γ oscillations. These effects were dose-dependently, but not entirely, inhibited by haloperidol treatment. **Conclusion**: The present study suggests that NMDAr antagonist-induced γ hypersynchrony represents an interesting biomarker to assess the action of antipsychotics.

ORAL-07-08

TRANSCRIPTIONAL INTERACTION BETWEEN AN ESTROGEN RECEPTOR SPLICE VARIANT AND ERBB4 SUGGEST CONVERGENCE IN MOLECULAR PATHWAYS CAUSING SCHIZOPHRENIA

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Purpose: We have previously found that variation in the estrogen receptor alpha (ERα) gene and mRNA are associated with schizophrenia. In particular, we showed that the delta-7 isoform of ERα significantly attenuates gene expression induced by wild-type ERα upon binding estrogen-response elements (ERE) demonstrating a dominant negative function. If aberrant ERα signaling in schizophrenia is causative in nature, it may interact directly with other causative pathways. The NRG1-ErbB4 pathway is a leading schizophrenia susceptibility pathway and reductions in the transcriptionally active form of ErbB4, the intracytoplasmic domain (ICD-ErbB4), has been shown in the brains of patients with schizophrenia. In this current study, we determine how the dominant negative ERα isoform, delta-7 interacts with ICD-ErbB4 to modulate gene transcription. Methods: Neuronal and non-neuronal cell-lines were transiently cotransfected with a 3x ERE luciferase reporter construct, ICD-ErbB4, wild-type and delta-7 ERα, and changes in ERE-mediated transcription were measured by luciferase reporter assay in triplicate. Confocal microscopy was to determine ERα-ICD localization within the cell. Results: We show that ICD-ErbB4 can potentiate the estrogen response through the wild-type receptor in neuronal and non-neuronal cell-lines (Interaction effect: $F(1, 8)=80.484$, $p=0.00002$). Overexpression of delta-7 interfered with this potentiation. Immunofluorescence microscopy revealed nuclear localisation of both wild-type and the delta-7 ERα with ICD-ErbB4. Conclusion: Our findings demonstrate synergy between ERα and ICD-ErbB4 in the transcriptional control of ER-target gene expression and suggests that this may represent a common molecular pathway which is disrupted in schizophrenia.

ORAL SESSION 8 – VISUAL PATHWAYS

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ORAL-08-01

UNIFORMITY DETECTOR RETINAL GANGLION CELLS RECEIVE ONLY INHIBITORY SYNAPTIC INPUT

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Uniformity detectors are an unusual type of retinal ganglion cell that were first discovered in the rabbit retina (Levick, 1967). They respond to steady illumination with a low maintained firing rate and are transiently suppressed by the introduction of bright or dark contrast within the receptive field. The purpose of this study was to characterise the dendritic morphology of the cells and to elucidate the synaptic mechanisms that underlie their receptive-field properties. **Methods:** Isolated preparations of the dark-adapted rabbit retina were viewed microscopically under infrared illumination and cells (n = 78) were targeted for electrophysiological recording on the basis of their soma size and shape. The physiological identity of the cells was confirmed by their extracellular spike responses to flashing spots of either contrast projected on the retina. The cells were then usually patched under voltage- or current-clamp; in some cases, the cells were labelled with Neurobiotin by semi-loose seal electroporation. **Results:** Uniformity detectors have a distinctive bistratified morphology, with recursive dendrites branching in the Off and On sublaminae, near the borders of the inner plexiform layer; the cells are coupled by gap junctions to a population of GABAergic amacrine cells. The transient stimulus-evoked inhibition of the maintained firing (18 ± 2 spikes/sec) is blocked by 1 μM strychnine, and the current-clamp recordings revealed that this glycinergic inhibition acts directly on the uniformity detector rather than on presynaptic bipolar cells. In fact, the uniformity detectors receive negligible excitatory input, and all of the synaptic currents in the cells were abolished in the presence of both glycinergic and GABAergic antagonists. **Conclusion:** The finding that uniformity detectors receive only inhibitory synaptic input is remarkable and this unique feature sets them apart from all other types of retinal ganglion cells, including the melanopsin ganglion cells.

ORAL-08-02

INFORMATION CARRIED BY THE SIGNALS OF NEURONS IN THE PRIMATE LATERAL GENICULATE NUCLEUS

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Purpose In primates two major pathways convey signals from the retina to visual cortex through the lateral geniculate nucleus (LGN): the parvocellular (P) and the magnocellular (M). We established the information carried by M- and P-cells by using their signals to reconstruct a flickering checkerboard. **Methods** Extracellular recordings were made from the LGN of anaesthetised marmosets (*Callithrix jacchus*) during presentation of a 256 element checkerboard. The luminance of each element was redrawn from a Gaussian distribution every 30 ms. A linear decoder reconstructed the time course of each checkerboard element from the train of impulses discharged by each neuron; to characterise the quality of reconstruction at the optimal element we estimated the signal-to-error ratios (SER) for 1 – 30 Hz. A lower bound on mutual information between the element and response is the integral of the SER. **Results** The peak SER of M-cells was on average 0.14 (at 7 Hz) and of P-cells was 0.10 (at 5 Hz); below 4 Hz P-cells showed higher SER than M-cells and above 5 Hz the SER of M-cells was greater than that of P-cells. When the receptive field was confined to one element the mutual information in M-cells was 3.8 bits/s (n = 33) and in P-cells was 2.2 (n = 41). When more than one element was in the receptive field, M-cells provided 1.5 bits/s (n = 59) and P-cells 0.7 (n = 45). **Conclusion** M-cells can provide more information about a flickering stimulus than P-cells, but because their receptive fields are smaller, for the same fine spatial scale P-cells surpass M-cells.

ORAL-08-03

CAT V1 CENTER-SURROUND INTERACTIONS CORRELATED TO THE ORIENTATION MAP

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Purpose: Orientation selectivity is a salient characteristic of the classical receptive field (CRF) of the great majority of cells in the striate cortex (V1, area 17). Furthermore it is well established that stimuli outside the classical receptive field modulate the center response. Yet the mechanisms that give rise to this phenomenon remain obscure. **Methods:** Here we used a combination of intrinsic optical imaging and single cell electrophysiology to target 27 V1 cells located in the pinwheels (PW) and domains (D) in four anesthetized and neuro-muscular blocked cats. The stimuli consisted of circular gratings optimized for aperture, orientation, contrast and temporal and spatial frequencies of the center presented in conjunction with an annulus varying in orientation extending 15 deg into the surround. **Results**: Our findings show that D-cells have sharp orientation tuning while PW-cells are more broadly tuned ($P < 0.0005$, Mann-Whitney U-Test). The extraclassical surround was broadly tuned for both Pinwheel and Domain cells at high center contrast while the surround was significantly ($P < 0.001$, Mann-Whitney U-Test) better tuned for domain cells at low contrast. **Conclusion:** In conclusion we show a functional correlation of the cell's location in the orientation map and it's center and surround tuning properties. The low contrast center may emphasize surround mechanisms that are cortically mediated supporting anatomical studies showing that long-range cortical inputs are better tuned to domains compared to pinwheel cells.

ORAL-08-04

PHASE- SENSITIVITY AND THE MAGNITUDE OF RESPONSES OF NEURONES IN THE CAT AREA 18

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The primary visual cortex of eutherian carnivores contains two subdivisions: cytoarchitectonic area 17 (area V1), and area 18, presumed homolog of the 'higher-order' area V2 of other mammals. In area 17, neurons are routinely classified as members of two, presumably functionally distinct classes, the 'simple' and 'complex' cells. **Purpose:** To examine to what extent distinct simple and complex cell classes can be distinguished in area 18. **Methods:** We analysed the spike-responses of single neurones recorded from area 18 of anaesthetized domestic cats to optimised patches of achromatic, drifting sine-wave gratings. For each cell we calculated the ratio of the modulated (F1) component of spike-responses to the mean discharge rate (F0). **Results**: Surprisingly, the cells which like classically defined simple cells had spatially distinct ON- and OFF-discharge regions and were strongly phase-sensitive ($F1/F0 \geq 1.5$) constituted almost half (36/73) of the sample ('simple+' cells). By contrast, presumptively 'higher-order', 'complex+' cells with overlapping ON- and OFF-discharge regions and very little phase-sensitivity ($F1/F0 \leq 0.5$) constituted only a small proportion (11/73) of the sample. As is the case of area 17 neurones (Bardy, Wang, Huang, FitzGibbon, Dreher unpublished observations), the mean peak firing rates to optimized gratings of complex+ (71.5 ± 26 spikes/s) and simple+ (43 ± 31.5 spikes/s) cells were significantly higher than these (20.5 ± 8 spikes/s) of 'intermediate' cells (characterized by F1/F0 of 0.5-1.5). **Conclusions:** The fact that V2 neurones with 'metabolically expensive' peak discharge rates (>25 spikes/s) dominate numerically complex+ and simple+ classes, suggest that these classes might play distinct roles in processing of visual information.

ORAL SESSION 8 – VISUAL PATHWAYS *(contin.)*

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ORAL-08-05

USING RANDOM WALKS TO REDEFINE SIMPLE AND COMPLEX CELLS IN THE VISUAL CORTEX

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Mammalian visual cortical cells are typically classified as 'simple' or 'complex' depending on their responses to moving contrast-modulated gratings. Responses of simple cells contain a large component that oscillates at the temporal frequency of the grating (amplitude, F1) and a smaller DC response (amplitude, F0) whereas complex cells are the opposite. The quantitative measure used to distinguish between simple and complex cells is the ratio of the amplitudes of these components, the F1/F0 ratio. Arbitrarily and independent of the number of spikes produced, those cells with F1/F0>1 are classified as simple and those with F1/F0<1, complex. **Purpose:** We sought to produce a less arbitrary classification scheme of simple and complex cells which takes into account the number of spikes produced. **Methods:** We used computational and analytic methods to calculate the probability distribution of F1/F0 ratios of an ideal complex cell that produces *n* spikes with equal probability over a single grating cycle. For each value of *n* we found the F0/F1 value that splits the probability distribution into a lower 95% and an upper 5% (x(*n*)). This value is then used to determine whether a cell is simple (F1/F0>x(*n*)) or complex (F1/F0≤x(*n*)). **Results:** When our F1/F0 data was reanalyzed using this scheme we found that a significant number of cells previously defined as complex were now simple. We also show that the distribution of F1/F0 ratios produced by our model is homologous to the distribution of distances produced by a random walk in two dimensions. **Conclusions:** Our novel classification scheme improves those currently used and provides a theoretical basis upon which simple and complex cells can be distinguished.

ORAL-08-06

ROLE OF INHIBITION IN STRIATE CORTICAL ORIENTATION SELECTIVITY REVEALED THROUGH A NOVEL PARADIGM

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Purpose: Using a novel protocol of electrical stimulation in the lateral geniculate nucleus (LGN), Kara et al. (*PNAS*,99(25),16261-66) recently claimed that the raw excitatory input to a striate cortical cell is already well tuned for orientation. However, such selectivity can be a consequence either of the excitatory convergence proposed by Hubel and Wiesel (*J.Physiol.*,160(1),106-54), or of simply sharpening LGN orientation biases by the enhanced intrageniculate inhibition from the electrical stimulation. **Methods:** We addressed this issue by recording from 13 cat LGN cells during application of the stimulation protocol (100µs pulses, every 133 msec) using two tungsten electrodes glued to each other (one recording, the other stimulating). Current strength was adjusted to levels below where local inhibition was just sufficient to suppress all spike responses to drifting visual stimuli, simulating the inputs arriving in the cortex via single geniculate afferents in Kara et al's design. **Results:** We found that (1)most LGN cells showed the well-known orientation biases (Circular Variance(CV) = 0.73±0.06 se. & Orientation selectivity index(OSI) = 0.72±0.09 se.) and (2)the electrical stimulation led to significant ($p<0.05$,Wilcoxon test) sharpening to levels comparable to cortical orientation selectivity (CV=0.89±0.02 & OSI=0.41±0.05). **Conclusions:** These results suggest that even local non-specific inhibition can sharpen geniculate orientation biases to cortical levels and support the model that intra-cortical inhibition usually provides such sharpening leading to the narrowing of the post-synaptic excitation in the cortex from biased geniculate inputs (Vidyasagar et al, *Trends Neurosci*,96(7),272-7). These experiments, while supporting Kara et al's finding that the geniculate afferent input is a critical determinant of cortical orientation selectivity, refute their conclusion that their findings support the model of excitatory convergence.

ORAL-08-07

STIMULUS SPEED SELECTIVITY AT THE PERIPHERAL REPRESENTATION OF VISUAL AREA V1

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Purpose: Estimating the speed of complex objects in motion, a crucial task for survival, is a non-trivial computational problem for the visual cortex because its retinal inputs are only sensitive to the rate of temporal change (or temporal frequency, TF) of spatial patterns. This provides an ambiguous estimate of speed for patterns of different spatial frequencies (SF). The extent to which cortical areas representing different hierarchical levels are responsible for the creation of robust neural representations of speed remains controversial. Here, we explored whether neurons in the primary visual area (V1) are selective to speed in a SF-invariant manner. Given the emphasis of detailed image analysis at the fovea, and the importance of detecting self-motion and fast approaching objects in the periphery, we hypothesized that the distribution of speed-tuned neurons in V1 is dependent on receptive field eccentricity. **Methods**: To test this hypothesis, we recorded extracellular activities of neurons in V1 of 7 marmosets, anaesthetized with Alfaxan followed by intravenous sufentanil (combined with nitrous oxide). Each neuron was stimulated with high-contrast, drifting sinusoidal gratings of optimal orientation, whose SF and TF varied independently over a range of 4.5 octaves. Speed-tuned neurons were identified as those whose responses were better fitted by a model in which peak TF responses increased with SF, compared to a model assuming independent SF and TF tunings. **Results**: We found that speed tuning was common in the peripheral representation of V1 (eccentricity > 40 degrees), where 25 of 72 recorded cells showed SF-dependent TF tuning. In contrast, only 3 of 90 cells recorded closer to the fovea (eccentricity < 20 deg) were speed-tuned, demonstrating a highly significant difference (chi-square= 25.41; $P<0.001$). **Conclusion**: This result highlights differences between neural substrates of central and peripheral vision, and provides evidence for the specialized role of motion processing in the peripheral representation of V1.

ORAL-08-08

CONJUNCTION CODING OF COLOUR AND MOTION IN HUMAN VISUAL CORTEX

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Purpose: Colour and motion serve as the prime examples of segregated processing in the visual brain. Here we examined where in the visual brain colour-motion conjunctions are represented. **Method**: Human volunteers (n = 5) viewed visual displays containing rotating coloured dots. The dots could be red or green, and rotate clockwise or counter-clockwise, leading to four possible stimulus combinations. Superimposed pairs of such stimuli provided two additional displays each containing both colours and both directions of motion but differing in their feature-conjunctions. We applied multivariate classifiers to voxel activation patterns obtained whilst subjects viewed such displays. **Results**: As well as confirming the presence of information on direction of motion across visual cortex, our analyses provide evidence of hue coding in all early visual areas except V5/MT+ and demonstrate the explicit representation of feature conjunctions in primary visual cortex and beyond. Within each visual area, information on colour, motion and their conjunctions appears to be coded in distinct sets of voxels. **Conclusions**: Our findings suggest that what has been taken as the prime example of the binding problem may be solved as early as V1.

ORAL-09-01

ANALYSIS OF THE PATTERNS OF AGGREGATION OF MUTANT SUPEROXIDE DISMUTASE 1 IN PRIMARY MURINE ASTROCYTIC CULTURES

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Previous studies have shown that inclusions of mutant superoxide dismutase 1 (mSOD1) are associated with motoneuron disease, although the exact cellular mechanisms mediating mSOD1-induced toxicity remain unclear. Recent evidence suggests that aggregation of misfolded SOD1 in astrocytes may contribute to the degeneration of motoneurons. **Purpose:** These studies investigated the patterns of mSOD1 aggregation *in vitro* in astrocytes. **Methods:** Cultures of astrocytes (n = 4 cultures) were established from forebrains of C57black6 mice, postnatal day 1.5 and maintained in DMEM with 10% (v/v) fetal calf serum and 1% (v/v) penicillin-streptomycin. At 21 days *in vitro*, cells were transfected for up to 72 hours with wild-type (WT) or mutant (A4V or G85R) SOD1-EGFP constructs. Neurochemical and cytochemical procedures were employed to analyse cellular responses to SOD1 species. **Results:** In cells expressing EGFP-tagged WT SOD1, the fluorescent protein was distributed evenly throughout the cell, whereas cells transfected with either species of mSOD1-EGFP showed bright intracellular inclusions. These apparent aggregates partially localized with GFAP. Western immunoblotting, probing with anti-SOD1 antibody, revealed the presence of hSOD1-EGFP monomers (~50kDa) in mSOD1 transfected species and endogenous SOD1 protein (~20kDa) in all samples. A4V, which is more toxic than G85R, produced significantly higher levels of aggregate-like inclusions than G85R, and aggregate formation in response to both forms of mSOD1 was time-dependent over 72 hours (all $P<0.05$). **Conclusion:** These data validate our model of mSOD1 aggregation and provide new evidence on the involvement of astrocytic mechanisms in motoneuron pathology.

ORAL-09-02

CU(ATSM) DELAYS DISEASE PROGRESSION AND INCREASES SURVIVAL IN A TRANSGENIC SOD1G93A MOUSE MODEL OF ALS

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Amyotrophic Lateral Sclerosis (ALS) is a common form of motor neuron disease. Although the aetiology of this debilitating disease remains unclear, more than 100 different mutations in the copper-zinc superoxide dismutase (SOD1) gene can cause familial ALS, implicating a gain in toxic function leading to the motor neuron degeneration possibly via oxidative stress. Transgenic mice overexpressing human mutant SOD1G93A (TgSOD1G93A) produce a phenotype that closely replicates both clinical and pathological hallmarks of human ALS. Diacetylbis(N4-methyl-3-thiosemicarbazonato) copper(II) (Cu(ATSM)) is a metal complex that crosses the BBB. **Purpose:** This study was carried out to evaluate the physical and biochemical effects of Cu(ATSM) treatment on the TgSOD1G93A murine model of ALS. **Methods:** TgSOD1G93A mice in C57B6 background were treated with Cu(ATSM) (n=14) or vehicle (n=18) orally commenced at the pre-symptom age of 140 days. Clinical assessment and motor function tests including rotarod and stride length were performed. Zymography and ELISA were performed to measure matrix metalloprotease-9 (MMP-9) activity and level in serum. Oxidative damage markers were investigated in spinal cords. **Results:** Disease onset was significantly delayed in TgSOD1G93A mice treated with Cu(ATSM) (mean onset age (±SEM) of 261±5.4 days, compared to 241±1.7 days for vehicle treated control mice, $p<0.001$). Cu(ATSM) also extended the life span of TgSOD1G93A mice by 37 days (14%, $p < 0.0001$). MMP-9 activity and level, which decrease as disease symptoms develop, were significantly restored to pre-symptomatic levels in Cu(ATSM) treated TgSOD1G93A mice. Protein carbonyl levels were reduced. **Conclusion:** Cu(ATSM) may prevent motor neuron deterioration caused by the SOD1G93A mutation. This may involve the compound's potential to restore MMP-9 activity and more importantly reduce oxidative stress in these mice. This study will facilitate the development of new therapeutic strategies.

ORAL-09-03

THE NEUROMOTOR SYSTEM IN A TRANSGENIC MOUSE MODEL OF MOTOR NEURONE DISEASE

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Purpose: Transgenic mice overexpressing a G93A mutation in the human superoxide dismutase 1 gene ($hSOD1^{G93A}$ mice) are a model of human motor neurone disease, and develop muscle weakness at 2-3 months, progressing to paralysis and death. These mice also show motor neurone hyper-excitability from birth, but whether this is associated with changes in neuromotor performance prior to the onset of muscle weakness is unknown. **Methods:** We investigated whether neuromotor performance in a narrow beam test was significantly altered in $hSOD1^{G93A}$ mice (n= 4) compared to wild type (WT) littermates (n= 6). Mice were placed on the edge of a Perspex strip (5 mm wide, 40 cm long) mounted 40 cm above surrounding surface in a dim, quiet environment and scored (by single scorer blind to genotype) for balance (-2/+2), balancing on hind legs (0/+2), movement (-2/+2), movement type (-1/+2), interest in surroundings (0/+1), falling off (0/-5) and cumulative score over a 2 minute session every 2-5 days from 20 to 90 days postbirth. Scores were analysed by a different person; scores for each genotype were blocked into 5 day groups and analysed with a 2 way ANOVA for age and genotype. **Results:** Cumulative, movement and movement type scores were all significantly different ($P<0.05$) for genotype; $hSOD1^{G93A}$ mice had lower scores. **Conclusion:** A simple narrow beam neuromotor performance test shows that $hSOD1^{G93A}$ mice have detectable neuromotor deficits compared to WT mice from 3 weeks onwards, well before onset of motor weakness at 8-12 weeks. This test may be useful for monitoring effects of early treatment strategies in this widely used mouse model of motor neurone disease.

ORAL-09-04

TRP CHANNELS REGULATE TRANSTHYRETIN-INDUCED CALCIUM INFLUX IN DRG NEURONS

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Familial amyloidotic polyneuropathy (FAP) is a peripheral neuropathy caused by the extracellular accumulation of insoluble, misfolded transthyretin (TTR) amyloid protein. TTR (a 48kD homo-tetrameric protein also known as pre-albumin) is normally synthesised in the liver and choroid plexus and is an important carrier of thyroid hormones throughout the body. Although relatively rare, FAP is invariably fatal with profound neurological disturbances to autonomic and sensory nervous system circuits. Common TTR mutants responsible for FAP show aberrant aggregation and cytosolic calcium dysregulation in cell lines. The exact mechanisms responsible for these effects are unknown at present and the aim of the present study was to investigate the actions of TTR in a peripheral cell model. Here we show the most clinically aggressive TTR mutant, L55P, disrupts calcium homeostasis in rat spinal neurons in a mechanism that is mediated by transient receptor potential cation channels (TRPs). Using dynamic light scattering, we show that L55P contains distinctive oligomeric species not present in wild-type forms. It is hypothesised that oligomers, rather than fibrils, interact with plasma membrane receptors, causing calcium influx. Using calcium imaging of dorsal root ganglion (DRG) neurons, we show that L55P induces a calcium influx sensitive to inhibitors of store-operated (5μM SKF96365, 53% of maximal response, n=6), voltage-gated calcium (5μM nifedipine, 32%, n=6), TRP (5mM lanthanum, 58% n=4) and sodium (2μM tetrodotoxin, 38% n=4) channels. These results suggest that TRPs and voltage-gated channels act in concert at the cell membrane to induce calcium influx in response to TTR. Significantly, such a mechanism for the action of TTR on cation-channels in neurons would greatly enhance our understanding of FAP pathogenesis as well as calcium dysregulation in other amyloidoses, such as Alzheimer's disease.

ORAL-09-05

REGULATION OF HUMAN ALPHA-SYNUCLEIN GENE EXPRESSION BY LIPIDS

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Parkinson's disease (PD) is an idiopathic neurodegenerative disorder. Lewy bodies are neuropathological inclusions found in PD brain and are composed of aggregated proteins including α-synuclein and other components including lipids. Alpha-synuclein is a 14 kDa intracellular neuronal protein that is found natively in its monomeric form but undergoes aggregation in PD. While its exact function is unknown, alpha-synuclein binds phospholipids, fatty acids, and cholesterol which has raised the question as to whether alpha-synuclein can be considered as a specialised apoplipoprotein. Previous work has shown that increased cellular alpha-synuclein predisposes towards aggregation, however, very little is known regarding the regulation of alpha-synuclein gene expression in the brain. We have recently identified putative binding sites for the nuclear hormone receptor liver X receptor (LXR) in the alpha-synuclein gene. Oxysterols regulate gene expression by activating LXR. **Purpose:** To determine whether oxysterols or other LXR ligands regulate alpha-synuclein expression. **Results:** Using western blotting and quantitative PCR analysis we have shown that neuroblastoma and oligodendrocyte cell lines, and primary human neurons treated with LXR ligands TO-901317, GW-3695 or 27-hydroxycholesterol significantly up-regulate their alpha-synuclein expression approximately 2-fold. **Conclusion:** These data indicate for the first time that alpha-synuclein gene expression may be at least partially regulated by LXR.

ORAL-09-06

AN IN VIVO MODEL OF LEWY BODY DEVELOPMENT IMPLICATES AUTOPHAGY AS CENTRAL TO PARKINSON'S DISEASE PROGRESSION

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Purpose: Parkinson's Disease (PD) is characterized by the accumulation of proteinaceous cytoplasmic inclusions (Lewy bodies, LB) followed by the degeneration of dopamine neurons in the Substantia Nigra pars compacta (SNpc). Currently no reliable model of PD including progressive LB formation exists. We identified that the Dopamine Receptor 2 knockout mouse (D2(-/-)) develops LB's in an age dependent manner and displays a loss of SNpc terminals in the striatum in association with accumulating α-synuclein protein. The D2(-/-) mouse therefore provides a model of LB development and potentially a model of progressive PD. We used this mouse model to investigate the mechanisms underlying the formation of LB's. **Methods:** The SNpc of young (3 months) and old (20 months) wild type and D2(-/-) mice were examined using real-time PCR, western blotting and immunohistochemistry. **Results:** In contrast to the accepted mechanisms of PD aetiology, treatment of D2(-/-) mice to increase oxidative stress or inhibit the ubiquitin proteosome system (UPS) did not increase LB formation or result in a loss of SNpc neurons. Furthermore, failure of Endoplasmic Reticulum (ER) to Golgi transport caused by α-synuclein accumulation was not supported as a major contributor to LB formation as only a partial ER stress response was induced in D2(-/-) mice. Investigation of the autophagic system revealed an upregulated response in aged D2(-/-) mice ($P<0.01$) suggesting autophagy is the primary mechanism of LB formation. **Conclusion:** These results suggest that autophagy and not the UPS is the primary protein clearance pathway contributing to the formation of LB's and supports the emerging role of the autophagic system in the clearance of aggregated protein in PD.

ORAL-09-07

DIVERGENT PHOSPHORYLATION PATTERN OF TAU IN P301L TAU TRANSGENIC MICE

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Purpose: Aggregates of hyperphosphorylated tau are prominent in brains of patients with Alzheimer's disease or frontotemporal dementia (FTD). They have been reproduced in animal models following the identification of tau mutations in familial cases of FTD. This includes our previously generated transgenic model, pR5, which expresses FTD (P301L) mutant tau in neurons. The mice are characterized by tau aggregation including tangle (NFT) formation, memory impairment and mitochondrial dysfunction. In 8-month old mice, S422 phosphorylation of tau is linked to NFT formation, however, a detailed analysis of tau solubility, phosphorylation and aggregation until a high age has not been done. **Methods:** Here, we undertook an analysis by immunohistochemistry, Gallyas impregnation and Western blotting of brains from 3 (n=6), 6 (n=3) and 20 (n=3) month-old mice. **Results:** NFTs first appeared at 6 months in the amygdala, followed by the CA1 region of the hippocampus. As the mice get older, the solubility of tau is decreased as determined by sequential extractions. Histological analysis revealed increased phosphorylation at the AT180, AT270 and 12E8 epitopes with ageing. The numbers of AT8-positive neurons increased from 3 to 6 months old. However, whereas S422 appeared only late and concomitantly with NFT formation, the only neurons left with AT8-reactivity at 20 months were those that had undergone NFT formation. **Conclusion:** As hyperphosphorylated tau continued to accumulate, the lack of AT8-reactivity suggests regulatory mechanisms in specifically dephosphorylating the AT8 epitope in the remaining neurons. Thus, differential regulation of phosphorylation is important for NFT formation in neurodegenerative diseases with tau pathology.

ORAL-09-08

METALLOMICS OF NEUROMELANIN IN PARKINSONIAN SYNDROMES

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Purpose: Changes in tissue metal content are suggested to contribute to neurodegenerative cascades in Parkinson's disease (PD). In this work, changes in neuromelanin (NM) structure and NM-associated metals were investigated using highly sensitive, high resolution methods. **Methods:** Twenty micron formalin-fixed sections of the substantia nigra (SN) were prepared from five controls, five idiopathic PD, three Incidential Lewy body disease (ILBD) and three Alzheimer's disease (AD) brains. X-ray absorption microspectroscopy (micro-XANES) determination of sulfur oxidation state within NM was performed and a quantitative description of the spectra was estimated by optimizing a linear combination of the set of known reference compounds from different sulfur species. NM-metal content was measured in 6-7 individual pigmented cells per case using synchrotron x-ray fluorescence and in 600x600 micron SN samples using particle-induced X-ray emission (PIXE). **Results:** The sulphur environment of the pigment polymer and concentrations of NM-associated calcium, manganese, iron, zinc and selenium did not differ between diagnostic groups. In contrast, using both X-ray fluorescence and PIXE on independent tissue sections, NM-associated copper (Cu) was found to be significantly decreased in PD and ILBD, but not in AD. **Conclusions:** This work reveals a novel decrease in NM-associated Cu content in PD. Decreased NM-associated Cu was also seen in ILBD, a disorder suggested to represent preclinical PD, suggesting this change occurs early in these synucleinopathies. Our findings may be relevant for Cu-containing proteins, such as superoxide dismutase and α-synuclein, thought to be involved in degenerative mechanisms in parkinsonian syndromes.

ORAL-10-01

HOW CAN TOO MUCH STARGAZIN CAUSE EPILEPSY?

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Transmembrane AMPA receptor regulatory proteins (TARPs) are critical in trafficking and anchoring AMPA receptors to synapses and therefore define neuronal excitability. Purpose: Linkage analysis in GAERS, a genetic rat model of absence epilepsy, identified a quantitative trait locus on chromosome 7 containing the TARP gene, stargazin. We have demonstrated that GAERS have increased stargazin expression in thalamocortical brain regions (Neurobiology of Disease 2008;31:261-5). Here we investigated the hypothesis that the mechanism by which this is pro-epileptogenic is via upregulation of AMPA receptor subunit plasma membrane expression. Methods: RNA was extracted from somatosensory cortex of juvenile and adult control and GAERS. Western Blots were done to examine stargazin and AMPA receptors protein expression in plasma membrane and cytosol. PCR exon scanning was performed to examine the presence of splice variants. Results: Stargazin protein expression was increased in the membrane of juvenile (n=4, $p<0.05$) and adult GAERS (n=4; $p<0.05$) and not in the cytosol. Additionally, in adult GAERS, there was an increase in membrane expression of GluR1 (n=4, $p<0.05$) and GluR2 (n=4, $p<0.05$) subunits of AMPA receptor. In juvenile GAERS, there was a trend for a decrease for GluR1 and no changes in GluR2 expression. No splice variants were detected. Conclusions: GAERS have increased stargazin membrane protein expression in the somatosensory cortex, the cortical focus of seizure generation. This is present in pre-epileptic juvenile GAERS and therefore is not merely a secondary consequence of the seizures. Abnormal expression of stargazin is associated with greater membrane expression of AMPA receptors, hence presumably enhanced responsiveness to glutamate and therefore a hyperxcitable, pro-epileptic somatosensory cortex.

ORAL-10-02

REGULATION OF RELEASE PROBABILITY AND FUNCTIONAL HETEROGENEITY AT DOPAMINERGIC SYNAPSES

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Purpose: Presynaptic function in glutamatergic neurons is heterogeneous across synapses. Relatively little is known about synapses formed by dopaminergic (DA) neurons. It is unclear whether DA synapses exhibit functional heterogeneity. We therefore examined this question *in vitro*. **Methods:** We studied synaptic vesicle exocytosis in cultured DA neurons using FM 1-43 and retrospective immunocytochemistry. Data modelling was used to examine the kinetics of vesicle release. **Results:** Synaptic probability of release (Pr) varied between individual DA synapses (n = 106). DA terminals generally exhibited lower Pr than hippocampal (Hpc) terminals (n = 81). DA terminals also exhibited less variation in Pr compared to Hpc terminals. We next investigated factors that caused DA terminals to exhibit lower Pr. We found that the recycling SV pool, a factor that is classically linked to Pr, was smaller in DA neurons than Hpc neurons. DA synapses demonstrated less overall heterogeneity in pool size than Hpc synapses. We also found that Pr was independently regulated by an additional novel factor, which was reflected in the time constant of FM 1-43 destaining, τ. τ differed significantly between DA and Hpc synapses. DA terminals exhibited a greater degree of heterogeneity in values of τ than Hpc terminals. **Conclusions:** These findings demonstrate that DA synapses are functionally heterogeneous. DA synapses exhibit lower Pr than glutamatergic Hpc synapses, which is attributable to differences in at least two independent factors. The degree of functional heterogeneity also differs between DA and glutamatergic synapses. These findings suggest that mechanisms regulating presynaptic function differ between DA and glutamatergic neurons at a molecular level.

ORAL-10-03

HOW CHANGES IN PRESYNAPTIC STIMULUS RATE DETERMINE THE TOTAL CURRENT GENERATED BY TYPE I AND II SYNAPSES

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Two functionally distinct synaptic types exist in somatosensory cortex: type I depress due to vesicle depletion and recover at a constant rate; type II depress independent of vesicle depletion and recover faster when stimulated at higher frequency. A model encapsulating these features was developed and biologically realistic parameters determined for both types. **Purpose**: To examine how different stimulus rates determine the total postsynaptic current generated by the synaptic types by comparing charge transfer characteristics. **Methods**: 1500 independent but identical synapses connect to a spherical cell, and are driven by random (Poisson) trains of action potentials at frequencies between 5 and 50 Hz. EPSC time course is calculated using an α-function with a peak of -40 pA at 1 ms and decay over 10 ms. The total current generated by simultaneously activating 1500 synapses is then determined. A thinning technique was used to generate inhomogeneous Poisson processes as a result of rate changes. **Results**: Instantaneous rate changes (5–50 Hz) produced large changes in currents, sustained by type II but not type I. Frequency changes at high rates (30–50 Hz) show small current changes at type I, but more significant ones at type II. Type II synapses followed linear, exponential or sinusoidal changes in frequency well. However, for all cases type I synapses could not follow changes of frequency >10 Hz on either the on or off response. When chirps were explored, only type II followed changes in frequency well but type I synapses demonstrated phase precession of ~40°. **Conclusion**: Biologically realistic type II synapses endow a connection with the ability to faithfully transmit action potential rates; in contrast, due to phase precession in serially connected neurons, frequency changes could be relayed much faster via type I than at type II synapses generating differential latencies.

ORAL-10-04

THE DYNAMIN 1 SPLICE VARIANTS HAVE DISTINCT IN VIVO PHOSPHO-REGULATION

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Purpose. Dynamin 1 (Dyn1) is crucial for the fission stage of synaptic vesicle endocytosis (SVE). The endocytic function of Dyn1 is phosphorylation-regulated. Dephosphorylation at two major sites Ser-774 and Ser-778 regulates endocytosis. The two main splice variants of dyn1 in the rat brain differ only in their C-terminus. Dyn1xa is 13 amino acids longer than dyn1xb and contains two extra phosphorylation sites, Ser-851 and Ser-857. We sought to determine how Ser-774 and Ser-778 are distributed on the splice variants, and to examine the potential role of Ser-851 and Ser-857 in Dyn1xa. **Methods.** We separated Dyn1xa and Dyn1xb with a modified SDS-PAGE procedure and employed iTRAQ-labelling to quantify their phosphorylation status using mass spectrometry. **Results. (1)** In the synaptosome, Dyn1xa has a higher phosphorylation stoichiometry than Dyn1xb for the Ser-774 and Ser-778 di-phosphorylation (n=3). This Dyn1xa di-phosphorylation is also strongly dephosphorylated during synaptosomal depolarization (n=3) compared to Dyn1xb. **(2)** Comparing whole brain and synaptosome, Dyn1xb phosphorylation stoichiometry remains equal (n=3). However, Dyn1xa had more Ser-774/Ser-778 double phosphorylation and Ser-851 and Ser-857 single and double phosphorylation (n=3) in the brain. **(3)** Comparing synaptosomal cytosolic and membrane fractions, significant enrichment of the aforementioned phosphorylation sites was observed in the cytosolic fraction, not in the membrane fraction where endocytosis occurs (n=3). **Conclusions.** Dyn1xa and xb have distinct phosphorylation profiles at different parts of the neuron. We propose that phosphorylation might differentially regulate localization of Dyn1xa and xb, and this raises the possibility of distinct functional roles for each splice variant in SVE.

ORAL-10-05

IDENTIFYING AND PREDICTING AXONAL DYSFUNCTION IN OXALIPLATIN-INDUCED NEUROTOXICITY

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Purpose Neurotoxicity is the major dose-limiting toxicity of oxaliplatin treatment, a platinum-derivative chemotherapy effective against colorectal cancer. Sensory and motor neurotoxic symptoms arise acutely during oxaliplatin infusion while chronic sensory neuropathy develops at higher cumulative doses. To clarify the pathophysiological mechanisms of acute and chronic oxaliplatin-induced neurotoxicity, axonal excitability studies were undertaken longitudinally in patients undergoing oxaliplatin treatment. **Method** Axonal excitability studies were undertaken in 25 patients across 90 cycles of oxaliplatin treatment, before and within 2-3 days of infusion. A subset of 17 patients were assessed longitudinally across treatment. Median nerve was stimulated at the wrist, recording sensory nerve action potentials from the 2nd digit and compound motor action potentials from abductor pollicis brevis. Excitability parameters were recorded including refractoriness (RF; marker of transient Na+ channels), superexcitability (SX; determined by fast K+ channels) and threshold electrotonus (TE; marker of internodal function). **Results** Following oxaliplatin infusion, sensory axons demonstrated reductions in refractoriness and superexcitability (RF 7.0± 3.5, p≤.005; SX -1.7±.46, p≤.001) while conversely, refractoriness increased (-12.0± 4.2, p≤.001) in motor axons. However, longitudinal assessment revealed changes only in sensory axons with cumulative changes across treatment (TE p≤.005; RF p≤.001; SX p≤.001). By final treatment, sensory amplitudes decreased (p≤.001), while motor amplitudes remained unchanged. **Conclusion** Oxaliplatin produces differential responses in motor and sensory axons, correlating to the clinical expression of symptoms. Axonal excitability studies provide information regarding ion channel function and axonal function in acute neurotoxicity and the development of chronic neuropathy. Results from the present series suggest that excitability techniques may successfully identify patients at-risk of developing neurotoxicity.

ORAL-10-06

NEUROTOXICITY IN ACUTE INTERMITTENT PORPHYRIA – UNDERSTANDING THE MADNESS OF KING GEORGE

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Purpose: Acute intermittent porphyria (AIP) is a rare metabolic disorder characterized by mutations of the porphobilinogen deaminase (PBGD) gene that leads to deficient enzyme activity and accumulation of porphyrins. Clinical manifestations of AIP include nerve dysfunction although the underlying pathophysiology of porphyric neuropathy (PN) remains unclear. The aim of the study is to investigate the mechanism of neurotoxic effects of porphyrins using axonal excitability techniques. **Methods**: 20 AIP patients were investigated through genetic screening, clinical biochemistry, nerve conduction studies, and multiple nerve excitability protocols. Excitability studies were undertaken on median motor axons included: stimulus-response curves, strength-duration time constant, threshold electrotonus (TE), recovery cycles and current-threshold (I/V) relationships. **Results**: Latent AIP patients who carried genetic mutations demonstrated normal nerve excitability. Patients who had porphyric attacks without neuropathy (AIPWN) showed significant differences in the hyperpolarizing I/V slope (AIPWN: 0.28±0.02, controls: 0.35±0.01; p<0.01). Mathematical modelling of these changes was consistent with reduced inward-rectifying conductance (I_H) by 27%. In contrast, nerve excitability studies undertaken during acute neuropathy demonstrated that axons were of high threshold and TE curves were "fanned-in", consistent with membrane depolarization, similar to post-ischaemic changes. **Conclusions**: It is proposed that porphyrin neurotoxicity is associated with reduction in IH in AIPWN axons, with subsequent dysfunction of the Na^+/K^+ pump contributing to the clinical features of acute porphyric neuropathy.

ORAL-10-07

NEUREGULIN MODULATES ACETYLCHOLINE RECEPTOR CLUSTERING VIA NON-TRANSCRIPTIONAL MECHANISMS

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Purpose: Neuromuscular synapse formation is driven by two nerve-derived molecules, agrin and neuregulin. Agrin signals through the Muscle Specific Kinase (MuSK) receptor to cluster existing acetylcholine receptors (AChRs) in the postsynaptic membrane. Neuregulin signals via ErbB kinase receptors to induce synaptic gene transcription. Studies suggest that neuregulin can regulate agrin-induced AChR cluster formation. We aimed to determine the mechanism by which neuregulin modulates this process. Methods: Mouse myotubes were cultured and treated with agrin and/or neuregulin. Specific inhibitors of selected second messengers were used to determine the effects on AChR cluster numbers. AChR clusters were quantified according to previous guidelines (Ngo et al., 2004). Real-time PCR and western blots were used to assess the mRNA and protein expression of synaptic molecules, and immunoprecipitation assays were used to assess MuSK phosphorylation. Results: Neuregulin potentiated agrin induced AChR clustering not by its known transcriptional mechanism, but rather by enhancing the phosphorylation status of MuSK (n=6). This enhancement appears to be by inhibiting Shp2 mediated de-phosphorylation of MuSK (n=3). This modulation, by neuregulin of agrin-induced AChR clustering was also observed following injections of neuregulin into embryonic muscles. Conclusion: This study shows that neuregulin induced signalling can modulate agrin/MuSK signaling via a new posttranslational manner to enhance agrin's ability to cluster postsynaptic AChRs.

ORAL-10-08

PATIENT AUTOANTIBODIES REVEAL A ROLE FOR THE POSTSYNAPTIC MUSCLE SPECIFIC KINASE IN SYNAPSE MAINTENANCE AT THE MATURE NEUROMUSCULAR JUNCTION

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Purpose: At the embryonic neuromuscular junction (NMJ), agrin secreted by the nerve terminal activates postsynaptic Muscle Specific Kinase (MuSK) to stabilize the acetylcholine receptor (AChR) cluster. Myasthenia gravis (MG) is an autoimmune disorder of the NMJ that causes muscle weakness in adults. Autoantibodies against MuSK are the cause of about 10% of MG cases. Here we investigate the effects of these antibodies on the NMJ. **Methods**: IgG from anti-MuSK-positive MG patients (anti-MuSK IgG) or control human IgG was added to cultured C2 myotubes or injected intraperitoneally into 6-week C57Bl6J female mice (45mg daily). Mice were killed for confocal analysis of endplates after 3-14 days. **Results**: When added to myotubes, anti-MuSK IgG caused disassembly of AChR clusters (n=3 experiments). Mice injected with anti-MuSK IgG from 2 out of 4 patients lost weight and became weak after ~10 days (n=3). Repetitive stimulation of the nerve (3Hz) led to decrement in the compound muscle action potential, suggesting synaptic failure. Confocal-based fluorescence resonance energy transfer (FRET) measurements revealed reduced postsynaptic clustering of AChRs. Nerve terminals (stained with synaptophysin and neurofilament) were displaced from what remained of the postsynaptic AChR cluster. IgG from all 4 anti-MuSK patients caused significant pre- and post-synaptic changes, but those that produced the greatest disassembly of synapses also caused muscle weakness. **Conclusion**: The results suggest that MuSK signaling regulates the mature NMJ and that patient anti-MuSK antibodies cause disassembly of the synapse by disrupting the normal pattern of homeostatic signaling by MuSK.

ORAL-11-01

HOMER1 EXPRESSION IN THE GROWTH CONE: REGULATING CALCIUM HOMEOSTASIS

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Homer post-synaptic proteins are known to function in axon pathfinding, although their function in the growth cone is unclear. We used targeted morpholino knockdown to reduce Homer1 expression in growth cones from embryonic dorsal root ganglia neurons. Homer1 knockdown reversed growth cone attraction (19.2±3.6 degrees) to repulsion (-18.4±2.2 degrees) in response to the calcium dependent guidance cues, BDNF (n=23) and Netrin-1 (n=16), suggesting a role for Homer in growth cone calcium signalling. Calcium imaging revealed significant perturbations in calcium dynamics including frequent spontaneous calcium transients within Homer1-morphant growth cones. The transients were abolished in calcium-free media (n=5), confirming they were a result of calcium influx. Pharmacological characterisation of calcium fluxes suggests they are mediated by store-operated channels, potentially transient receptor potential cation channels (TRPCs) rather than voltage gated calcium channels (VGCCs). Inhibition of store operate channels with SKF-96365 (5μM) randomized turning (angle -1.4±2.0) and reduced the transient calcium fluxes from 1.51 ± 0.19 transient/min in Homer morphant (n=18) growth cones to control levels of 0.29 ± 0.08 transient/min (SKF n=8). Similarly Lanthanum, a TRP inhibitor also radomized turning (3.36±2.2 degrees, n=11). In contrast VGCC blockers, such as the L-type inhibitor nifedipine (5μM), had no significant effect on growth cone turning (turning angle -10.36±2.8, n=10). Furthermore, we show a close association between Homer1 and store-operated channels in key functional areas of growth cones. Taken together, our data provides evidence that the frequency of calcium transients is mediated by a Homer1-TRPC interaction, suggesting that Homer1 orchestrates the activity of TRPC and cytosolic calcium, thereby regulating growth cone calcium homeostasis and motility.

ORAL-11-02

NETRIN ACTS DIRECTLY ON AN IDENTIFIED SENSORY NEURON TO SPECIFY SITE AND DIRECTION OF DENDRITE OUTGROWTH

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Neurons generally possess a single long axon and one or more branched dendrites, which extend from stereotypic locations around the cell body. The developmental mechanisms that determine the location and orientation of outgrowth of those neural processes are poorly understood. We have previously identified a role for NetrinA/DCC signalling in specifying the site of emergence and direction of outgrowth of the dendrite of an identified sensory neuron, v'ch1, in the *Drosophila* embryo. **Purpose:** To elucidate the mechanism by which NetrinA/DCC signalling regulates dendrite outgrowth from the v'ch1 sensory neuron. Methods: Full length versions of *netrinA* or *frazzled* (the Drosophila DCC homologue) were expressed in specific tissues in a wild type, *netrinA* or *frazzled* mutant background using the Gal4-UAS system. The morphology of the v'ch1 neuron in these embryos was established by mAb 22C10 immunohistochemistry. **Results:** Misexpression of NetrinA in oenocytes, which surround the v'ch1 cell body, leads to the formation of additional, short neurites from this neuron, but only mild defects in dendrite growth. Misexpression of Frazzled in the epidermis leads to dendrite defects which phenocopy the *netrinA/frazzled* mutant phenotypes. Expression of NetrinA in oenocytes and the epidermis rescues the dendrite defects of *netrinA* mutants. Expression of Frazzled in v'ch1 rescues the dendrite defects of *frazzled* mutants. All of these results were shown to be statistically significant using Fisher's exact test. **Conclusion:** Our findings suggest that NetrinA, released from the epidermis, binds to Frazzled receptors on the nearby v'ch1 neuron. Activation of Frazzled receptors leads to dendrite outgrowth from the v'ch1 cell body from a specific location and in a stereotypic direction.

ORAL-11-03

BDNF EXERTS CONTRASTING EFFECTS ON PERIPHERAL MYELINATION OF NGF-DEPENDENT AND BDNF-DEPENDENT DRG NEURONS

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While Brain Derived Neurotrophic Factor (BDNF) has been shown to promote peripheral myelination during development and re-myelination after injury, the precise cellular mechanisms mediating this effect remain to be elucidated. **Purpose and Methods**: here we examine the influence that BDNF exerts on the capacity of Schwann cells to myelinate distinct sub-populations of dorsal root ganglion (DRG) neurons using in vitro myelination assays, compartmentalized cultures and genetic analysis. **Results**: we find that BDNF promotes myelination of Nerve Growth Factor (NGF)-dependent (TrkA+) neurons, an effect that is dependent on neuronal expression of the p75 neurotrophin receptor (p75NTR), as BDNF failed to exert pro-myelinating effect upon p75NTR knockout neurons. We have also identified a complementary role for BDNF as an inhibitor of myelination of BDNF-dependent (TrkB+) DRG neurons. Interestingly, this inhibitory influence is also mediated by axonal signals. Our data implicate that neuronally expressed full-length TrkB receptors mediate this inhibitory effect. Thus, these results demonstrate that BDNF exerts contrasting effects upon Schwann cell myelination, depending upon the complement of BDNF-receptors that are expressed by different sub-populations of DRG neurons. These data suggest that Schwann cell do not directly respond to BDNF during myelination in vitro. We are currently developing a strategy for the specific knockdown of p75NTR in Schwann cell to address this issue. **Conclusions**: Together, our data demonstrate that BDNF exerts contrasting modulatory roles in peripheral nervous system myelination, and that its mechanism of action is acutely regulated and specifically targeted to neurons. The cellular basis of this previously unrecognised dichotomy in BDNF function is highly receptor specific.

ORAL-11-04

ROLE OF ATP SIGNALLING IN THE DIRECTIONAL MIGRATION OF NEOCORTICAL INTERNEURONS

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Purpose: Selective loss of cortical interneurons has been associated with at number of neurological disorders such as schizophrenia and epilepsy. Coordinated neuronal migration plays a fundamental role in neocortical development, thus raising the question of whether the decreased numbers of interneurons is instigated by disrupted migration. It is well established that cortical interneurons originate from the ganglionic eminence and migrate into the neocortex. Previous studies have identified the importance of intracellular calcium fluctuations during this process, however, little is known about the signalling pathways that regulate this dynamic event. We have investigated the role of extracellular ATP signalling in modulating intracellular calcium and influencing migration. **Method:** Immunohistochemistry and Western blotting was used to analyze the expression profile of P2X receptors. Real-time imaging of embryonic brain slice cultures from glutamate decarboxylase-(GAD)-67 GFP knock-in mice was performed to monitor the migratory kinetics under normal and perturbed conditions. **Results:** Our investigation of interneurons entering the neocortex at embryonic day E12-13 has revealed the multidirectional nature of migration. Although extracellular ATP is ubiquitously expressed throughout the telencephalon, its specificity of action is regulated at the receptor level. We performed a developmental profile for the P2X2, P2X3 and P2X4 receptors and show regionalized expression patterns within the neocortex and ganglionic eminences during the period of migration. We have discovered that ATP signalling affects the directionality of migration and demonstrate that slice cultures exposed to P2X antagonist, TNP-ATP, have altered migratory kinetics. **Conclusion:** The guidance-directed migration of interneurons is dependent upon localized cues in the surrounding milieu. We have discovered a novel role for extracellular ATP signalling in directing the trajectory of migrating interneurons at early stages of development.

ORAL-11-05

WNT5A-RYK INTERACTIONS IN CALLOSAL AXON GUIDANCE

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Purpose: Wnt5a-Ryk interactions are involved in axon guidance during formation of the corpus callosum (CC). 25% of *Ryk*$^{-/-}$ mouse embryos display the Ryk phenotype, a novel CC malformation whereby callosal axons approach and cross the midline but are unable to escape into the contralateral hemisphere, instead forming axon bundles on the contralateral side of the midline. At present, the signaling pathway downstream of Wnt5a-Ryk interactions is unknown. This project aims to investigate the role of Ryk in neurite outgrowth and guidance in cultured cortical neurons and uncover the Ryk signaling pathway. **Methods:** Cortical neurons from embryonic day (E) 18 *Ryk*$^{+/+}$ (n=3) and *Ryk*$^{-/-}$ (n=2) mouse embryos were cultured for 24 hours in the presence or absence of Wnt5a. Analysis of neurite length and branching was performed using ImageJ software. **Results:** *Ryk*$^{+/+}$ cortical neurons grown in the presence of Wnt5a display a 14% reduction in longest neurite length compared to *Ryk*$^{+/+}$ neurons grown in the absence of Wnt5a (p=0.043). In addition, Wnt5a has no effect on neurite length in Ryk$^{-/-}$ neurons. **Conclusions:** E18 cultured cortical neurons display a Wnt5a-Ryk-dependent reduction in neurite outgrowth. In the absence of Ryk however, E18 cultured cortical neurons do not respond to Wnt5a demonstrating that in this context, Wnt5a is acting through Ryk. This assay will now be used to identify signaling molecules downstream of Wnt5a-Ryk interactions. The activity of members of the planar cell polarity and divergent atypical protein kinase C/PAR3/PAR6 Wnt signaling pathways will be investigated.

ORAL-11-06

EMX1 REGULATES MIDLINE CROSSING OF A SUB-POPULATION OF CALLOSAL AXONS

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PURPOSE: To investigate a discrepancy in the literature regarding different Emx1 mutant mice and variations in their phenotypes, we have analysed the development of the corpus callosum in *Emx1* knockout mice backcrossed onto a C57Bl/6 background. **METHODS**: Gold chloride staining, immunohistochemistry, *in situ* hybridisation, transmission electron microscopy and magnetic resonance imaging (MRI) techniques were used to analyse the phenotype of both embryonic and adult *Emx1*/C57Bl/6 mice. **RESULTS**: We observed small rostral Probst bundles in both embryonic (n=6) and adult (n=5) *Emx1*/C57Bl/6 knockout mice, thus demonstrating that Emx1 regulates the development of a subpopulation of callosal axons. Further analysis of adult *Emx1* brains demonstrated a reduction in brain size in caudal regions (p=0.003, Student's t-test) and cortical axon pathfinding defects, as analysed by diffusion weighted MRI and q-ball tractography (n=3). The size of the corpus callosum and hippocampal commissure (measured together) was increased in caudal regions in *Emx1* knockouts compared to wildtype mice (p=0.003, Student's t-test). The hippocampus was significantly reduced in *Emx1* knockout (n=5) compared to wildtype mice (n=6; p=0.0001, Student's t-test). **CONCLUSION**: These results correlate with the graded expression of Emx1 (high-caudal to low-rostral) across the mouse cerebral cortex and indicate that Emx1 does regulate cortical development and the formation of at least a subset of callosal axons.

ORAL-11-07

THE IMPORTANCE OF BEING ORDERED

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Purpose: Ten-m3 knockout (KO) mice exhibit altered topographical distribution of ipsilateral projections in the visual thalamus. Geniculocortical connections are unchanged in these mice, meaning the initial topographical error remains uncorrected. Behavioural data reveals pronounced visual deficits in KO mice, which can be overcome by monocular inactivation. This study therefore assesses the functional effect of this misrepresentation of visual space on the primary visual cortex (V1). Methods: Single unit *in vivo* electrophysiological recordings in anaesthetised mice and c-fos immunoreactivity were used to assess activity levels in V1 under monocular and binocular visual conditions. Results: Immunoreactivity for c-fos in layer 4 of V1 shows markedly less staining in KO (n=7) than wild-type (WT) equivalent (n=7). Monocular inactivation of KOs reveal marked changes in V1, with the distribution of ipsilaterally driven cells no longer restricted to classical binocular areas (n=8). V1 contralateral to the unaffected eye also shows an increase in staining in KOs, compared to binocularly active conditions. Response properties of V1 cells were similar between KOs and WTs during monocular stimulation. Binocular stimulation resulted in a marked suppression of responses in KOs (n=5); a phenomenon not seen in WTs (n=8). KO mice also displayed the presence of cells in medial V1 (normally monocular) that had spatially divergent receptive fields driven independently by each eye (2 of 5 cells). Conclusion: The erroneous representation of visual space from the ipsilateral eye in Ten-m3 leads to suppression of V1 activity during binocular stimulation. This mechanism most likely underlies the visual deficits observed in Ten-m3 KOs and their visual recovery during monocular inactivation.

ORAL-11-08

PRIMARY AND SECONDARY DEGENERATION OF THE OPTIC NERVE FOLLOWING PARTIAL TRANSECTION: THE BENEFITS OF LOMERIZINE

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Purpose: Following initial (primary) injury to the CNS, intact tissue is vulnerable and may undergo a form of 'bystander' damage termed secondary degeneration. Partial optic nerve (ON) transection is an excellent model in which to unequivocally differentiate events that occur during primary and secondary degeneration and we have recently shown that the CNS-specific L- and T-type calcium channel blocker lomerizine protects retinal ganglion cells (RGCs) from secondary death at 4 weeks (p≤0.05). **Methods:** Here we used immunohistochemistry of the ON at 1 and 4 weeks (n = 7-12/group) to examine events during primary degeneration within the injury site and secondary degeneration in adjacent tissue, and further study the protective effects of lomerizine. We assessed optokinetic nystagmus to determine whether lomerizine rescued visual function. **Results:** Primary injury showed morphological disruption, loss of β-III tubulin axonal staining, reduced myelinated axon density, increased proteoglycan expression (phosphacan), increased microglia and macrophage numbers and increased oxidative stress (p≤0.05 for each parameter). Similar changes were seen in tissue undergoing secondary degeneration (p≤0.05). Lomerizine reduced morphological disruption, oxidative stress and phosphacan expression, and limited early increases in macrophage numbers in tissue undergoing primary and secondary degeneration (p≤0.05), but failed to prevent progressive de-myelination of optic axons and did not fully restore visual function. **Conclusion:** Blockade of calcium channels with lomerizine limited some primary and secondary degenerative changes following CNS injury. However such an approach should be combined with other treatments to prevent progressive demyelination and ensure long-term maintenance of full visual function.

ORAL-12-01

UNIQUE RECEPTOR DEPENDENT, PLASMIN MEDIATED DISRUPTION OF THE BLOOD BRAIN BARRIER (BBB) BY TISSUE-TYPE PLASMINOGEN ACTIVATOR (T-PA)

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Purpose: t-PA is a thrombolytic agent used in patients with ischaemic stroke but has been reported to damage BBB integrity. We have studied this phenomenon using an in vitro model of the BBB. **Methods:** Transwell tissue-culture inserts harbouring porous (3.0µm) membranes were seeded with transformed human astrocytes on their abluminal side and human primary brain endothelial cells (BECs) on their luminal surface. After co-culturing for 72hr, t-PA (10nM-1µM) was added to the BEC containing inner well for 24hr and BBB integrity assessed by passage of FITC-labelled BSA from luminal to the abluminal compartment. **Results:** t-PA caused a marked concentration dependent increase in BBB permeability (P<0.001). Similar effects were seen using the t-PA variant tenecteplase, while a truncated t-PA variant (reteplase) and urokinase were without effect. The effect of t-PA was plasmin-mediated as it could be fully blocked by α_2 anti-plasmin or aprotinin (P<0.001). Surprisingly, plasmin alone did not enhance extravasation suggesting that t-PA needs to selectively target plasminogen-activation to the cell surface to influence the BBB. Consistent with this, an inactive t-PA variant blocked the t-PA effect (P<0.05) implying the involvement of a t-PA specific receptor. We also found that t-PA induced marked shape-change and reduced viability of astrocytes, and potentiated OGD-induced endothelial cell death. **Conclusion:** t-PA-mediated plasminogen activation affects BBB permeability in a unique, highly targeted and specific fashion. This highlights t-PA as a protease not only important for plasminogen activation, but also for precise orientation of plasmin to its neurovascular targets. By identifying the domain of t-PA responsible for engaging the cell surface, it may be possible to block the damaging effect of t-PA on the BBB, while preserving its thrombolytic action.

ORAL-12-02

GLUTATHIONE UPTAKE INTO RAT BRAIN MITOCHONDRIA

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Purpose: Glutathione is a major cellular antioxidant that is found in the cytosol and mitochondria. The mitochondrial pool, although typically less than 15% of the total, is an important determinant of cellular viability following oxidative stress. Synthesis of glutathione only occurs in the cytosol so the generation and maintenance of the mitochondrial pool requires transport across the inner membrane. The dicarboxylate and 2-oxoglutarate carriers have been implicated in glutathione transport in mitochondria from liver and kidney. This process has not been investigated in most other tissues including brain. Thus, the aim of the present study was to characterize the properties of [^{3}H]-glutathione transport into isolated brain mitochondria. **Methods:** Mitochondria were isolated from rat forebrain and incubated (usually for 30 seconds) with [^{3}H]-glutathione. Some incubations contained known substrates and inhibitors for mitochondrial anion transporters. Incorporated radioactivity was measured following recovery of the mitochondria by filtration. **Results:** [^{3}H]-glutathione was rapidly incorporated into mitochondria by a process that slowed markedly during the first minute, was apparently influenced by the mitochondrial glutathione content and resulted in net glutathione accumulation (n = 3-21). [^{3}H]-glutathione incorporated into the mitochondria was not rapidly released (n=3). Uptake was competitively inhibited by substrates and inhibitors for several mitochondrial anion transporters (p<0.01). Citrate, isocitrate and benzene-1,2,3-tricarboxylate were particularly effective (approx. 70% inhibition at 1 mM; n = 3-8), suggesting a possible role for a tricarboxylate carrier in the glutathione transport. **Conclusion:** Brain mitochondria are able to rapidly incorporate glutathione via a transport process that is unidirectional and possibly involves a tricarboxylate carrier. The properties of this uptake differ substantially from those reported previously for transport into kidney and liver mitochondria.

ORAL-12-03

FRACTALKINE/CX3CL1 SIGNALLING IN THE MOUSE OLFACTORY EPITHELIUM POSITIVELY CONTRIBUTES TO A MICROENVIRONMENT FOR OLFACTORY SENSORY NEURON SURVIVAL AND REPLACEMENT

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The olfactory epithelium is a site of massive adult neurogenesis where olfactory sensory neurons are continuously turned over. Tissue macrophages have been implicated in phagocytosis of degenerating cells but the molecular mechanisms that allow for their recruitment while maintaining a neurogenic microenvironment are poorly understood. We report that the neuroprotective chemokine fractalkine (CX3CL1) is expressed by olfactory sensory neurons and that monocyte-derived cells depend on CX3CL1-signalling for migration and apical dendrite expression within the olfactory epithelium. After injury, there was increased macrophage infiltration into the olfactory epithelium of mice that were deficient for the fractalkine receptor, CX3CR1, when compared to their wild-type counterparts (in each group, n≥3). Epithelia of the former mice also contained significantly higher levels of the pro-inflammatory cytokines e.g. tumour necrosis factor-alpha and interleukin-6 while expression of olfactory marker protein, a marker for mature olfactory sensory neurons, was significantly decreased as determined by qPCR (n≥5). Histological counts confirmed aggravated loss of olfactory sensory neurons in fractalkine receptor knock-out mice (in each group, n=3). Furthermore, pulse labelling with 5-bromo-2-deoxyuridine showed impaired proliferative responses in the germinal zone of the olfactory epithelium in CX3CR1-deficient mice after injury (in each group, n=3). Thus, our data indicate that signalling through the CX3CR1 receptor is neuroprotective and contributes to an environment that allows for olfactory sensory neuron replacement from endogenous stem/progenitor cells.

ORAL-12-04

INVESTIGATION OF THE FUNCTIONAL ROLE OF PARKIN CO-REGULATED GENE (PACRG) IN RESPONSE TO CELLULAR STRESS

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PACRG shares a common bi-directional promoter with *parkin*, a gene associated with early onset Parkinson's disease (EO-PD). Although the function of PACRG is currently unknown, the protein has been implicated in the unfolded protein stress response and neurodegenerative disorders. We have recently demonstrated that PACRG is regulated by the ubiquitin-proteasome system and is a component of Lewy bodies (*Taylor et al.,* 2007). **PURPOSE:** To investigate the potential protective role of PACRG against toxicity induced by a variety of cellular stressors. **METHODS:** BE-(M17) neuroblastoma cells stably overexpressing PACRG were generated. Parental and PACRG overexpressing cells (n=3) were treated with reagents known to induce several different cellular stress responses, including MG-132, H2O2, dopamine, tunicamycin, staurosporine and nocodazole. Cell viability was determined by LDH assay and PACRG localisation by immunohistochemistry. **RESULTS:** Overexpression of PACRG conferred significant protection against cellular toxicity associated with staurosporine, H2O2 and nocodazole treatments, but not dopamine or tunicamycin. Immunohistochemistry demonstrated PACRG-positive aggresomes following MG-132, dopamine and H2O2 treatment. The most significant effect was observed in response to proteasomal inhibition, where PACRG-positive aggresomes were observed in 38.8± 4.9 % of parental cells compared with 63.0±2.4 % of cells overexpressing PACRG (p=0.002, n=3). In addition to protecting cells against nocodazole-mediated toxicity, aggresomes in PACRG overexpressing cells were resistant to microtubule destabilisation. **CONCLUSION:** These results support a role for PACRG in protecting cells against toxicity mediated by different stress pathways. This protective effect may involve the formation and stabilisation of aggresomes. Further studies are needed to determine the potential role of PACRG in Lewy body biogenesis and PD.

ORAL-12-05

MODIFYING REACTIVE GLIOSIS POST ISCHEMIA BY NRF2 ACTIVATION

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Stroke results in oxidative stress and reactive gliosis. NF-E2-related factor 2 (Nrf2) is a transcription factor required for the induction of phase II detoxification providing resistance to oxidative stress. The Nrf2 system is strongly expressed in astrocytes. Sulforaphane (SFN) and tert-butyl hydroquinone (tBHQ) are potent stimulators of the Nrf2 system. We aimed to explore whether Nrf2 stimulation could modify reactive gliosis after focal ischemia. **Methods:** Permanent focal ischemia was induced in six groups of mice (n=10) by the Rose Bengal photothrombotic method. 15 minutes after stroke onset animals were treated with either tBHQ (10mg/kg), SFN (5mg/kg) or vehicle (corn oil). Neurobehavioural assessment was performed at 1, 3, 7, 14 and 28 days post ischemia. Animals were killed 1 day and 1 month post ischemia and infarct volume and reactive gliosis determined. Activation of the Nrf2 system was confirmed by western blot. **Results:** The animals all demonstrated significant motor impairment resulting from the stroke for the first 3 days, then regained motor functions until day 28. This improvement was independent of Nrf2 stimulation. Infarct volumes did not differ at 24 hours. The Nrf2 system was increased after administration tBHQ and SFN. Activation of the Nrf2 system reduced reactive gliosis. **Conclusion:** Modification of reactive gliosis by stimulation of the Nrf2 system may be beneficial in enhancing neuroregeneration after stroke.

ORAL-12-06

THE UBIQUITIN E3 LIGASE INTERACTING PROTEIN, NDFIP1 IS ASSOCIATED WITH NEURONAL SURVIVAL FOLLOWING CEREBRAL ISCHEMIA IN VIVO

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Purpose: Ndfip1 functions as an adaptor for the E3 ubiquitin ligase Nedd4 family to promote ubiquitination of protein targets that cannot interact directly with Nedd4. Recently Ndfip1 has been shown to be associated with survival following traumatic brain injury *in vivo* suggesting Ndfip1 may promote neuronal survival in response to cerebral ischemia via Nedd4-dependent ubiquitination. The aim of this study is to investigate the role of Ndfip1-mediated ubiquitination as a novel mechanism of endogenous protection against stroke. **Methods:** Ndfip1 and Nedd4-2 endogenous expression was assessed following Endothelin-1 induced transient middle cerebral artery occlusion (MCAo) in conscious rats using immunohistochemistry and immunoprecipitation assays. **Results:** Ndfip1 demonstrated significant neuronal upregulation ($p<0.05$) in stroke animals (n=17) compared to sham (n=4). Furthermore, the majority of neurons with increased Ndfip1 did not stain for the apoptotic marker TUNEL, but showed a significant correlation with apoptosis ($p<0.0001$) suggesting Ndfip1 is associated with survival against delayed cell death. Double immuno-labelling revealed Nedd4-2 is upregulated following stroke and colocalises with Ndfip1 upregulation in cortical neurons. Immunoprecipitation assays also confirmed Ndfip1 binds directly with Nedd4-2 following cerebral ischemia. Over expression of Ndfip1 *in vitro* conferred complete protection of PC12 cells against oxygen and glucose deprivation (OGD) (n=5 independent experiments; $p<0.001$) suggesting that the Ndfip1 upregulation observed *in vivo* in response to stroke is involved in endogenous protection. **Conclusion:** This is the first known study to identify an endogenous role for a HECT domain class E3 ligase in stroke and suggests Ndfip1-mediated ubiquitination is associated with neuronal survival in cerebral ischemia.

ORAL-12-07

EARLY MOLECULAR EVENTS OF THE CEREBRAL INFLAMMATION RESPONSE FOLLOWING ACUTE TRAUMATIC BRAIN INJURY IN HUMAN BRAIN TISSUE

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Introduction: Little is known on the molecular events following severe traumatic brain injury (TBI) in humans and to date there are no efficient therapies for the treatment of TBI patients. The availability of human brain tissue from the Neurotrauma Tissue/Fluid Bank is a unique opportunity to analyse the numerous biochemical and molecular changes following TBI. **Methods**: In this study, a total of 21 trauma brain samples were analysed. Age and sex matched samples were used as controls. To explore the cerebral inflammation within the human brain tissue, we measured the level of expression and the concentrations of various inflammatory mediators by enzyme-linked immunosorbent assay and Bioplex cytokine assays. **Results**: In the samples of individuals who died within 5 minutes of the brain injury there was marginal or no difference in the levels of IL-6 (2.38±0.84, n=14 vs. 0.19±0.10 pg/mg protein, n=11, $p<0.02$) and IL-1β (0.03±0.03, n=14 vs. 0.03±0.01 pg/mg protein, n=17). However, those levels were dramatically increased in the samples of individuals who died more than 6 hours following brain injury: IL-6 (31.08±7.95 pg/mg protein, n=7, $p<0.008$) and IL-1β (2.36±0.61 pg/mg protein, n=14, $p<0.01$). TNF-α was undetectable in both control and trauma brain samples. **Conclusion**: These results show for the first time that the inflammatory response starts within hours of acute TBI in the human brain tissue. Furthermore, real-time PCR experiments will indicate if these elevated protein levels are associated with upregulation of the transcription. A better understanding of the molecular mechanisms following TBI in humans will improve the diagnosis and the future treatment of patients.

ORAL-12-08

THE TLR ADAPTER PROTEIN MYD88 PLAYS A KEY ROLE IN THE REGULATION OF NEURAL INJURY

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Myeloid differentiation factor 88 (MyD88) is an adaptor protein that is integral to many immune linked pathways including certain Toll Like Receptors (TLR). Data generated through this study suggests a complex role for MyD88 during ischemic insult, involving both pro-survival and pro-death signalling. Primary neuronal and glial cultures were isolated from wild type (WT) and MyD88 knockout mice (MyD88-/-). A novel membrane-based method of culture was used to study neurons and glia separately, whilst still being able to communicate with one another. WT and MyD88-/- neurons were cultured in the presence of both WT and MyD88-/- glia, these combinations of cells were then exposed to 4 hours of oxygen glucose deprivation (OGD). Cellular survival was assessed with a mitochondrial viability assay. Activation by phosphorylation of the TLR system downstream signaling components ERK, JNK, TPL2 and NFkB was measured by Western blot. Cellular viability following OGD shows that cells lacking MyD88 are more susceptible to ischemic injury. MyD88-/- mice were then exposed to mid-cerebral artery occlusion and found to exhibit a two fold increase in infarct volume when compare to WT mice (n=12). The presence of glia increases the survival of neurons after OGD by 30%, and MyD88-/- glia potentate this increase in survival of neurons, suggesting a pro-death signalling role for MyD88 in glia. MyD88 -/- neurons show delayed phosphorylation of mediators (ERK, JNK, TPL2 & NFkB) known to play a role in the determination of cellular fate as evidenced by Western blots. Together these data suggest that there is a dual role for MyD88-dependant signaling.

ORAL-13-01

INCREASED CELL PROLIFERATION AND ANGIOGENESIS IN THE FETAL SUBVENTRICULAR ZONE IS CORRELATED WITH THE SEVERITY OF GROWTH RESTRICTION

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Purpose: Adverse prenatal factors can result in abnormal brain development, contributing to the aetiology of several neurological disorders. Intrauterine insults could occur during neurogenesis and gliogenesis, disrupting these events. Here we investigate the effects of chronic placental insufficiency (CPI) on cell proliferation and the microenvironment in the subventricular zone (SVZ). **Methods:** At 30 days of gestation (dg; term~67dg), CPI was induced in pregnant guinea pigs via unilateral uterine artery ligation to produce growth-restricted (GR) fetuses (n=7); controls (n=6) were from the unoperated horn. At 60dg, fetal brains were stained immunohistochemically to identify proliferating cells (Ki67), immature neurons (PSA-NCAM), astrocytes (glial fibrillary acidic protein, GFAP), microglia (Iba-1) and the microvasculature (von Willebrand Factor) in the SVZ. **Results:** There was no difference ($p>0.05$) in the a) number of Ki-67-immunoreactive (IR) cells, b) density of Iba-1-IR microglia or c) percentage of SVZ occupied by blood vessels in control versus GR fetuses. Regression analysis revealed that the a) number of Ki67-IR cells and b) percentage of SVZ occupied by blood vessels, increased ($p<0.05$) with the severity of growth restriction. The percentage of SVZ occupied by blood vessels was also correlated ($p<0.05$, $r^2=0.61$) with the number of Ki67-IR cells in the SVZ in GR fetuses. PSA-NCAM-IR was present in the SVZ in control and GR brains, whereas GFAP-IR was negligible. **Conclusion:** CPI increases cell proliferation and promotes angiogenesis in the fetal SVZ when growth restriction is severe. These proliferating cells are likely to be neurons; their long-term survival is being assessed. Furthermore, the microvasculature within the SVZ influences cell proliferation.

ORAL-13-02

ASTROCYTE ARCHITECTURE IN THE HYPOXIC/ ISCHEMIC BRAIN

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Purpose: Hypoxic/ischemic (H/I) brain damage is a common problem in the human neonate and can lead to detrimental outcomes ranging from cerebral palsy to death. Currently we have a limited understanding of the mechanisms that cause brain damage, and thus limited treatments. Astrocytes function to maintain the extracellular environment, regulating molecules such as neurotransmitters, glucose, water and ions. Thus, normal astrocyte function is required for normal neuronal function. We have investigated whether neuronal damage in some brain regions might be related to changes in the morphology of astrocytes. **Methods:** Neonatal pigs (N=15) were anaesthetised and exposed to 4% oxygen for 30min, including 10min of ischemia, and allowed to recover for 72hr. Control animals (N=9) were littermates exposed to anaesthesia, but not hypoxia/ischemia. Pigs were then euthanased and brain tissues fixed in paraformaldehyde. Grey and white matter astrocyte morphology was examined using multiple methods, such as iontophoretic injection of Lucifer Yellow, Golgi staining, and immunohistochemistry for glial markers including GFAP. **Results:** In response to hypoxia/ischemia, significant anatomical changes occur in grey and white matter astrocytes at 72hr post-insult, including decreases in overall size of astrocytes, decreased number and length of processes and decreased complexity of branching. These changes occurred in regions of the brain that suffered neuronal damage. **Conclusions:** We suggest that changes in glial architecture may play an important role in consequent neuronal damage in the H/I brain. As such, therapies targeted at either preventing or reversing astrocytic changes may offer an alternative pathway to neuroprotection in the neonatal H/I brain.

ORAL-13-03

THE TRANSCRIPTION FACTOR NFIA REGULATES CORTICAL GLIAL DEVELOPMENT VIA ANTAGONISM OF THE NOTCH EFFECTORS HES1 AND HES5

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Radial glia act as multipotent progenitors during development of the central nervous system (CNS). Radial glia give rise to neurons first, followed by glia (astrocytes and oligodendrocytes). This 'gliogenic switch' plays a fundamental role in enabling appropriate cortical function and is conserved throughout vertebrate phylogeny, yet how it is regulated remains unclear. The Nuclear Factor One (Nfi) transcription factors play a key role in cortical development. Mice deficient in Nfia, for example, display aberrant cortical development, including agenesis of the corpus callosum (ACC), the largest fibre tract in the brain. The underlying cause of ACC in Nfia-deficient mice appears to be the failure of midline glial populations, such as the glial wedge (GW) and the indusium griseum glia (IGG), to form. However, the mechanism by which Nfia regulates glial development and maturation is completely unknown. Here we demonstrate that neither excessive apoptosis, nor aberrant proliferation, are the causes of the phenotypic absence of the GW or the IGG in Nfia mutant mice (n = 3 independent replicates for all experiments). Instead, we show that a suite of glial-specific markers are downregulated at the cortical midline of Nfia-deficient mice, suggestive of a role for Nfia in regulating glial differentiation directly. Furthermore, we demonstrate that the basic helix-loop-helix transcription factors Hes1 and Hes5 are significantly upregulated within the cortical ventricular zone of Nfia knockout mice. Hes1 and Hes5, key effectors of the Notch signalling pathway, play a pivotal role in maintaining radial glia as multipotent progenitors. As such, their upregulation in Nfia mutants implies that differentiation of radial glial progenitors is aberrant in these mice. Collectively, these data indicate that Nfia may play a central role in enabling radial glia to differentiate into mature glial lineages, a finding that significantly enhances our understanding of the molecular mechanisms regulating the gliogenic switch.

ORAL-13-04

TRANSCRIPTIONAL CONTROL OF NEURONAL MIGRATION IN THE DEVELOPING CEREBRAL CORTEX

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How cell migration is co-ordinately regulated with other aspects of neuron production is not well understood. Here we show that the proneural protein Neurogenin2 (Neurog2), which controls neurogenesis in the embryonic cerebral cortex, directly induces the expression of the small GTP-binding protein Rnd2 in newborn cortical neurons before they initiate migration. *Rnd2* silencing leads to a defect in radial migration of cortical neurons similar to that observed when the *Neurog2* gene is deleted. Remarkably, restoring *Rnd2* expression in *Neurog2*-mutant neurons is sufficient to rescue their ability to migrate. Our results identify *Rnd2* as a novel essential regulator of neuronal migration in the cerebral cortex and demonstrate Rnd2 to be a major effector of Neurog2 function in the promotion of migration (Heng et al, *Nature* 2008).

ORAL-13-05

SEIZURE-RELATED GENE SEZ-6 REGULATES EXCITATORY CONNECTIVITY OF CORTICAL NEURONS

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Development of appropriate dendritic arbors and connectivity is crucial for neuronal information transfer. Seizure-related gene 6 (Sez-6) is up-regulated in cortical neurons during development, by pentylenetetrazole (PTZ) treatment and environmental enrichment. In hippocampal long-term potentiation, Sez-6 mRNA was up-regulated in an NMDA receptor-dependent manner suggesting involvement in the activity-dependent plasticity of learning and memory. Using immunocytochemistry and immunoelectron microscopy, we have localized Sez-6 expression to the somatodendritic compartment of cortical neurons. In sez-6 null-mutant mice, abnormal dendritic arborization of cortical neurons is observed. Deep-layer pyramidal neurons in the somatosensory cortex of sez-6 null mice exhibit an excess of short dendrites, as assessed by Golgi-Cox impregnation and embryonic cortical neurons in culture display excessive neurite branching in the absence of Sez-6. Overexpression of individual Sez-6 isoforms in knockout neurons reveals opposing actions of membrane-bound and secreted Sez-6 proteins with membrane-bound Sez-6 exerting an anti-branching effect under both basal and depolarizing conditions. Layer V pyramidal neurons in knockout brain slices show reduced excitatory post-synaptic responses to layer II/III stimuli and a reduced dendritic spine density on their apical dendritic arbors correlated with a reduction in punctate staining for the asymmetric synapse marker post-synaptic density 95 (PSD-95). Activity of signalling pathways involving CaMKII, a key enzyme for synaptic plasticity, and Erk is affected by Sez-6 treatment. In conclusion, cell-surface protein complexes involving Sez-6 help to sculpt the dendritic arbor, in turn enhancing synaptic connectivity.

ORAL-13-06

SPATIAL LEARNING DEFICITS IN TEN_M3 KNOCKOUT MICE

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Purpose: The entorhinal cortex (EC) is topographically connected to the ipsilateral hippocampal formation (HCF), both of which are implicated in spatial learning. The maintenance of topographic relationships is thought to aid in the association of neural representations between related areas of the brain. Both EC and HCF express the novel transmembrane protein Ten_m3. Previous work from our lab has shown that Ten_m3 is a functionally important axonal guidance molecule in the visual system in vivo. **Methods:** Performance in the Morris water maze (MWM) was compared between wildtype (WT; n=8) and knockout (KO; n=8) groups in light conditions. Subjects had to associate a hidden platform's location with environmental stimuli. Subjects were trained over 7 days, and probed on the 8th by moving the platform location. This protocol was repeated for naive WT (n=8) and KO (n=8) groups in dark conditions. Escape latency was compared for both groups in both conditions. Analysis of swim speed and time spent in the 4 pool quadrants was performed for day 7 and probe. **Results:** Both groups acquired the task, but latency was significantly higher in KOs versus WTs in both light and dark conditions ($p < 0.01$). WT acquisition did not change from light to dark conditions, while KOs showed a trend towards improvement. Differences in swim speed did not solely account for the observed differences. Qualitative analysis suggested that groups navigate using different strategies. **Conclusion:** These data suggest that spatial learning is defective in *Ten_m3* KOs, consistent with the suggestion that *Ten_m3* is required for the normal development of the circuitry responsible for spatial learning. These results call the visual nature of the MWM into question.

ORAL-13-07

DEVELOPMENTAL VITAMIN D DEFICIENCY AND DOPAMINE ONTOGENY

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Purpose: Evidence is accumulating that normal levels of vitamin D are important for brain development. Developmental vitamin D (DVD) deficiency results in altered behaviour in adult offspring, such as a heightened response to psychomimetic-induced locomotion. In the current study we examined the effect of DVD deficiency on dopamine ontogeny in prenatal and adult rats. **Methods:** Female Sprague-Dawley rats were fed a vitamin D deficient diet from 6 weeks prior to mating until birth, when vitamin D was added to the diet. Tissue was collected at E12 or E15, or P1 or P70. Dopamine transcription factors (Nurr1, Pitx3, limx1b, TH and p57kip2) were examined in E12 and E15 midbrain by qPCR. Dopamine, related enzymes and metabolites were quantitated in P0 brain. Dopamine receptors and transporters in adult brain were examined using radioligand binding assays. Tyrosine hydroxylase (TH) staining was quantified in P70 brain using unbiased stereological counting. **Results:** There was a 20% reduction in Nurr1 expression in DVD-deficient embryos ($p<0.05$). None of the other transcriptional factors studied were altered. Dopamine levels were unaltered in neonatal DVD-deficient brains but the ratio of DOPAC/HVA was increased consistent with a reduction in COMT ($p<0.05$). In the DVD-deficient adults DAT density and affinity were increased in female but not male striatum and nucleus accumbens consistent with the enhanced locomotor response to amphetamine seen in these animals ($p<0.05$). Finally TH stained cells were reduced in substantia nigra in DVD-deficient adult males ($p<0.05$). **Conclusion:** DVD deficiency alters dopamine ontogeny. We believe that down-regulation of Nurr1, a major transcription factor in dopaminergic cell maturation, differentiation and maintenance may be a key factor in these outcomes.

ORAL-13-08

DEVELOPMENTAL VITAMIN D DEFICIENCY IS ASSOCIATED WITH DEFICITS IN SOCIAL BEHAVIOUR IN SPRAGUE-DAWLEY RATS

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Purpose: Evidence from animal experiments demonstrate that developmental vitamin D (DVD) deficiency influences brain development and behaviour, including alterations in psychomimetic induced hyperlocomotion, which is often used to model the positive symptoms of schizophrenia. The negative symptoms of schizophrenia are often subtle from a clinical perspective, but nevertheless, well described animal models of social behaviour are available. The aim of this study was to examine the behaviour of DVD-deficient rats in a social interaction test. Methods: Sprague-Dawley rats were fed a vitamin D deficient diet or control diet six weeks prior to mating until birth and then fed a vitamin D replete diet until testing at postnatal day 70. The rats were housed under reversed lighting conditions for 10 days prior to a 10 minute social interaction test. Social behaviour was measured between pairs (n=10 pairs per group) of unfamiliar rats (matched according to sex, age and body weight) in an open field under low light (30 lx). The social interaction test was scored using both a manual event recorder (the Observer) and an automated video tracking system (Ethovision). Results: Male and female DVD-deficient rats had significantly reduced social interaction compared to controls. Microanalysis indicated that DVD-deficient rats had decreased anogenital sniffing. There was no significant effect of maternal diet on aggressive or non-social behaviour. Conclusions: DVD deficiency is associated with specific impairments in social investigation in male and female rats. These results suggest that DVD deficiency in the rat models aspects of both positive and negative symptoms of schizophrenia.

ORAL-14-01

NEURON MIGRATION IN THE DEVELOPING PERIPHERAL NERVOUS SYSTEM

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Although neuronal migration is common in the developing CNS, it has been assumed that peripheral neuron precursors (neural crest cells) cease migration prior to the onset of neural differentiation. Enteric neurons arise from vagal level neural crest cells that migrate into and along the developing gut. **Purpose:** To examine whether immature enteric neurons migrate. **Methods:** We used mice in which only immature enteric neurons express GFP (TH-GFP mice) and mice in which all enteric neural crest-derived cells express a photo-convertible fluorescent protein. Immature neurons were imaged in intact explants of embryonic gut using confocal or conventional fluorescence time-lapse microscopy for 2-11 hours. **Results:** Around 50% of immature neurons (n = 80) migrated during the imaging period with an average speed of 15 µm/h. This is slower than the speed at which the population of neural crest-derived cells advances along the developing gut. Most migrating immature enteric neurons migrated caudally by extending a long leading process followed by translocation of the cell body. In many migratory neurons, a swollen structure of variable size would detach from the cell body and move along the leading process in the direction in which the neuron was migrating. Immunohistochemistry using pericentrin antibodies revealed that the swelling did not contain the centrosome. This mode of migration is different from that of undifferentiated enteric neural crest cells and neural crest cells in other locations, but has some similarities to that of migrating neurons in many regions of the developing CNS. **Conclusion:** Immature enteric neurons can migrate and this appears to be the first report of neuronal migration in the developing vertebrate peripheral nervous system.

ORAL-14-02

DEVELOPMENT OF MOTILITY IN MOUSE DUODENUM

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Purpose Motility in adult duodenum is controlled by enteric neurons and interstitial cells of Cajal (ICC-MY), which mediate slow waves. Little is known about mechanisms controlling motility during gut development. Enteric neural precursors and immature neurons are present in the mouse duodenum at E11.0, but when neural control of motility develops is unknown. **Methods** Spatiotemporal maps were generated from video recordings of embryonic mouse duodenum in vitro. Intracellular recordings were made from the circular muscle in the presence of nicardipine. Immunohistochemistry was performed using markers for ICC (Kit) and neurons (Hu). **Results** Spontaneous motility was absent from E12.5 duodenum (n=3). At E14.5, spontaneous contractions propagated orally, anally or bi-directionally. These were unaffected by TTX (n=4) and resembled 'ripples', myogenic contractions seen in neonatal mouse colon. Morphological ICC-MY were not detected. Contractions in E16.5 duodenum were more frequent than at E14.5 and were seen in wild-type mice and mice lacking enteric neurons (Ret -/-). Kit+ ICC-MY were present, but did not form networks. At E18.5, mature ICC-MY networks and electrical slow waves were present. Neurally mediated complexes were also observed. These were blocked by TTX and enhanced by blocking nitric oxide synthase (n=8). In E14.5, E16.5 and E18.5 duodenum, all contractile activity was blocked by nicardipine. Conclusions In embryonic mouse duodenum there are prominent spontaneous contractions that are independent of neurons or ICC-MY, and are therefore myogenic. Thus, the control of motility in the embryonic gut differs markedly from the mature gut.

ORAL-14-03

PANCREATIC PREGANGLIONIC NEURONS: DIFFERENTIAL RESPONSES TO CCK1 AND 5-HT3 RECEPTOR STIMULATION

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Purpose: The dorsal vagal nucleus (DVN) is the main source of the vagal innervation of the pancreas. *In vitro* studies have demonstrated that the DVN contains a heterogeneous population of pancreatic preganglionic neurons (PPNs) However, little is known about their morphological and electrophysiological characteristics *in vivo*. The aims of this study were (i) to identify DVN PPNs in vivo and (ii) to characterize their responses to stimulation of cholecystokinin (CCK_1) and serotonin ($5\text{-}HT_3$) receptors which are major regulators of pancreatic secretion. **Methods:** Male Sprague-Dawley rats anaesthetised with isoflurane (1.7%/100% O_2) were used in all experiments. Extracellular single unit recording techniques were used to record the activity of PPNs within the DVN and their locations were marked using Pontamine Sky Blue and juxtacelluar labelling. PPNs were identified by antidromic activation in response to stimulation of the pancreatic vagus nerve. **Results:** Forty-four PPNs were identified within the rostral, medial and caudal DVN and thirty eight were tested for responsiveness to CCK-8 (CCK_1 receptor agonist) and phenylbiguanide (PBG; $5\text{-}HT_3$ receptor agonist). These had axonal conduction velocities in the C-fibre range (< 1 m/s). CCK and PBG (0.1-10 µg/kg, i.v.) produced three types of response: (i) PPNs in the medial DVN were completely inhibited by CCK (n = 18) and PBG (n = 10), (ii) PPNs in the caudal DVN were activated by CCK (n = 5) and PBG (n = 2) and (iii) CCK (n = 9) and PBG (n = 7) had no effect on PPNs in the rostral DVN. **Conclusion:** CCK has complex actions on PPNs that may be related to its effects on pancreatic exocrine and endocrine secretion.

ORAL-14-04

MANY DIFFERENT NEUROCHEMICAL TYPES OF NERVES DISAPPEAR FROM THE PREGNANT RAT UTERUS

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Purpose: Constriction of blood vessels has been linked to the reduced blood flow characteristic of pre-eclampsia, which affects 5-8% of pregnancies. This suggests that nerves controlling blood vessel diameter may contribute to the pathophysiology of pre-eclampsia. **Methods:** We examined uterine horn whole mounts from virgin female rats in late estrous/early diestrous and 20-day pregnant rats after immunoperoxidase staining (n=at least 4/group). Six neurochemicals in uterine nerves were visualized: tyrosine hydroxylase (TH) and neuropeptide Y (NPY) for sympathetic nerves, vesicular acetylcholine transporter (VAChT) and nitric oxide synthase (NOS) for parasympathetic nerves, and substance P (SP) and calcitonin gene-related peptide (CGRP) for sensory nerves. Stained whole mounts were dehydrated, resin-embedded and mounted on glass slides. **Results:** The non-pregnant uteri showed significant densities of axons immunoreactive for TH, NPY, SP, CGRP, VAChT or NOS. Dense plexuses of TH- and NPY-containing axons and a few SP-, CGRP-, VAChT- and NOS-containing axons occurred around blood vessels. All six types of axons were present in the muscle, where the densities of VAChT- and NOS-positive fibers were low compared to TH, NPY, SP or CGRP fibers. SP- and CGRP-immunoreactive axons occurred in the mucosal layer and were found close to the villi. Pregnant uteri had very significantly reduced numbers of TH-, NPY-, VaChT-, NOS-, SP- and CGRP-immunoreactive axons. In the pregnant uterus, rare nerves immunoreactive for each of these markers were present on the mesometrial side whereas there were no nerves on the anti-mesometrial side. **Conclusion:** This study describes for the first time the innervation of the complete non-pregnant rat uterus and shows that pregnancy causes parasympathetic and sensory as well as sympathetic denervation.

ORAL-14-05

CHANGES IN MITOGEN-ACTIVATED PROTEIN KINASES (MAPKS) IN RAT AUTONOMIC PELVIC GANGLION NEURONS UNDERLYING ERECTILE DYSFUNCTION IN DIABETES

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Diabetic autonomic neuropathy results in erectile dysfunction in clinical and experimental diabetes. The molecular mechanisms perturbed by diabetes underlying autonomic nerve failure are unknown. Pelvic ganglia (PG) provide the parasympathetic innervation to the erectile tissue (corpus cavernosum). A loss of parasympathetic innervation has been shown in erectile tissue from streptozotocin-induced diabetic rats (STZ-rats), while sympathetic innervation remains intact. The MAPKs, extracellular signal-regulated kinase (ERK), c-Jun n-terminal kinase (JNK) and p38, have been implicated in the aetiology of diabetic sensory neuropathy. **Purpose:** The aim of this study was to elucidate the mechanism(s) leading to autonomic nerve failure underlying diabetic autonomic neuropathy. The effect of diabetes on ERK and JNK activation was examined. **Methods:** Adult male rats were given an intraperitoneal injection of STZ and rats maintained for 12 weeks. Tissues from freshly euthanased rats were processed for Western blotting. Immunoblots with PG protein were probed with antibodies raised against phosphorylated (i.e. active) MAPKs or MAPKs irrespective of phosphorylation state (i.e. total MAPK). Band densities were scanned and a ratio of phosphorylated to total protein (P/T) determined to indicate activation of JNK and ERK isoforms. **Results:** Diabetic rats (n=5) had low body weights (346.0±19.7g vs. 531.7±16.0g; *P*<0.0001) and high blood glucose (25.7±2.4g/l vs. 6.6±0.4g/l; *P*<0.0001) compared to age-matched controls (n=6). P/T ERK decreased (p44ERK 0.92±0.12 arbitrary density units vs. 0.53±0.23, *P*=0.011; p42ERK 2.79±0.03 vs. 1.69±0.3, *P*=0.013) and JNK activation increased (p54/56JNK 0.66±0.09 vs. 1.09±0.09, *P*=0.02; p46JNK 0.48±0.12 vs. 0.88±0.08, *P*=0.007) in diabetic PG compared to controls. **Conclusion:** Changes in MAPK signalling in pelvic ganglion neurons may, as in sensory neurons, contribute to the aetiology of pelvic autonomic diabetic neuropathy.

ORAL-14-06

CHEMICAL CODING OF THE MAJOR NEURON TYPES IN THE MOUSE SMALL INTESTINE

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Chemical and morphological identification has been achieved for all neuron types in the guinea-pig small intestine, but such identification is lacking for mice, which are increasingly being used in enteric neuroscience because of the ease of genetic modification in this species. **Purpose**: The aim was to determine the chemical coding of all major neuron types in the mouse small intestine. **Methods**: Tissues were from 36 adult BalbC mice. A range of antibodies to different chemical markers was investigated, using single and double staining methods. Neurons were distinguished by chemistry, shape and projections to targets. **Results**: Over 90% of myenteric and 80% of submucosal neurons have been accounted for in identifiable functional and neurochemical classes. Notably, intrinsic primary afferent neurons were identified as cholinergic neurons with neurofilament, calbindin and CGRP immunoreactivity that were 26% of myenteric neurons. Excitatory and inhibitory muscle motor neurons, secretomotor neurons and interneurons have also been identified. Excitatory muscle motor neurons contained acetylcholine, tachykinins and calretinin, inhibitory neurons contained nitric oxide synthase and VIP and some contained NPY, secretomotor neurons were non-cholinergic and contained VIP and NPY or were cholinergic containing somatostatin and CGRP. Surprisingly, some VIP secretomotor neurons expressed tyrosine hydroxylase. **Conclusion**: The types of myenteric neurons, their chemical coding and their proportions are similar in the mouse and guinea-pig. There are substantial differences in the chemistries of submucosal neurons and their functional identifications in the mouse are still being resolved.

ORAL-14-07

MECHANISMS UNDERLYING ASCENDING EXCITATION IN THE HUMAN COLON

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Purpose: The intrinsic neural circuitry underlying propulsion in the human colon is poorly understood. Here, we have investigated the effects of selective activation of ascending neural pathways on motor activity of the circular muscle (CM) in specimens of human colon. **Methods:** Specimens (~6x4cm) were obtained with prior informed consent from patients undergoing elective surgery and dissected to remove the mucosa and submucosa. A two-chambered bath was sealed with silicon grease and perfused with oxygenated Krebs solution at 36°C. An array of microhooks (25mm) monitored circular muscle contractility in the oral chamber, via an isometric transducer. **Results:** Spontaneous contractions in Krebs solution were separated by a mean time of 1.52min±0.4 (mean± SEM, n=20, N=5) and occurred at amplitudes of 5.8g±0.5 (n=50, N=5). Spontaneous contractions persisted in the presence of hexamethonium (1mM) and terodotoxin (TTX, 1µM). Electrical nerve stimuli (0.4ms, 80V, single pulses, or at 10Hz) applied selectively to the aboral chamber elicited contractions of the CM, whose amplitude and duration were dependent upon the timing of stimulation. The mean amplitude of contractions to single stimuli was 10.5g±5.9 and 11.6g±5.4 for trains of stimuli. Hexamethonium (1mM) applied to both chambers had little effect on the responses to activation of the ascending nerve pathway (n=5). However application of TTX(1µM) to both chambers blockaded the response to neural stimulation in 4 of 5 preparations. DMPP (1mM) applied to the aboral chamber evoked premature hexamethonium-sensitive contraction in 2 of 3 preparations. **Conclusions:** Spontaneous myogenic contractions occur rhythmically in human colon. Selective activation of ascending neural pathways modulates the timing and amplitude of these contractions.

ORAL-14-08

TRANSCUTANEOUS ELECTRICAL STIMULATION SPEEDS UP COLONIC TRANSIT IN CHILDREN

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Direct electrical stimulation of sacral nerves with implanted devices speeds up colonic movement in adults [1]. A pilot study showed that transcutaneous electrical stimulation (TES) over the abdomen increases defecation in children with chronic constipation [2]. **Purpose:** Test if TES speeds up colonic transit in children with slow transit constipation in a randomized placebo-controlled trial. **Methods:** 41 children (8-18 yrs) were randomly assigned into sham (n=19) or full stimulation (n=22) groups and given stimulation (pulse-modulated current, carrier frequency 4kHz, beat frequency 80-150 Hz, intensity <40 mA; 4 adhesive electrodes on belly and back covering spinal outflow T9-L2, current crossed left-to-right and front-to-back) by physiotherapists for 20mins, 3/wk for 4 weeks, then 2 months follow-up. Analysis: daily diaries of defecation and soiling, radioisotope transit studies (scintigraphy) and QOL questionnaire (PedsQL) before and 2 months after stims. Six children received stimulation daily. **Results:** There was significant speeding in colonic transit rates (p=0.029), reduction in soiling (p=0.004), and improved QOL (p=0.04) in the group given full stimulation vs placebo. There was no change in defecation in children stimulated 3/week, but defecation increased from 1 to 5 episodes per week with more frequent stimulation (p= 0.03). **Conclusion:** Colonic transit, soiling and QOL improved in children given TES 3/week. Defecation increased in children given daily stimulation. Transcutaneous stimulation is non invasive and an attractive option for treating chronic constipation in children. Further studies are needed to determine optimal stimulation parameters and to test on other types of constipation. [1] *Dinning et al 2007, Colorectal Dis 9:123-32.* [2] *Chase et al, 2005, J Gastroenterol Hepatol 20:1054-61.*

ORAL SESSION 15 – RETINAL ANATOMY AND DISEASE

Sponsored by ARC Centre of Excellence in Vision Science

ORAL-15-01

SYNAPTIC INPUTS ONTO LARGE SPARSE AND BROAD THORNY GANGLION CELLS IN MARMOSET RETINA

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Purpose: The functional characteristics of many ganglion cell types in primate retina remain poorly understood. Here, we investigated the density of synaptic inputs onto two types of wide-field cells, the "large sparse" and "broad thorny" type cells. **Methods:** Ganglion cells were retrogradely labeled and photofilled. Presumed bipolar input was identified with antibodies against the C-terminal binding protein 2 or the glutamate receptor 4 subunit. Presumed amacrine cell input was identified with an antibody against the glycine receptor anchoring protein gephyrin. The density of immunoreactive (IR) puncta (presumed synaptic input) onto cell dendrites was determined. **Results:** The average density of presumed bipolar cell input onto large sparse ganglion cells was 4.3 ± 2.0 IR puncta / 100 μm^2 dendritic surface area (n = 19 dendritic regions from 3 cells). Results for presumed amacrine cell input onto large sparse ganglion cells yielded an average density of 4.8 ± 1.4 IR puncta / 100 μm^2 (n= 9 dendritic regions from 3 cells). Direct comparisons of presumed bipolar and amacrine cell inputs for two large sparse cells yielded similar average densities for the two types of input. Much like the large sparse cells, average densities of presumed bipolar inputs onto two broad thorny cells was 5.4 ± 2.0 IR puncta / 100 μm^2 (12 dendritic regions from 2 cells). **Conclusion:** Presumed bipolar cell inputs are at approximately equal density in large sparse and broad thorny ganglion cells. Thus, any functional differences between these cell types are unlikely to arise from variation in synaptic densities.

ORAL-15-02

ALTERATION IN RGC DENDRITIC MORPHOLOGY FOLLOWING AAV TRANSDUCTION WITH DIFFERENT GROWTH FACTORS

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Background: Adeno-associated virus (AAV) delivered genes promote neuronal viability and axonal regeneration, but it is unknown whether sustained expression of virally produced proteins affects the structure of transduced neurons. Products of transgenes used to promote axonal regeneration (CNTF, BDNF and GAP43) affect dendritic growth in cultured neurons. Here we examine retinal ganglion cell (RGC) dendritic morphology after long-term AAV2 vector transduction. **Methods**: Adult rats received an intravitreal injection of saline, AAV-GFP, AAV-CNTF-GFP, AAV-BDNF-GFP or AAV-GAP43-GFP. Two weeks later, rats (n=21) received an autologous peripheral nerve graft onto the cut optic nerve of the AAV-injected eye. After 6-9 months, live retinas were wholemounted and regenerating (fluorogold labelled) RGCs injected iontophoretically with 2% lucifer yellow. Morphology was documented using Neurolucida. Values were obtained for each RGC type (RI, RII, RIII or unclassifiable). **Results**: There were no differences between saline and GFP groups in the frequency distribution or morphology of RGC types. However, AAV-CNTF-GFP or AAV-BDNF-GFP increased the proportion of unclassifiable RGCs to nearly 50%. BDNF expression did not alter relative frequencies of RI, RII and RIII cells, whereas CNTF expression increased the relative proportion of RI and RIII cells. In AAV-CNTF-GFP injected retinas, all cell types had significantly increased soma size and RII cells showed increased dendrite length and higher branch complexity. After AAV-BDNF-GFP injections, RI cells were larger and had more complex dendritic branches. RII cells had increased tortuosity and larger dendritic fields, while RIII cells had longer dendrites and larger field volumes. **Conclusions**: Long-term transgene expression affects the morphology of transduced neurons, potentially altering their input and physiological characteristics.

ORAL-15-03

ANGIOTENSIN TYPE-1 RECEPTOR INHIBITION IS NEUROPROTECTIVE TO AMACRINE CELLS IN A RAT MODEL OF RETINOPATHY OF PREMATURITY

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Aim: Retinopathy of Prematurity (ROP) is a disease characterized by abnormal growth of inner retinal blood vessels as well as neuronal and glial cell changes. The aim of this study was to characterize in detail the neuronal deficits that occur in a rat model of ROP, and whether the angiotensin II type-1 receptor (AT1R) inhibitor, valsartan, alters retinal neurochemistry and cell survival. Methods Newborn Sprague-Dawley rats were exposed either to 80%:21% O2 (22:2 hours/day) until postnatal day 11 (P11), then 7 days room air, or room air for the entire experimental period. Valsartan (40 mg/kg/day) was administered from P12-P18. Neuronal changes were assessed using post-embedding immunocytochemistry, thickness of retinal layers, quantification of TUNEL positive cells, and immunocytochemical analysis of using markers of known retinal circuits. Results: ROP was associated with a reduction in thickness of the INL and IPL, due to cellular loss of inner retinal neurons as determined by a significant increase in TUNEL labelling from P11-P13. Amino acid immunocytochemistry revealed widespread changes in virtually all classes of neurons, with a significant reduction in the density of glycine immunoreactive amacrine cells. Immunolabelling for parvalbumin, revealed a significant reduction in AII amacrine cell density during ROP. Treatment with valsartan, partially ameliorated these neuronal changes. Conclusions: This study shows that neurons within the rod pathway are particularly affected by ROP, and that treatment with an AT1 receptor antagonist, is partially protective.

ORAL-15-04

CALORIC RESTRICTION REDUCES AGE-RELATED SUSCEPTIBILITY OF THE OPTIC NERVE TO INTRAOCULAR PRESSURE CHALLENGE

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Purpose: To investigate the effect of aging and calorie restriction on the optic nerve response to sub-ischaemic levels of acute intraocular pressure (IOP) elevation in aging mice. **Methods:** The dark adapted scotopic electroretinogram (ERG) was measured before, during and after (1 hour) acute IOP challenge (50 mmHg for 30 minutes) in the following cohorts of wildtype C57/BL6 mice (i) 3 month old mice n = 8 (ii) 18 month old mice fed ad libitum (AL n = 10) and (iii) 18 month old mice that had alternate day fasting commenced at 12 months of age (CR n = 10). Signals were collected for dim and bright flashes (-4.54 and -2.23 log cd s/m^2) and analyzed in terms of ganglion cell (positive scotopic threshold response pSTR) and postreceptoral (P2) responses. Statistical comparison of parameters was performed using ANOVA. **Results:** 18 month old AL mice showed significantly greater pSTR loss ($p=0.004$) and slower recovery ($p<0.001$) compared to 3 month old mice. 18 month old CR mice were not significantly different to 3 month old mice in terms of pSTR loss during acute IOP challenge ($p = 0.87$), but showed slower functional recovery ($p = 0.02$). Direct comparison between 18 month old AL and CR mice showed a protective effect of calorie restriction against pSTR loss during IOP elevation ($p=0.009$) but did not improve recovery ($p=0.58$). Similarly, calorie restriction also showed protective effect against P2 loss ($p=0.008$). **Conclusion:** Calorie restriction is shown to have a protective effect against age related susceptibility of ganglion cells to acute intraocular pressure challenge in mice. The findings will hopefully help elucidate the mechanisms as to why aging is a risk factor for neurodegenerative disease.

ORAL SESSION 15 – RETINAL ANATOMY AND DISEASE *(contin.)*

Sponsored by ARC Centre of Excellence in Vision Science

ORAL-15-05

MULTIFOCAL PUPILLOGRAPHIC PERIMETRY IN GLAUCOMA

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Purpose: To investigate 4 variants of multifocal pupillographic perimetry in glaucoma. **Methods:** We tested 40 normal and 39 glaucoma subjects. Glaucoma patients had mild to severe visual fields in at least one eye. All subjects were examined with HFA achromatic, SWAP and Matrix 24-2 perimetry, Stratus OCT, slit lamp and tonometry. Informed written consent was obtained from all subjects. Multifocal stimuli having 44 test regions/eye, extending to 30 deg eccentricity, were presented concurrently to both eyes. Recording duration was 4 minutes, divided into 8 segments of 30 s. Pupil diameter was monitored under infrared illumination. Four stimulus protocols were examined which differed in terms of mean presentation intervals (MPI) of 1 or 4 s per region, or maximum stimulus brightness. Maximum luminances were 150, 290 or 340 cd/m^2, and the background was at 10 cd/m^2. Measures of field loss included the N-worst amplitudes, response delays, or pair-wise linear combinations of those regardless of location in the field, and also clustered amplitudes, between-eye asymmetry, and super versus inferior visual field (SIVF) asymmetry. **Results:** Diagnostic performance was assessed by areas under ROC curves (AUCs). For all field severities the best AUCs were produced by the 44 region 4 s MPI, 150 cd/m^2 stimulus protocol. For severe fields the N-worst amplitudes gave an AUC of 0.97. The best AUCs for the other measures were also obtained by this protocol: clustered amplitudes 0.958, between-eye asymmetry 0.929, SIVF asymmetry 0.946. **Conclusions:** Simple measures, such the sum of N-worst response measures, and measures extracting features of glaucomatous damage: e.g. clustering and asymmetries, each provided excellent diagnostic power. The method eliminates problems associated with subjective testing as employed in conventional perimetry.

ORAL-15-06

A ROLE FOR POTASSIUM IN OUTER RETINAL CONTROL OF CHOROIDAL STRUCTURE AND FUNCTION

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Purpose Although activation of potassium (K) channels on endothelial cells and vascular smooth muscle cells and fluctuations in potassium concentration [K] within the vessels are recognized as important in the regulation of mammalian blood pressure and flow1, the interrelationship of [K] with sodium [Na] and chloride [Cl] in vascular beds is less understood especially in the choroid of the eye. Thus we examined temporal changes in [K] [Na] and [Cl] in choroid in recovery from Form Deprivation Myopia (FDM). We predicted that extravascular [K] would be indirectly related to vessel diameter and blood flow changes. Methods Monocular occlusion of hatchling chickens (n=52) for 11days was examined with elemental microanalysis. Results. FDM induced significant increases in [K], [Na] and [Cl] ion abundance in the extravascular space of the choroid and within the lymphatic vessels. Occluder removal initiated vascular dilation within 30 minutes. After 24 hours, [K] level, choroidal thickness and blood flow were close to control levels. However [K] continued to decrease and remained low until 120 hours whereas the Na and Cl levels stayed high until refractive normalization occurred ~120 hours post- occlusion. Occlusion reduced the number of fenestrations on the retinal side of the choriocapillaris and on the lymphatic sinusoids of the outer choroid for 48 hours. As extracellular [Na] and [Cl] fell, the concentrations within the lymphatics increased. Conclusion Reintroduction of temporally modulated light initiates reversal of occluder induced increase in [K], [NA] and [Cl] in the outer retina and initiates changes in distribution of these ions in choroid. The reductions in extravascular choroidal [K], is inversely related to rapid dilation of the choriocapillaris and lymphatics and increase in blood flow. 1Edwards, etal Nature 1998.

ORAL-15-07

DIFFERENTIAL EXPRESSION OF ANTI-ANGIOGENIC FACTORS IN THE PRIMATE FOVEA AND MACULA DURING DEVELOPMENT

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Purpose: To determine the cellular distribution of three antiangiogenic factors - natriuretic peptide precursor B (NPPB), pigment epithelium-derived factor (PEDF) and collagen type IVα2 (COL4A2) - found by microarray analysis to be differentially expressed in the macula of developing human retinas. **Methods:** Differential expression was confirmed by quantitative RT-PCR (Q-PCR), using RNA samples from the developing macula and periphery of 3 foetal human retinas aged 17-20 weeks (WG). Proteins and mRNA expression were localised by immunohistochemistry and *in situ* hybridisation, respectively, in macaque and human retinas. **Results:** Q-PCR analysis confirmed the microarray findings. COL4A2 protein was localised to blood vessel walls in nasal retina and in vascularized regions of temporal retina, but not in the macula which is avascular until late in development. NPPB protein was localised to small puncta, mainly in the ganglion cell layer (GCL), which were more numerous in the macula than in peripheral locations of macaque retinas. PEDF mRNA was expressed in the GCL of central retina, particularly at the developing fovea. PEDF mRNA labelling in the pigment epithelium increased with gestational age. **Conclusion:** Lower levels of COL4A2 mRNA in macular samples are explained by its localisation to retinal vessels, which are absent from the macula until 25 WG. Localisation of both PEDF and NPPB to the GCL is consistent with a role in slowing development of macular vessels which initially form in the GCL. Peak expression of PEDF in the developing foveal GCL is consistent with a role in defining the foveal avascular area.

ORAL-15-08

EXPRESSION OF COMPONENTS OF THE COMPLEMENT SYSTEM IN THE DEVELOPING AND ADULT MAMMALIAN RETINA

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Purpose: Recent studies have suggested that C1q, which is a component of the classical complement system, is induced in neurons by astrocytes and may modulate synaptogenesis. This study examined expression of elements of the classical and alternate pathways of the complement cascade in the developing rat retina to determine whether other proteins in these two pathways might be implicated in regulating synapse formation or maintenance. **Methods:** Immunohistochemistry using antibodies against the C5a receptor, the C5L2 receptor, C3, C4, C9 and C1q, was performed on retinae of Dark Agouti rats (n=16) aged 7 days to adult. **Results:** Labelling for the C5a receptor, which is traditionally described as being largely expressed by myeloid-derived cells (such as mast cells, macrophages, basophils, platelets, monocytes, granulocytes and endothelial cells) was developmentally regulated, being strongly expressed in the P7 retina. Labelling was particularly prominent in the inner plexiform (synaptic) layer, where two strongly labeled sublaminae were evident. The punctate labelling was suggestive of expression in synaptic terminals. In older animals the labeling was less conspicuous in the inner plexiform layer, but labelling became more overt in somata of cells in the ganglion cell layer along with some somata in the inner nuclear layer and in horizontal cells in the outer plexiform layer. Immunolabelling with the other antibodies confirmed that the retina may express multiple components of the complement system. **Conclusions:** The association of the C5a receptor with regions containing newly formed synapses in the developing retina suggests that this component of the complement pathway may play a role in CNS development, maintenance and/or degeneration. This is currently being investigated.

ORAL-16-01

DO HOMOZYGOUS SODIUM CHANNEL BETA1 MUTANT (C121W) MICE MODEL DRAVET SYNDROME, A SEVERE FORM OF EPILEPSY?

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Rational: The *SCN1B* (C121W) mutation is described in an Australian family with generalised epilepsy with febrile seizures plus. We have developed mice that harbour the C121W mutation in order to investigate cellular and network mechanisms underlying this disease. **Methods:** EEG recordings were made from epidural electrodes. Thermal seizures were induced by a hot stream of air during which body temperature was measured rectally. Acute slices were cut from P14 animals. Whole-cell recordings were made in current clamp mode from CA1 pyramidal and inhibitory neurons. **Results:** The phenotype of homozygous mice is severe leading to premature death by P20. The mice have aberrant EEGs consistent with seizure activity. Mice also display an ataxic gait (n=3). Further, a thermal insult results in seizures at a lower temperature threshold, that are distinct in their expression, involving full tonic-clonic seizures for mutant mice that are never observed in wild type mice (n=20). Single-cell electrophysiological measurements revealed a deficit in the CA1. An input-output relationship that measures action potential (AP) firing with progressively larger current injections in pyramidal neurons shows a collapse of AP trains at higher current injections (n=10). This collapse was not evident for inhibitory interneurons (n=8). **Conclusions:** Homozygous C121W mice display several of the key behavioural phenotypes noted in humans and other mice models of Dravet syndrome. In contrast to Scn1a mouse models AP collapse occur in pyramidal neurons and not inhibitory interneurons. These results are particularly interesting in the context of the recently described patient with Dravet syndrome who is homozygous for the *SCN1B* (R125C) mutation.

ORAL-16-02

LONG TERM NPY OVER-EXPRESSION FOCALLY IN THALAMUS SUPPRESSES GENERALISED ABSENCE-LIKE SEIZURES IN A GENETIC RAT MODEL

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Purpose: NPY may be an endogenous anti-convulsant. We have demonstrated a suppressive effect of acute administration of NPY in a genetic rat model of generalised, thalamocortical based epilepsy. This study assessed whether long term focal over expression of NPY using recombinant adeno-associated virus (rAAV) in the thalamus suppresses genetic generalised absence-like seizures. Aim: Male, 7 week old, Genetic Absence Epilepsy Rats of Strasbourg (GAERS) were injected under anaesthesia with either rAAV-NPY or saline (0.6ul over 30 min) bilaterally into the nucleus reticularis thalami (nRt). After injection epidural EEG electrodes were implanted for seizure recording. Seizure activity was quantitated weekly for 11 weeks post-treatment by a 90-minute EEG recording, analysed by a blinded observer. Results: The GAERS injected with saline (n=4) showed an age dependent increase in seizures, while the rAAV-NPY treated rats (n=4) showed sustained seizure suppression over the 11 week period (repeated measures ANOVA, F=1.9, p=0.029). At 11 weeks post-treatment, 64% seizure suppression was observed in the rAAV-NPY injected rats compared to control injected rats; also the individual burst duration was reduced by 55%. Conclusion: Adenoviral over-expression of endogenous NPY into the thalamus resulted in a sustained reduction in seizure expression in GAERS, a spontaneous genetic model of absence epilepsy. Long-lasting focal over-expression of NPY could be a potential treatment for generalised thalamocortical epilepsies.

ORAL-16-03

HIPPOCAMPAL NEUROPLASTICITY IN TRAUMATIC BRAIN INJURY: RELATIONSHIP TO POST-TRAUMATIC EPILEPSY

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Introduction: Traumatic brain injury (TBI) is a major community health problem with high incidence and long-term morbidity. The lateral fluid-percussion injury (LFPI) model has been widely used as a model of closed-head TBI and for the study of late recurrent post-TBI epilepsy seizures. Purpose: As the hippocampus (HC) is associated with seizure onset, neuronal counting was performed in key HC regions (dentate gyrus (DG) and CA3c hilar region). Methods: 14 animals were included in the study (8 LFPI, 6 sham); LFPI was induced using 3.5 atm pressure. 6 months post-TBI, brains sections were prepared after paraffin-embedding and thionin staining was performed for stereological counting of the DG and CA3c and Timm staining for assessment of mossy fibre sprouting (MFS) in the stratum moleculare. Results: Neuronal numbers were not different between the TBI group and shams for either the DG nor the CA3c region (both P>0.05). However, TBI animals that developed epilepsy had increased neuron numbers compared to sham in both the DG (31% increased, P=0.02) and the CA3c region (27% increased, P=0.02) and compared to the non-epileptic TBI animals (DG, 61% increased, P=0.04; CA3c, 63% increased, P=0.04). No significant difference was observed between the epilepsy TBI and either of the sham or non-epileptic TBI animals on the contralateral side (P>0.05). In the epilepsy TBI animals, for both sides of the HC, MFS was greater in the stratum moleculare compared to non-epileptic TBI animals. Conclusion: Neuroplastic changes that occur ipsilaterally in the hippocampus post-TBI in the rat LFPI model are associated with both MFS and epilepsy outcomes at 6 months post-trauma.

ORAL-16-04

ELECTROPHYSIOLOGICAL CHARACTERISATION OF THE MODULATION OF CORTICO-THALAMO-CORTICAL OSCILLATIONS BY CARBAMAZEPINE IN VIVO

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Purpose : Corticothalamic 5-9 Hz oscillations and sleep-related thalamocortical spindle oscillations (7–15 Hz) share common frequency bands but are underlain by distinct intracellular characteristics (Pinault et al, J. Physiol. 2006). 5-9 Hz oscillations are more pro-epileptogenic than sleep spindles. The present study investigates in cortico-thalamo-cortical systems the cellular and network of carbamazepine (CBZ), an anti-focal seizure drug that aggravates absence seizures. **Methods:** Extracellular and microEEG recordings were performed in the thalamic reticular nucleus (TRN) and in the ventrobasal complex (VB), along with the simultaneous frontoparietal surface electrocorticogram, in neuroleptanalgesied non-epileptic control rats (n = 8). The location and morphology of the recorded neurons were identified histologically following juxtacellular application of Neurobiotin. **Results:** Subcutaneous injection of CBZ (10 mg/kg) significantly increased both the frequency of occurrence and the power of delta, 5-9 Hz and spindle-like oscillations in both the cortex and the thalamus. Simultaneously, TRN cells developed a recurrent, stereotyped, sequence of two rhythmic burst firing patterns characterized by a few cycles (2.6 ± 0.16) of corticothalamic 5-9 Hz oscillations immediately followed by a thalamocortical spindle-like oscillation. **Conclusion:** These findings suggest that CBZ promotes the triggering of thalamocortical spindle-like oscillations by the pro-epileptogenic corticothalamic 5-9 Hz oscillations. This CBZ-induced promotion of thalamic spindle activity is likely the manifestation of an increase in the rhythmic hyperpolarisation of the thalamocortical neurons in the VB by enhancement of $GABA_A$ receptors activity.

ORAL-16-05

AN IMMUNOHISTOCHEMICAL STUDY EXAMINING THE REVERSIBILITY OF NEUROPATHOLOGY IN THE MUCOPOLYSACCHARIDOSIS IIIA MOUSE BRAIN

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Mucopolysaccharidosis type IIIA (MPS IIIA) is a neurodegenerative lysosomal storage disorder resulting from a lack of the lysosomal enzyme sulphamidase (SGSH). Heparan sulphate accumulates within lysosomes along with GM2 and GM3 gangliosides and cholesterol. Activation of micro- and astroglia and axonal spheroid formation has been described. There is presently no treatment and children often die before age 20. **Purpose:** To explore direct sulphamidase replacement as a potential therapy option. **Methods:** MPS IIIA mice (n = 4-8/group) received fortnightly intra-cerebrospinal fluid injections of recombinant human SGSH beginning at either six, 12 or 18 weeks of age. Additional MPS IIIA and unaffected mice received vehicle. Following euthanasia at 21-22 weeks of age, mice were fixation-perfused with 4% paraformaldehyde and blinded quantification of the % stained area of a lysosomal/endosomal membrane marker (LIMP-II) and the astroglial marker, GFAP (MetaMorph software), plus quantification of the number of activated microglia (isolectin B4; BSI-B4) and the number of ubiquitin-positive inclusions (AnalySIS Lifescience software) was undertaken. **Results:** All four markers were statistically significantly elevated in vehicle-treated MPS IIIA mouse brain ($p<0.05$, ANOVA, post-hoc Bonferroni). Early intervention significantly ($p<0.05$) reduced LIMP-II in the colliculi, hippocampus and cerebral cortex. GFAP and BSI-B4 were reduced in the colliculi, cortex and thalamus and the number of ubiquitin-positive lesions was reduced in the colliculi, periaqueductal gray and hypothalamus but not cuneate. Mice treated from 12 weeks exhibited restricted improvements (LIMP-II in hippocampus, cortex; GFAP in cortex). Mice treated from 18 weeks showed no improvement. **Conclusion:** Early intervention is critical in order to maximise therapeutic outcomes.

ORAL-16-06

AXONAL DYSFUNCTION AND CONDUCTION FAILURE IN DIABETIC NEUROPATHY

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Purpose: Diabetes may be complicated by the development of a symmetrical length-dependent polyneuropathy. In addition to causing sensory symptoms, patients with diabetic neuropathy (DN) may complain of weakness and fatigue. The present study was undertaken to evaluate the role of axonal Na+/K+ pump dysfunction in the development of motor symptoms in DN. Methods: Nerve excitability techniques, which provide information about membrane potential and axonal ion channel function, were undertaken in 15 patients with established DN, 10 patients with diabetes who had no evidence of neuropathy (DWN) and 15 healthy controls. Excitability parameters were recorded following stimulation of the median nerve at the wrist. Recordings were obtained at baseline, and then before and after one minute of maximal voluntary contraction (MVC) of abductor pollicis brevis. Results: Compared to control values, baseline motor amplitude was significantly decreased in DN patients with associated reductions in Na+ channel parameters There was an increase in threshold following MVC in DN patients, DWN patients and controls, consistent with activity-dependent hyperpolarization due to increased Na+/K+ pump activity. The magnitude of the threshold increase was less in DN patients compared to either DWN patients or controls (DN group 13.1 ± 2.2%; DWN group 24.6 ± 5.5%; controls 20.4 ± 1.9%; $P<0.05$). DN patients with significant baseline reductions in Na+ channel dependent parameters, developed activity-dependent conduction block following MVC ($r = -0.65$, $P <0.05$). Conclusions: The reduced threshold change and post-contraction reductions in motor amplitude are likely to be secondary to Na+/ K+ pump dysfunction. Alteration in Na+/ K+ pump function, coupled with reductions in nodal Na+ currents, may be sufficient to trigger conduction failure in DN patients and are likely to contribute to the clinical symptoms of weakness and fatigue in this condition.

ORAL-16-07

CEREBROCORTICAL PATHOLOGY IN CANINE FUCOSIDOSIS

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Introduction Canine fucosidosis is an inherited lysosomal storage disorder caused by deficiency of alpha-L-fucosidase. Affected dogs display clinical signs of neurological deterioration and have extensive cytoplasmic vacuolation and degeneration in neural and visceral tissue. Apoptosis, astrocytosis, spheroid formation and microgliosis are a feature of lysosomal storage disorders which also occur in neurodegenarative diseases like Alzheimer's they have not been reported in fucosidosis. Investigation of these markers of inflammation and neurodegeneration provides a deep understanding of the progressive pathophysiology of this disease and insights into a likely success of therapy. This study investigated pathological markers from preclinical to late disease in fucosidosis. **Methods** Animals were grouped into young control (n=3), adult control (n=8), preclinical (n=3), early clinical (n=6), late clinical (n=7). At necropsy frontal cortex was paraffin embedded and stained with haematoxylin and eosin, Luxol Fast Blue /Periodic Acidic Schiff, glial fibrillary acidic protein, Ubiquitin and Ricinus Communis Agglutinin 1 Lectins. Using image analysis these neuroinflammatory markers were quantified. **Results** Vacuolation, perivascular storage, pyramidal neuronal loss, astrocytosis, myelin loss, microgliosis and axonal spheroid formation were observed in early affected brain prior to clinical signs of disease. There was a significant increase in % of vacuolation, astocytosis, perivascular storage, axonal spheroid formation among all the groups ($P<0.001$). In aging controls there was a significant demyelination, astrocytosis, axonal pathology compared to young brain ($P<0.05$). There was a progressive reduction in myelin, increased vacuolation, astocytosis, axonal spheroid formation, perivascular storage, pyramidal neuronal loss and microgliosis as the fucosidosis advanced. **Conclusions** Understanding of these early storage events, inflammatory markers in the brain and the possibility of reversing these changes with early treatment provide findings relevant to this and other neurodegenerative diseases.

ORAL-16-08

RELATIONSHIP BETWEEN PCREB EXPRESSION AND MORPHINE PREFERENCE IN RATS

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Purpose: Long-term exposure to drugs of abuse results in dependence and long-term neuroadaptations that have been associated with changes to the intracellular cAMP transduction cascade, with cAMP-response-element-binding protein (CREB) playing a key role in this cell signaling cascade. Previous results from our laboratory have shown that following chronic compulsory morphine consumption and withdrawal about 20-30% of rats will voluntarily choose to consume morphine in their drinking water sufficient to reinstate their dependence. We have previously termed these rats high morphine preferring (HMP). The present study examined changes in phosphorylated-CREB expression in morphine preferring rats when given a choice or a compulsory no choice situation to self-administer morphine. Methods: Male Sprague Dawley rats (n = 40) were housed individually and exposed to increasing concentrations of morphine HCl in their flavoured (sucrose) drinking water for 3 weeks. Following a one week drug free period, rats were then given a choice between a morphine containing sucrose solution and a sucrose solution only. Based upon their morphine intake they were classified as high morphine preferring (HMP) or low morphine preferring (LMP). One week later, half the population from each group (HMP or LMP) were given a second choice phase or a compulsory no choice situation. 8 days later, rats were heavily anaesthetized and brains were fixed for pCREB immunohistochemical analysis. Results: HMP rats which were given a second choice phase showed a significantly higher expression of pCREB positive cells in the nucleus accumbens and striatum compared to LMP rats. HMP rats which were given a compulsory no choice situation showed no significant differences in the striatum but higher pCREB expression in the nucleus accumbens region. Conclusion: pCREB expression in morphine preferring rats does not occur as a consequence of morphine exposure, rather pCREB expression occurs only in rats that choose to prefer morphine.

ORAL-17-01

COMPARISONS OF DORSAL HORN NEURONES TO NOCICEPTIVE AND TACTILE INPUTS

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Purpose: We have recently shown that the small diameter afferent fibres that innervate the humerus are sensitive to vibro-mechanical stimuli. In this study, we have characterised the vibro-tactile and electrical response properties of cells in the dorsal horn that relay cutaneous and bone derived sensation to the brain. **Methods:** In anaesthetised rabbits microelectrode recordings were obtained from 32 spinal (C5-T1) dorsal horn neurones. Sinusoidal vibration (20-300µm, 5-300Hz) was applied to the ipsilateral paw. Stimulus epochs of 1s were repeated 5 times at 10s intervals. Electrical stimuli (2mA, 2ms, 5-300Hz) were applied to the 'bone' nerve bundle arising from the humerus. **Results:** In response to cutaneous vibration in general, cells (n=10 of 32) acted as band-pass filters over the range of 5-100Hz. All cells responded to onset of an epoch, before markedly declining within and across successive epochs. At frequencies >~100Hz prolonged suppression (>10 minutes) or abolition of responsiveness was observed. Electrically evoked response latencies were consistent with activation of Group III & IV nerve fibres (n=22). At low frequencies, 5-10Hz, responses were intermittent during an epoch but become more reliable at frequencies of 5-50Hz. At frequencies > ~100Hz, responses were usually suppressed. Both short term (within an epoch) and long term (lasting at least 15 min) suppression of spike activity was observed. Similarly short-term suppression of spontaneous activity was observed. **Conclusion:** Long-term depression, long considered a cortical or cerebellar phenomenon was demonstrated in the spinal dorsal horn following high frequency primary afferent barrages initiated by innocuous vibro-tactile stimulation or activation of Group III and IV nociceptive afferents.

ORAL-17-02

ACUTE CHANGES IN THE BODY SCHEMA: DEVELOPMENT OF A PHANTOM HAND

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Purpose: These studies investigated the neural mechanisms underlying phantom hand sensations. **Methods:** In 10 subjects we applied acute ischaemic paralysis and anaesthesia to the right arm by inflating a cuff around the upper arm above arterial pressure. The wrist and fingers were held extended and the arm was covered so subjects could not see it. Development of a phantom hand (including its perceived posture and size) and the impairment of different sensory modalities (including light touch, warmth, cold and painful heat) were tracked over 30-40 minutes in several experimental sessions. Development of the phantom was tracked by moving joints on a remote arm and by selection of variably-sized templates of the hand. **Results:** Although held immobile, all subjects developed a phantom of their ischaemic hand that moved into flexion as the block progressed. The final perceived position of the wrist was on average flexed by 31°±1.2° compared to the starting position and finger joints were flexed by 51°±3.5°. The perceived shifts from full extension began at 10-20 minutes after cuff inflation, which was the same time that von Frey thresholds for touch began to increase on the thumb (~10 minutes) and the wrist (~20 minutes). The final position of the phantom was reached at ~30 minutes. Sensation of cold was also impaired beginning at 15 minutes. **Conclusion:** The perceived posture of a phantom hand evoked by deafferentation does not occupy the position in which it was produced. Changes in its posture arise when large myelinated axons (and some smaller axons) are beginning to be blocked.

ORAL-17-03

VESTIBULAR AND SOMATOSENSORY INTERACTIONS FOR HUMAN SPATIAL ORIENTATION

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Purpose: Galvanic Vestibular Stimulation (GVS) at the mastoid processes evokes a vestibular afferent signal unbridled from feedback from other sensory systems. With the head pitched nose-down GVS evokes a virtual motion about an earth-vertical axis [1]. This study investigates firstly, how the perception of rotation induced from prolonged bilateral binaural GVS equates with natural kinetic stimulation and, secondly, how the CNS incorporates vestibular and somatosensory input into a coherent perception of orientation. **Methods:** During 2 minutes of DC GVS (1.5mA) and whole-body rotation we measured, (in 12 healthy subjects 21-54yrs) perceived rotation about the vertical axis, by verbal report during standing and by measuring rotation when attempting to step in place. The second study consisted of two phases (i) a conditioning phase of sustained GVS with eyes open, (ii) a response phase where GVS was turned off and blindfolded subjects attempted to step in place. **Results**: The reported rotation during standing decayed with a time constant τ=50sec. However, there was no habituation in the stepping response toward the anode. Following conditioning with GVS subjects walked in the direction of the conditioning cathodal electrode. These effects were incorporated into a systems control model, in which vestibular and somatosensory signals of heading direction summate. A nonlinear least squares fit revealed the time constants of decay for each of the peripheral and central vestibular processes. **Conclusions**: Constant bilateral GVS is equivalent to an increasing rotational velocity. During prolonged vestibular input the signal transmission gain does not change; rather the zero response reference level is altered through central adaptive mechanisms. Vestibular and somatosensory signals are dynamically combined for our orientation sense. 1. Day BL & Fitzpatrick RC (2005), J Physiology 567, 591-597.

ORAL-17-04

THE ROLE OF VESTIBULAR EFFERENTS ON THE VESTIBULO-OCULAR REFLEX (VOR)

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Purpose: The aim of this study was to compare the VOR between the normal C57Bl/6 mouse and the alpha9 knockout mouse. The alpha9 knockout mouse lacks the alpha9 subunit on the nicotinic acetylcholine receptor – in essence removing vestibular efferent feedback. **Methods:** Using the video technique developed by the first author we measured vestibular-evoked 3D (horizontal, vertical and torsional) eye rotations in 5 C57Bl/6 and 5 alpha9 knockout mice. The stimulus consisted of horizontal sinusoidal and transient whole-body rotations (sinusoids: 0.02 – 10Hz, peak velocities of 20, 50 and 100deg/s; transients: 3000deg/s/s acceleration steps from 0 to 150deg/s). **Results:** Sinusoids: For both mouse types there was no difference in VOR gain and phase between eyes (P=0.14, P=0.65, respectively) and between positive and negative cycles of the sinusoids (P=0.63, P=0.22). There were no naso-temporal asymmetries (P=0.07, P=0.50). Transients: In both mouse types there was no difference in acceleration or velocity gain between eyes (P=0.28, P=0.28, respectively) and there were no naso-temporal asymmetries (P=0.40, P=0.58). Thus all the data were pooled accordingly. The comparison between mouse types revealed three major differences: 1) low velocity (20deg/s) sinusoidal VOR gains of the C57Bl/6 were significantly lower (~50%) than those of the alpha9 knockout (P<0.05), 2) non-linear gain increase was absent in the alpha9 knockout, and 3) the VOR time constant (measured from sinusoidal and transient data) was significantly shorter in the alpha9 knockout (~ 90%). **Conclusion:** These results, plus those from previous vestibular afferent recordings in these mouse types, suggest that efferents affect the sensitivities and ratios of regular versus irregularly firing afferents.

ORAL-17-05

THE ROSTRO-TEMPORAL AUDITORY AREA (RT) PROJECTS TO BRAIN REGIONS ASSOCIATED WITH EMOTIONAL PROCESSING

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Current models of primate auditory cortical functional organization include 3 primary-like "core" areas, characterized by koniocellular cytoarchitecture and direct projections from the ventral subdivision of the medial geniculate body (MGBv). The "core" areas are surrounded by "belt" and "parabelt" regions in a roughly concentric arrangement, representing additional hierarchical levels of processing. Of these, only "parabelt" regions are reported to have direct connections outside the temporal lobe. The rostral-temporal auditory field (RT) is the most recently recognized, and least characterized of the 3 "core" areas. In order to understand its place in the auditory processing network, we injected anterograde and retrograde tracers in RT and adjacent fields in 4 marmosets (*Callithrix jacchus*), under Alfaxan anaesthesia. Supporting its classification as a "core" auditory field, we found that RT exhibited core-like reciprocal connectivity with the MGBv and with other auditory cortices, including dense connections with lateral "belt" areas. However, different from what has been reported for other "core" fields, we observed a focused projection from RT to the caudal medial prefrontal cortex, a region that has been implicated in processing affective context in sensory information. In addition, and RT sends dense projections to the temporal pole, ventral caudate and lateral amygdala, structures that have also been linked to emotional processing. Thus, while RT belongs in the auditory core according to the classical criteria, it also has connectivity with limbic structures. These results are consistent with the view RT may represent a transitional level in the hierarchy of auditory processing, which may could play a role in processing the affective content auditory information. This interpretation is also consistent with a proposed functional role for RT as an area where the structure of sounds is integrated over large time windows, in comparison with other "core" fields.

ORAL-17-06

NEURAL TIMING THROUGH MICRO-STIMULATION OF THE VENTRAL COCHLEAR NUCLEUS

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Purpose: Penetrating Auditory Brainstem Implants (PABIs) have recently been used to stimulate the ascending auditory pathway showing their potential in hearing restoration. Previous animal PABI studies have investigated neural threshold and intensity responses, although timing may be a more important feature of neural coding. We therefore investigated neural timing from PABI stimulation. **Methods:** Male Hooded Wistar rats (n=15) were anaesthetised, fitted with hollow ear bars, and implanted with a 32-channel microelectrode in the VCN. Extracellular single neurons (n=42) were recorded in the central nucleus of the inferior colliculus (CIC) using quartz microelectrodes, in response to acoustic and VCN electrical stimulation. **Results:** A high correlation was observed from all CIC neurons that showed excitatory responses to VCN stimulation between their characteristic frequencies (CFs) and the CFs of the VCN sites that excited them with lowest threshold ($r=0.80$, $p<0.005$), and lowest first spike latency (FSL, $r=0.86$, $p<0.005$). In addition, a rise in threshold and FSL was found with an increase in frequency difference between the CFs of recorded CIC and stimulated VCN sites. **Conclusion:** These results indicate that micro-stimulation of the VCN has some similar temporal characteristics to acoustic stimulation. However, characteristics such as the increase of FSL with increasing frequency difference between the CIC and VCN showed large differences. Through an increased understanding of neural timing from electrical stimulation, advantageous stimulation strategies for PABIs incorporating essential timing information will be able to be developed.

ORAL-17-07

ORIGIN OF HYPERACTIVITY IN GUINEA PIG INFERIOR COLLICULUS AFTER ACOUSTIC INJURY

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Tinnitus, a phantom auditory sensation, is prevalent in the human population and often associated with a mild hearing loss. Several lines of evidence suggest that tinnitus is accompanied by hyperactivity in central auditory pathways, and the current view is that this is a centrally generated plastic response to altered input. **Purpose:** We investigated in guinea pigs the acute and chronic effects of a loud tone exposure, which results in mild hearing loss, on the development of spontaneous hyperactivity in the inferior colliculus. We also investigated whether the hyperactivity in this nucleus is dependent on integrity of the peripheral auditory receptor. **Methods:** Anaesthetized guinea pigs (n=37) were exposed for 1 hr to a loud pure tone. After different recovery times cochlear thresholds were measured and spontaneous activity in inferior colliculus was assessed by single neuron recordings before and after cochlear silencing. **Results:** Our over-exposure paradigm resulted in a small, permanent, frequency-restricted threshold loss in the cochlea up to almost 6 weeks post-exposure (maximum survival time in our study). We also found an increase in spontaneous firing rates ($p<0.001$ compared to controls) in restricted frequency areas of the inferior colliculus, broadly corresponding to the frequency of the cochlear lesion. Hyperactivity in the inferior colliculus disappeared after treatments abolishing cochlear activity. **Conclusion:** This novel finding seems to be conflict with the present view that the hyperactivity is centrally generated. However, dependency on the peripheral receptor need not indicate that the hyperactivity in the central pathways is a direct result of increased spontaneous activity in the periphery. We hypothesize that hyperexcitability within the central nervous system could result in greater neuronal firing to normal levels of spontaneous input from the periphery.

ORAL-17-08

EXCEPTIONAL HIGH-FREQUENCY HEARING IN AUSTRALIAN PYGOPOD LIZARDS

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Pygopod lizards are legless geckos that occur almost exclusively in Australia. We studied the hearing capacity of species of the genus *Delma* in the field using a mobile laboratory. All apparatus was battery operated and under computer control. *Delma pax, D. desmosa* and *D. haroldi* were wild caught under licence in the Pilbara of Western Australia and generally quickly released after experimentation. Under Isoflourane gas anaesthesia, a silver wire electrode was introduced into the mouth cavity and compound auditory-nerve potentials (CAP) averaged in response to repeated short pure tone stimuli (10ms, 1ms rise/fall, 6/s; 30 to 42 repeats). Extremely quiet conditions allowed a measurement threshold of 1μV. Input-output functions (I/O) and forward-masking thresholds (CAP amplitude reduction) using noise bursts as a masker (1kHz bandwidth, 30ms, 3ms rise/fall; ending 3ms before the tone) were also measured. CAP audiograms show very poor sensitivity below 1kHz, a broad area of best hearing at 30dB SPL (2 to 6kHz), a poor threshold near 8kHz but, remarkably, a second area of improved hearing at higher frequencies up to 13 to 15kHz. Such high frequency responses were previously unknown in lizards. The I/O functions were steepest at 8kHz, suggesting recruitment there. Forward-masking thresholds for mid- and high-frequency tones showed minima at both mid- and high frequencies, suggesting that the exceptional responses to frequencies above 8kHz are mediated by the same sensory cells that respond to the mid-frequency range. These animals and gecko relatives have highly specialised hearing organs and these very unusual response patterns need to be related to the unique features of their basilar papillae.