ORAL-14-01

EXPRESSION OF THE NEURITE OUTGROWTH INHIBITOR NOGOA FOLLOWING FOCAL ISCHEMIA OF PRIMATE NEOCORTEX IS GREATER IN THE NEONATE COMPARED TO THE ADULT

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Purpose: The primary visual cortex (V1) is commonly affected by ischemic stroke with molecular and cellular responses differing between the developing and adult brain. Inhibitory molecules such as NogoA may have key roles in repair inhibition following neocortical injury. We investigated the difference in the laminar and cellular expression profile of NogoA at the penumbra following ischemic injury of adult and neonate marmoset monkey V1. **Methods:** To induce ischemia, injections of 0.3µL (1mg/ml) endothelin-1 were performed over 4 sites surrounding posterior cerebral artery of operculum V1 (PD14; n=3) and adult (>1 year; n=2) marmoset monkeys. Following 3 weeks recovery immunohistochemistry for NogoA expression was performed on non-lesioned and lesioned hemispheres. Immunohistochemistry was used to measure NogoA expression and define cell subtypes expressing the molecule. **Results:** Discrete NogoA expression was observed throughout uninjured neonatal and adult V1, particularly in layers 4 and 6. Post-injury, higher levels of NogoA expression were detected in the lesion penumbra in neonates compared to adults. Interestingly, a small population of neurones (NeuN+) were detected as NogoA+, especially in adult V1, post-injury. This is in addition to the expected localisation of NogoA on oligodendrocytes (Olig2+) and myelin in white matter. Further characterisation revealed this subpopulation of NogoA+ neurones as parvalbumin-expressing interneurones. **Conclusion:** We postulate that neuronal expression of NogoA may play modulatory roles following ischemic injury either through redirecting putative regenerating neurites away from the metabolically compromised injury site or inhibiting the formation of new connections in the lesion penumbra. Hence, post-injury neuronal expression of NogoA may prove neuroprotective by maintaining the integrity of surviving visual connections.

ORAL-14-02

THE ROLE OF THE CYTOSKELETON AND CASPASE ACTIVATION IN AXONOPATHY FOLLOWING EXCITOTOXICITY IN VITRO

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Purpose: Axon degeneration is a key pathological feature of neurodegenerative conditions including amyotrophic lateral sclerosis and Alzheimer's disease, although the cause remains uncertain. Our investigations have demonstrated that axon degeneration can result from excitotoxic insult, a pathogenic process implicated in neurodegeneration. Mechanisms of excitotoxin induced axon degeneration are not clear. The current study investigated the role of the cytoskeleton and caspase activation in axonopathy following kainic acid exposure. **Methods:** Cortical neurons were cultured from C57/Bl6 or mice with a knockout of the neurofilament-L gene (NFL-KO) and grown in compartmented microfluidic chambers to examine the role of axon and soma. Neurons at 10 days were exposed to 100µM kainic acid (18hours) in the absence or presence of taxol (1µM) in the axon or soma compartment. Axonal fragmentation was determined from phase contrast images of axons. Immunohistochemical analysis was performed using antibodies to active caspase-3 and MAP2. N=5 repeats from 3 separate cultures. **Results:** Kainic acid applied to soma induced a 41.3% (+/-8.5 SEM) increase in axon degeneration in the axon compartment, which was associated with axonal caspase-3. Pretreatment of axonal or somal compartments with taxol both reduced subsequent kainic acid-induced caspase activation and axonopathy, with a more marked effect following taxol applied to the axonal compartment (12.2+/-2.3% fragmentation, $p<0.05$) as compared to the somal compartment (25.8+/-5.2% fragmentation). Axon degeneration was significantly ($p<0.05$) reduced (15.3%+/-2.1%) in cultured cortical neurons derived from NFL-KO mice. **Discussion:** Cytoskeletal elements such as neurofilaments and microtubules are involved in excitotoxin-induced axon degeneration. However, unlike Wallerian degeneration, this axonopathy involves activation of caspases. Microtubule stabilization may represent a potential therapeutic strategy to minimize degeneration following neuronal insults.

ORAL-14-03

THE POTENTIAL OF NK1 ANTAGONISTS AS ANTI-CANCER AGENTS IN BRAIN TUMOURS

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Purpose: Recent studies have implicated Substance P (SP) and its NK1 receptor in cancer development, and specifically in angiogenesis, acceleration of cell growth and evasion of apoptosis. However, few studies have examined the role of SP using in vivo experimental models of brain tumours. The present study investigates the potential of NK1 antagonists as novel anti-cancer agents using in vitro and in vivo model of brain tumours. Methods: A375 human melanoma cells were treated with the NK1 antagonists Emend or N-acetyl-L Tryptophan (NAT), and markers of cell viability and cell death assessed. For the in vivo model, A375 cells were directly injected into the striatum of male Balb/c nude mice and the effect of NK1 antagonist treatment on tumour growth examined. Results: The NK1 antagonist Emend resulted in a significant ($p<0.001$) decrease in the number of viable cells in vitro. Furthermore, both NAT and Emend treated cells had significantly ($p<0.05$) elevated LDH levels when compared to the non-treated cells, suggesting increased cell death following NK1 antagonist administration. Treatment with an NK1 antagonist in vivo supported the in vitro results, with Emend treated animals demonstrating a significant ($p<0.05$) decrease in tumour volume when compared to the controls. Conclusion: Administration of an NK1 antagonist resulted in a reduction of cell viability and a corresponding increase in cell death markers in vitro. In addition, blockage of SP in vivo caused a significant decrease in tumour growth when compared to controls. Thus, we have confirmed that SP does play a role in cancer growth, and that NK1 antagonists may provide a novel therapeutic intervention in the treatment of brain tumours.

ORAL-14-04

ROLE OF CHEMOKINE SIGNALING IN AN ANIMAL MODEL OF ATROPHIC AGE-RELATED MACULAR DEGENERATION

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Purpose: Recruitment of inflammatory cells into the injured retina is thought to exacerbate photoreceptor death in retinal degenerations such as age-related macular degeneration (AMD). Monocyte/microglia recruitment is dependent on expression of chemo-attractants, such as chemokines, although their role in AMD is yet to be clarified. Using microarray analysis, we investigate the expression and localization of prominent chemokines and chemokine-regulators in a light-induced model of atrophic AMD. **Methods:** SD rats were exposed to 1000lx of light for up to 24hrs. At specific time-points during and following exposure, animals were euthanized and retinas processed. Photoreceptor apoptosis was assessed using TUNEL (n=5) and counts were made of monocytes/microglia immunolabeled with IBA1 (n=4 per). Expression of chemokines were assessed by microarray analysis, and qPCR (n=3-4). Some chemokines were also selected for spatiotemporal analysis by in situ hybridization (n=3 per time point). One-way ANOVA was used for statistical analysis. **Results:** Using qPCR, significant up-regulation ($P<0.05$) of chemokines (Ccl3, Ccl4, Ccl7, Cxcl1, Cxcl10, Cxcl11) and chemokine-regulators (Adam17, IL1B, Myd88, Tlr2, Tnfa) was observed at 24hrs, which correlated with the increase ($P<0.05$) in photoreceptor death. In situ hybridization on retinal cryosections revealed that Cxcl1 and Cxcl10 are expressed by Muller cells and RPE, while Ccl3, Ccl4, and Ccl7 are expressed by microglia - predominately in regions of heavy photoreceptor degeneration. In conjunction, a localized recruitment of IBA-expressing monocytes/microglia ($p<0.05$) to the degenerating ONL was observed. **Conclusions:** Our data indicate that the retina actively contributes to the guidance of the neuroinflammatory response following retinal injury, through local expression of multiple inflammatory factors from chemokine pathways. Characterization of the retinal immune response is crucial in clarifying the underling pathogenesis of inflammation in retinal degenerations, such as AMD.

ORAL-14-05

IN VIVO SUTURELESS MEDIAN NERVE REPAIR

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Purpose. Photochemical tissue bonding (PTB) is an innovative sutureles technique for tissue repair that uses visible laser light to activate photosensitive dyes, such as Rose Bengal (RB), that crosslink collagen fibres, with minimal temperature increase. Our aim in this study was to compare a novel RB-chitosan adhesive repair upon transected rat median nerve to the gold standard end-to-end suture repair. **Methods**. In Long Evan rats (n=60) the left median nerve was transected, repaired and allowed to recover. Three experimental groups were used; end-to-end suture repair, RB-chitosan PTB repair and a sham control. The RB-chitosan adhesive was activated using a green laser (λ=532nm, Fluence~133J/cm2). A tensiometer was used to test and compare the bonding strength of PTB to the suture repair. Histological assessment and electrophysiological recording were used to determine the impact of laser irradiation on the nerve, and an infrared pyrometer measured temperature change of the nerve. **Results.** RB-chitosan adhesive PTB repair achieved acute tensile strengths of 0.37±0.15 N, that was unchanged 1-week after transaction (n=30), with minimal heating (~<6°C, n=10). The RB-chitosan adhesive tensile strength was comparable to the suture group, however histological damage was more apparent in the suture group. When the laser was not used (control), tensile strength dropped to 0.015±0.015 (n=15). **Conclusion.** The laser-activated RB-chitosan adhesive is a simple and promising sutureless procedure for peripheral nerve repair, and is well suited to tissue repair because of its biocompatibility, strength and flexibility in situ along with the absence of thermal and cytotoxicity.

ORAL-14-06

VARIABILITY IN α- AND β-SYNUCLEIN IN PARKINSON'S DISEASE AND MULTIPLE SYSTEM ATROPHY

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Purpose: α-Synuclein is considered the basis for neurodegeneration in Parkinson's disease (PD) and multiple system atrophy (MSA), with pathological diagnosis being made on the presence of α-synuclein positive inclusions in the brain. However, the main cell type containing inclusions is different (neurons for PD, oligodendrocytes for MSA), and MSA shows a higher abundance and greater degree of associated neuronal loss, suggesting involvement of distinct pathological mechanisms in disease progression. β-Synuclein is suggested to be a negative regulator of α-synuclein inclusion formation, and reduced β-synuclein levels are seen in the cortex of dementia with Lewy body cases compared to PD. However these proteins have not been compared between PD and MSA. **Methods:** Following study approvals, frozen brain tissue from controls (n=6), PD (n=6) and MSA (n=6) were obtained from the NSW Brain Banks. Crude soluble and insoluble proteins were extracted from the putamen, and α- and β-synuclein protein levels measured by western blotting. Non-parametric Mann-Whitney U tests were used to determine differences in synuclein levels between the groups, and in the annual rate of change between PD and MSA. **Results/ Conclusions:** PD and MSA showed increased levels of pathological insoluble α-synuclein compared to controls (increased 168% in PD and 175% in MSA from controls, p=0.002), however there was a 765% increase in levels of β-synuclein in PD (p=0.015) compared to MSA. The annual rate of change in insoluble α-synuclein was higher in MSA (increased 15% per year from PD, p=0.025), suggesting a more rapid rate of disease progression in MSA and a potentially protective effect of β-synuclein in PD.

ORAL-14-07

CORTICAL PYRAMIDAL NEURONS DEMONSTRATE RESILIENCE TO DEGENERATION FOLLOWING MILD TRAUMATIC BRAIN INJURY IN THE ADULT MOUSE

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Traumatic brain injury (TBI) is a leading cause of death and disability, frequently resulting in long-term impairments in cognitive and motor function. Adaptive remodelling of neuronal circuitry may contribute to recovery of function. However, the cellular mechanisms linking structural alterations with functional recovery are not fully understood. **Purpose:** To develop a clinically relevant mouse model of TBI in which injury-induced cellular alterations can be monitored over time. **Methods:** Adult male mice (C57 or YFPH) underwent mild to moderate lateral fluid percussion brain injury (FPI; n=3 per time point). Sham-operated and naïve animals were processed concurrently. For immunohistochemical analysis animals were perfused at 1, 2, 4 and 8 weeks post-injury. To investigate dynamic structural alterations in layer 5 pyramidal neurons the cortex of YFPH mice was imaged using in vivo two-photon microscopy. **Results:** Immunohistochemical analysis in fixed tissue demonstrated neurofilament and amyloid precursor protein accumulation in disrupted axons. A stereotypical reaction in astrocyte and microglial populations was also observed. Layer 5 YFP-expressing pyramidal neurons exhibited resilience to neurodegeneration with the majority surviving the injury and maintaining relatively normal cytoarchitecture, albeit with aberrant 'clipped' apical dendrites. Degeneration of YFP-expressing axons was observed within the lesion penumbra, corpus callosum and internal capsule. In vivo two-photon imaging in the cortex of YFPH mice revealed that surviving neurons within the injury site and penumbra survived for at least 4 months following FPI and underwent dendritic spine addition and elimination on their apical dendrites. **Conclusion:** Together our data demonstrate that cortical neurons in the adult mouse brain exhibit resilience to structural injury and an ongoing capacity for adaptive remodelling.

ORAL-14-08

A RARE FUNCTIONAL HAPLOTYPE OF THE *P2RX4* AND *P2RX7* GENES LEADS TO LOSS OF INNATE PHAGOCYTOSIS AND CONFERS INCREASED RISK OF AGE RELATED MACULAR DEGENERATION

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Background: Age-related macular degeneration (AMD) is a leading cause of blindness in Western countries and is diagnosed by the clinical appearance of yellow subretinal deposits called drusen. **Purpose:** To investigate the novel scavenger role of P2X7/P2X4 receptors in AMD. **Methods:** We performed a genetic association study of functional polymorphisms in the *P2RX7* and *P2RX4* genes in a cohort of 744 patients with AMD and 557 age-matched Caucasian control subjects. **Results:** The P2X4 Tyr315Cys variant was two-fold more frequent in AMD cases compared to controls with the minor allele predicting susceptibility to disease. Pairwise linkage disequilibrium was observed between Tyr315Cys in the P2RX4 gene and Gly150Arg in the P2RX7 gene. Genotyping revealed an unique and rare haplotype containing the P2X4 315Cys plus P2X7 150Arg variants overrepresented in AMD (n=17) compared with controls (n=3) (Odds Ratio = 4.05, P=0.026). Expression of P2X7 (wild type or variant 150Arg at this position) in HEK 293 cells conferred robust phagocytosis towards latex beads whereas co-expression of the P2X7 150Arg and P2X4 315Cys variants completely inhibited phagocytic capacity. In the primate eye, immunohistochemistry indicated P2X7 and P2X4 receptors were co-expressed on microglia and macrophages but neither receptor was seen on retinal pigment epithelial cells. **Conclusion:** These results demonstrate that a haplotype including two rare variants in P2RX7 and P2RX4 confers a functional interaction between these two variant receptors which impairs the normal scavenger function of macrophages-microglia. Failure of this P2X7 mediated innate phagocytic pathway prevents clearance of subretinal debris and predisposes individuals towards AMD.

ORAL-15-01

MOTIONS ADD, ORIENTATIONS DON'T, IN THE HUMAN VISUAL SYSTEM

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Purpose: A variety of experiments suggest that human orientation detectors have narrower bandwidth than do detectors for motion direction. My aim was to use (previously published) data from rapid serial visual presentation experiments to explicitly test for bandwidth. **Methods:** Nine human subjects were presented with a rapid stream of stimuli; the stream comprised at least 30 stimuli per second. Where the stimuli were gratings differing in orientation, the task was to respond when a target orientation was seen. Otherwise, the stimuli were dot patterns coherently moving in a variety of directions: the task here was to respond to a target direction. The effects of two consecutive stimuli will sum if the detector's tuning curve is broad enough to encompass both stimuli. Conversely, tuning bandwidths can be determined by finding the smallest angle between consecutive stimuli that facilitates detection. The data analysis therefore determined the interaction between consecutive stimuli in producing detection. **Results:** For the orientation task, subjects were less likely to respond when two preceding orientations bracketed the target orientation, presumably due to a failure of facilitation. For the motion data, by contrast, observers were more likely to respond when the vector sum of two previous directions was in the target direction. I fitted these data with a model consisting of an array of detectors whose peak sensitivities were evenly distributed across the stimulus range. Adjustment of tuning bandwidth allowed the model to fit both sets of data. **Conclusion:** Motion sensors have a broad bandwidth, thereby providing for vector summation of consecutive motions. Orientation sensor bandwidth is less than half of the motion bandwidth, preventing cross-orientation summation.

ORAL-15-02

COMBINATION OF ANTIPSYCHOTICS WITH A POSITIVE ALLOSTERIC MODULATOR OF THE M1 MUSCARINIC RECEPTOR YIELDS SYNERGISTIC EFFICACY IN ANIMAL MODELS OF SCHIZOPHRENIA

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Purpose: Conventional antipsychotics are largely ineffective in treating negative symptoms and cognitive impairment in schizophrenia. Allosteric enhancement of acetylcholine activity at the M1 muscarinic acetylcholine receptor (mAChR) has emerged as a promising strategy for improving these impairments. We investigated the effects of combining an allosteric M1 mAChR modulator with different antipsychotics. **Methods**: The NMDA receptor antagonist MK-801 was used to disrupt prepulse inhibition (PPI) or memory formation in mice (n=10/group). The M1 mAChR allosteric modulator, BQCA, was combined with sub-effective doses of clozapine, haloperidol, olanzapine or aripiprazole prior to MK-801 treatment, and animals were subjected to PPI testing (to test sensorimotor gating) or a Y-maze training session (to test spatial recognition memory). **Results**: BQCA alone did not restore MK-801-disrupted PPI (BQCA + MK-801 = 5.4±3.1% vs. control = 36.7±2.5% & MK-801 = 10.0±2.0%), but enhanced the reversal produced by clozapine (clozapine + MK-801: 24.3±4.0%; + BQCA: 34.4±5.1%), olanzapine (olanzapine + MK-801:21.1±2.7%; + BQCA: 36.6±3.3%), haloperidol (haloperidol + MK-801: 21.2±2.6%; + BQCA: 35.0±3.2%) or aripiprazole (aripiprazole + MK-801 :18.9±3.3%; + BQCA: 25.7±3.3%). In the Y-maze, BQCA alone had no effect, whereas BQCA and clozapine was the only combination that restored memory loss (novel: 38.8±2.0%, familiar: 29.0±2.0%) in MK-801 treated mice (novel: 32.0±1.7%, familiar: 34.1±2.1%) to a similar level as in the control group (novel: 38.7±1.1%, familiar: 28.1±1.8%). **Conclusion**: We provide proof-of-concept that judicious combination of a positive allosteric modulator of the M1 mAChR with current antipsychotics may be a viable add-on treatment for improving the pharmacological treatment of the schizophrenic syndrome.

ORAL-15-03

RESPONSE SENSITIVITY IN THE AUDITORY BRAINSTEM ALTERED BY FEAR CONDITIONED FREQUENCY DISCRIMINATION

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The cochlear nucleus (CN) is a first order auditory processing brainstem structure which can be divided into dorsal and ventral sub-nuclei (DCN and VCN). Intrinsic inhibitory and excitatory projections help determine the response sensitivity of output cells in the VCN believed to be responsible for frequency coding. This investigation seeks to understand whether this finely balanced sensitivity can be altered when rats are classically conditioned to fear the addition of a tone of a different frequency to a repeatedly presented tone (the conditioned stimulus). Change in heart rate was used as a measure of conditioned fear response. In six hooded wistar rats, heart rate change to the discriminating tone was typically characterised by a drop followed by a rise. Wireless neural recording was conducted to assess the level of neural firing in each of the CN sub-nuclei to the discriminating tone and how this is altered through conditioning. Electrode sites (n=128) were located in DCN and VCN. Multi-unit clusters in the DCN showed a significant reduction ($p<0.05$) in the level of firing to the discriminating tone when it was fear conditioned, compared to neural firing seen during an acclimatisation period in the absence of conditioned fear. The opposite response was seen in multiunit clusters located in the VCN which displayed a significant increase neural firing rate in response to fear conditioned stimulus ($p<0.05$). This altered firing pattern suggests that fear conditioned responses are more widespread that initially thought and can alter sensory processing at early stages of the neural pathway. Given that the DCN and VCN are intricately linked a change in the balanced state of inhibition and excitation may underlie this process driven by higher order top-down mechanisms.

ORAL-15-04

DEVELOPMENT OF A CELLULAR-RESOLUTION CONNECTIVITY ATLAS OF THE PRIMATE CEREBRAL CORTEX

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Purpose: Understanding the network of connections between areas of the cerebral cortex is of fundamental importance for understanding physiological and pathological brain function. One promising approach is the development of digital resources that allow co-registration of the results from many individuals onto a common template, as well as correlations with histology and quantitative analyses. This entails generating connectivity atlases (CAs) for the whole brain, with large-scale efforts currently under way for the mouse brain. Here we report on the first results of a program aimed at creating such a resource for the brain of a primate, the marmoset. **Methods:** The development of the marmoset CA is anchored on a large database of retrograde tracer injections (>200 cases), which have been stored in a digital format that preserves the location of each labelled neurone, relative to anatomical landmarks in coronal sections. We have developed techniques for creating 3-d volumes from these materials, and then co-registering these to a template brain in which the cortical cytoarchitecture has been mapped in detail. **Results:** The feasibility of this approach has been demonstrated, through the creation of a site where detailed information from tracer injections in the frontal lobe can be analysed interactively, and downloaded for offline analyses. The histological characteristics of the cortex where injection sites and labelled neurones are located can be visualised interactively, for each individual animal. **Conclusion:** Using current technology, it is feasible create a CA of the primate brain that preserves information with cellular resolution. This will allow future population-based analyses, including quantitative models of network interactions between areas.

ORAL-15-05

GENETIC ANALYSIS OF HDAC4 FUNCTION IN LONG-TERM MEMORY

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PURPOSE: An emerging body of evidence suggests that memory formation is associated with increased histone acetylation of plasticity-related genes. This initiates changes in gene expression that facilitate the synaptic growth required for storage of long-term memories (LTM). The Class IIa histone deacetylase HDAC4 is expressed in neurons, and its subcellular localisation is regulated by CaMKII in response to Ca2+ influx, therefore we hypothesised that it may play a role in regulation of LTM. **METHODS**: HDAC4 was overexpressed (FLAG-HDAC4) or knocked down (via inverted repeat RNAi) in the adult mushroom body (a region of the Drosophila brain critical for memory). Associative memory was assessed using the repeat-training courtship assay (n=20/group). **RESULTS**: Immunohistochemical analysis of FLAG-HDAC4 revealed a predominantly extranuclear subcellular localisation in the lobes and calyx (dendritic field) of the mushroom body. Overexpression of HDAC4 resulted in a deficit in LTM ($p<0.05$, ANOVA) however, no effect on STM was observed. Knockdown of HDAC4 also impaired LTM ($p<0.05$, ANOVA). **CONCLUSION**: Both an increase and a decrease in HDAC4 expression abrogated normal LTM formation in Drosophila, indicating that the role of HDAC4 in regulation of LTM formation is likely more complex than inhibition of plasticity-related gene expression via its deacetylase activity. Indeed, the subcellular localisation of HDAC4 suggests it may also play a synaptic role during LTM formation. Using genetic screening in Drosophila, we aim to identify genes that regulate HDAC4 subcellular localisation and/or genetically interact with HDAC4 during memory formation.

ORAL-15-06

NOVEL METHOD FOR DETERMINING FREQUENCY DISCRIMINATION ABILITIES OF CATS WITHOUT USING NEGATIVE REINFORCEMENT

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Purpose: Animal behavioural studies make a significant contribution to research and provide vital information regarding physiological aspects in ways not possible with human subjects. However, behavioural experiments in animals can be prohibitively time consuming, difficult and stressful to the animal. **Methods:** We developed a novel behavioural experimental system to allow efficient animal training in response to audio-visual stimuli, without employing negative re-enforcers such as electric shocks or food deprivation. Cats were required to perform a relatively simple task of moving toward and away from the device in accordance to the stimuli (go/no-go task). **Results:** Our new experimental setup proved to be effective with all subjects (n=15) performing at above 90% correct on an easy task. Subjects were trained within several weeks and then generated ~200 trials within ~5 sessions per day. A frequency discrimination threshold of 330 Hz (8 kHz reference) in one normal hearing cat measured with the current system was comparable with previously published results. An automated threshold detection technique was also developed and yielded comparable thresholds from another 2 normal hearing control cats. **Conclusion:** The system is relatively simple to set up and animals can generate numerous valid trials after few weeks of training. This method can be generalised to test a variety of different perceptual abilities such as rate and electrode discrimination. Correlation of data generated by this system with electrophysiological data from the same animal is also possible. Ability of testing in home cage and lack of negative reinforcement makes the process faster and less stressful for the animal.

ORAL-15-07

HOW MANY SCENES ARE SEEN? ATTENTION ALLOCATION IN MULTIPLEX DISPLAYS

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Purpose: There is an increasing prevalence of multiplex displays in modern life for both personal and professional use. While a great deal of research has examined attention allocation in single scene viewing, little is known about what these paradigms can tell us about how people attend to a multiplex. **Methods:** In a series of experiments we examine attention allocation across the multiplex and tease apart several potential causes of processing difficulty. Using a modified version of the flicker paradigm with multiple scenes containing a single changed item, we use change detection performance as an index of attention allocation. **Results:** In Experiment 1, participants (n=16) were required to detect changes in monoplex, quadraplex and nonaplex displays. Unsurprisingly, change detection performance decreases as scene number increases (p <.001). There are many potential reasons for this difficulty with multiplex arrays. In Experiment 2 (n=15) and Experiment 3 (n=16) we show that performance is influenced by the information content of the multiplex rather than semantic similarity between scenes or the physical continuity of content across scenes. **Conclusion:** The underlying factors governing attention allocation in multiplex displays appear surprisingly similar to those for single scene viewing, raising questions about whether a multiplex of scenes is treated perceptually as a single scene.

ORAL-15-08

NEURAL ENCODING OF COMPETITIVE EFFORT IN THE ANTERIOR CINGULATE CORTEX

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Purpose: In social environments animals often compete to obtain limited resources. Strategically electing to work against another animal represents a cost-benefit decision, but it is not well-understood how the brain encodes competitive effort costs. Here we tested whether competitive effort tasks recruit the anterior cingulate cortex (ACC), an area previously implicated in cost-benefit decision-making. **Methods:** Single-units in ACC were recorded (n=68) in freely moving rats (n=5) as they performed a competitive foraging choice task involving two goal trajectories. The amount of cost/competition and reward/benefit were manipulated for each trajectory. **Results:** In the baseline cost-benefit configuration, the majority of ACC neurons exhibited heightened and differential firing between the goal trajectories ($p<0.05$, t-test; n=50/68 cells). Inter- and intra-session manipulations indicated that differential firing was not attributable to effort or reward in isolation, but rather ACC encoding patterns appear to indicate net utility assessments of available choice options. When at least one trajectory involved competitive effort, ACC firing rate differentials exhibited a linear relationship with choice behaviour. **Conclusion:** This study demonstrates that in rats, the ACC registers competitive effort costs, and likely uses this information to inform course of action selection.

ORAL-16-01

LEAD (PB) MODULATES HUMAN MICROGLIA INFLAMMATORY RESPONSES

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Purpose: Microglia, the brain resident macrophages, play important role in homeostasis of the CNS as both inflammatory and neuroprotective cells. Microglia activation may occur in response to toxins and environmental pollution. Lead is an environmental persistent pollutant with potent neurotoxic effects even at low concentration. To date, little is known about the role of human microglia in lead induced toxicity. The aim of this study was to investigate inflammatory effects of low concentration of lead acetate on human microglia *in vitro*. **Methods**: Human microglia (M-MG), derived from blood monocytes (n=30), were cultured according to an established protocol [1]. Following exposure to 10 uM lead (Pb) acetate for 24 hours, M-MG were investigated for changes in morphology, phenotype, cytokine secretion patterns and function. **Results**:Lead was taken up by M-MG and visualized intracellularly using fluorescent probes, and fluorescence microscopy and flow cytometry. Lead induced minor, but significant morphological changes. However, at 10uM, lead was not toxic and did not influence cell viability. In the presence of lead, M-Mg showed no changes in phagocytosis and T-lymphocyte stimulation. Most interestingly, down-regulation of chemokine receptors (CCR1, CCR2 and CXCR1) was seen in M-MG in presence of Pb, with the exception of CX3CR1 which was significantly up-regulated *(p<0.05)*. M-MG exposed to lead increased significantly expression and secretion of Il-8 (CXCL8). **Conclusion**: Lead exposure at 10uM modulates inflammatory responses in human microglia. [1]Etemad S et al. A novel *in vitro* human microglia model: Characterization of human monocyte-derived microglia. *J Neurosci Methods*, 2012.

ORAL-16-02

REGULATION OF ZINC TRANSPORTERS IN NEURONS AND ASTROCYTES

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Zinc transporters (ZnTs) are essential for maintaining zinc (Zn) homeostasis in the brain. An alteration in Zn distribution has been suggested to play a key role in Alzheimer's disease (AD), with research showing that Zn accumulates both within and around amyloid-β (Aβ) plaques and neurofibrillary tangles. Furthermore, there is a dysregulation in multiple ZnT's in the AD brain. **Purpose:** This project aims to determine whether the presence of metals and/or Aβ effect the expression and regulation of ZnT1 (responsible for cellular Zn efflux) and ZnT3 (responsible for synaptic vesicle Zn content). We investigated the effects of Zn, copper (Cu) and $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ on protein levels of both ZnT1 and ZnT3 in cortical neurons and astrocytes. **Method:** Cells were cultured from C57/BL6 mice, with neurons derived from E14 embryos and astrocytes from P0 (<24 h old) pups. Cells were dissociated and plated at 1.5 x 10^5 density. Experiments were performed at 21 days in culture (neurons) and 15 days in culture (astrocytes). A range of concentrations of Zn, Cu, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were utilised. Total protein was quantified by BCA and ZnT expression measured by Western Blot. All individual experiments were done in triplicate and repeated (n=3-4/treatment). **Results:** Preliminary results show that ZnT1 is present at a lower concentration in untreated astrocytes compared to untreated neurons. ZnT3 is not present in untreated astrocytes. **Conclusion:** There is a cell-type specific regulation of ZnT proteins, and our ongoing analyses will determine whether this is also modulated by factors relevant to the pathogenesis of AD.

ORAL-16-03

ASTROCYTES MAINTAINED ON 3D NANOSCAFFOLDS EXHIBIT ALTERED PHENOTYPIC RESPONSES TO RHO KINASE INHIBITORS

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Astrocytes are dynamic cells and have well documented roles in astrogliosis. We recently investigated pharmacological manipulation to induce a pro-survival phenotype in astrocytes and used bioengineering to assess astrocytic responses to biomatrices. **Purpose:** To investigate effects of cytoskeletal drugs on astrocytes cultured on nanoscaffolds. **Methods:** Random and aligned nanoscaffolds were engineered from poly-ε-caprolactone. Primary astrocytes (postnatal day 1.5 C57Bl6 mice) were subcultured and plated after 10 days(*div*) in 96-well plates, on random or aligned scaffolds in 96-well plates (8,000 cells/well), or on glass coverslips in 24-well plates (20,000 cells/well). Astrocytes were treated 8 *div* later with vehicle, dibutyryl cAMP (100 μM), or Rho kinase inhibitors Y27632 (30 μM) or Fasudil (100 μM) for another 3 *div* (all n=5) when biochemical and morphological analyses were undertaken. **Results:** Astrocytes were cobblestoned in culture plates, but dramatically different in phenotype on scaffolds: tight clusters formed on random scaffolds, with more elongated processes on aligned scaffolds. Drug treatments decreased the intensity of F-actin staining, increasing that for G-actin (disassembly of actin stress fibres), consistent with decreased GFAP staining. Labelling found for Ahnak (enlargeosome marker) was similar to GFAP but more widespread with Y27632 and Fasudil. Processes infiltrated both types of scaffolds, showing more growth along aligned nanofibres. On random scaffolds Y27632 and Fasudil elevated cell viability and glutamate transport relative to control (P<0.05). **Conclusion:** Astrocytes flourished on biomatrices and phenotypes induced by the Rho kinase inhibitors are of interest for brain repair.

ORAL-16-04

ASTROCYTES IN TDP-43 PATHOLOGY

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Purpose: Changes in the TAR DNA binding protein TDP-43 has recently been suggested an aetiological factor in motor neuron disease (MND). TDP-43 is a DNA and RNA binding protein regulating transcription and splicing and is also involved in transport and local post-transcriptional modification of mRNAs. The protein is abundantly expressed in motor neurons and astrocytes. TDP-43 pathology is triggered by abnormal processing and cytosolic aggregation of the protein or by mutations in the TDP-43 gene. The pathology is similar and the outcome is directly linked to cell death. Mutant TDP-43 causes familial forms of human MND, MND-like disease in transgenic animals and kills motor neurons in primary culture. TDP-43 pathology is also found in astrocytes: a cell type that plays critical roles in the pathology of MND. The mechanisms behind TDP-43-mediated pathology are not known but likely involve non-cell autonomous injury. Thus a clear understanding of normal TDP-43 function and how mutant TDP-43 abrogates this function will provide insight into the basis of MND. Methods: We have established cellular models of TDP-43 proteinopathies by expressing fluorescently tagged TDP-43 (wild-type and mutants) in astrocytes in primary cultures. We have also silenced TDP-43 expression in these cells. We have used these models to investigate the role of TDP-43 and its mutants on normal cell function and on the response of these cells to injury. Results: Presence of TDP-43 mutations affected proliferation of these cells in a p53-dependent manner, caused reorganisation of the cytoskeleton and lead to impaired wound healing in an in vitro injury model (n=5). Moreover, astrocytes carrying TDP-43 mutations had decreased expression of GLT-1 and GLAST glutamate transporters (n=3) and displayed changes in mitochondrial membrane potential as well as in the intracellular transport of these organelles (n=5). Finally, the presence of mutant TDP-43 increased the activity of the Rho family GTPases Rho A and Rac-1 while significantly reduced Cdc42 activity suggesting a direct role for TDP-43 in the regulation of the Rho-family GTPases.

ORAL-16-05

THE ROLE OF BDNF IN CNS MYELINATION AND REMYELINATION

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Purpose: Oligodendrocytes myelinate CNS axons in a stereotypical and highly regulated manner. We have previously demonstrated that brain derived neurotrophic factor (BDNF) promotes oligodendrocyte myelination, via activation of oligodendrocyte-expressed TrkB receptors. Here we investigate the influence that TrkB exerts *in vivo* by generating mice with a conditional deletion of TrkB in oligodendrocytes ($TrkB^{fl/fl}$ MBPcre). **Results:** Analyses of $TrkB^{fl/fl}$ MBPcre mice during development revealed significant reductions in myelin protein expression and myelination of CNS white matter tracts (n=3). This hypomyelination was not due to a reduction in oligodendrocyte number nor the number of myelinated axons, but a significant reduction in myelin thickness, as determined by ultrastructural analyses (n=3). These data suggest that oligodendrocyte-expressed TrkB receptors exert a specific influence to promote myelin membrane extension and myelin thickness. To investigate the potential influence that BDNF exerts on CNS remyelination, we examined TrkB receptor expression in mature mice. We identified that TrkB receptor expression in oligodendrocytes significantly decreased with aging, and that by adulthood TrkB was no longer detected in oligodendrocytes *in vivo* (n=3). Interestingly, the TrkB receptor was strongly re-expressed in oligodendrocytes in mice subjected to a cuprizone-mediated demyelinating challenge (n=3), suggesting that BDNF could be an important factor in promoting remyelination. **Conclusion:** BDNF activates oligodendrocyte-expressed TrkB receptors to promote myelin membrane extension and myelin thickness during development. We are currently investigating whether exogenous BDNF can also promote CNS remyelination *in vivo*.

ORAL-16-06

TRANSCRIPTIONAL CONTROL OF MYELIN MAINTENANCE IN THE ADULT CNS

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Purpose: Although the transcription factors required for the generation of oligodendrocytes and CNS myelination during development have been relatively well established, it is not known whether continued expression of the same factors is required for the maintenance of myelin in the adult. Here we use an inducible conditional knockout (iCKO) strategy to investigate whether continued oligodendrocyte expression of the recently identified transcription factor Myelin gene Regulatory Factor (MRF) is required to maintain the integrity of myelin in the adult CNS. **Method:** We generated $MRF^{fl/fl}$ PLP-CreERT+ve iCKO mice in which MRF can be ablated in myelinating cells via 4-Hydroxytamoxifen administration. Adult (8 weeks) MRF iCKO and control ($MRF^{fl/fl}$ CreERT–ve) mice were given intraperitoneal injections of 1mg 4-Hydroxytamoxifen per day for 5 days. **Results:** Genetic ablation of MRF in mature oligodendrocytes resulted in delayed but severe CNS demyelination, with clinical symptoms beginning at 5 weeks and peaking at 8 weeks following ablation. Demyelination was accompanied by microglial/macrophage infiltration and axonal damage (n=5, $p<0.05$). Transcripts for myelin genes such as PLP, MAG, MBP and MOG were rapidly down-regulated following MRF ablation, indicating an ongoing requirement for MRF in the expression of these genes (n=3, $p<0.05$). Subsequently, a proportion of recombined oligodendrocytes undergoes apoptosis over a period of weeks (n=5, $p<0.05$). Surviving oligodendrocytes gradually lose the expression of mature markers such as APC/CC1 and their association with myelin, without re-expressing OPC markers or re-entering the cell cycle (n=3-5, $p<0.05$). **Conclusion:** These results demonstrate that ongoing expression of MRF within the adult CNS is critical in order to maintain mature oligodendrocyte identity and the integrity of CNS myelin.

ORAL-16-07

OLIGODENDROCYTE DYNAMICS IN THE HEALTHY ADULT CNS: EVIDENCE FOR MYELIN REMODELLING

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Oligodendrocyte progenitor cells (OPCs) continue to proliferate and generate myelinating oligodendrocytes (OLs) well into adulthood. **Purpose:** The function of continued oligodendrogenesis is unclear and it is not known whether adult-born OLs ensheath previously unmyelinated axons, or remodel pre-formed myelin. **Methods:** Using transgenic lineage tracing (with PDGFRa-CreERT2 transgenic mice) and immuno-electron microscopy we examined the “myelination profiles” of OLs generated across the lifespan. **Results and Conclusions:** In the optic nerve individual OLs born between P30 and P60 possessed 21 ± 7 internodes (mean ± s.d., n=18 OLs; range 11-35) of length 76 ± 2 µm (mean ± s.e.m, n=271 internodes; range 12-234 µm), whereas OLs born between P120 and P185 possessed 77 ± 7 internodes per OL (mean ± s.d., n=15 OLs; range 41-125) of length 22 ± 1 µm (mean ± s.e.m., n=702 internodes; range 6-293 µm). We conclude that adult-born OLs in the optic nerve are engaged in remodelling pre-existing myelin - either by replacing OLs that die in service or by intercalating among existing myelin sheaths.

ORAL-16-08

PERI-INFARCT GLIAL CELL RESPONSES IN A PHOTOTHROMBOTIC MODEL OF STROKE IN RATS

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Microglial and astrocytic responses in tissue surrounding an infarct are likely to be important determinants of the extent of neuronal plasticity and functional recovery following a stroke. However, the development of these peri-infarct glial cell responses is not well defined, particularly in the photothrombotic models of stroke that have been widely used to study neuronal plasticity. **Purpose:** To define the development of glial cell activation in response to infarction as a basis for testing interventions to potentially promote neuronal plasticity. **Methods:** Infarcts were induced by photothrombosis in male Sprague-Dawley rats and the brains fixed for analysis 3 hours to 3 days later. **Results:** Tissue infarction identified by loss of immunoreactivity for the neuronal marker, NeuN, and pale cresyl violet staining was well advanced by 3 hours. Peri-infarct microglial activation was already detectable at this time based on changes in cellular morphology. Circularity of the microglial cells, assessed as an indicator of these morphological changes, was significantly increased compared with microglia in the contralateral hemisphere (0.106 ± 0.013 vs 0.046 ± 0.003, n=3, $p<0.01$). By 24 hours, the circularity of the peri-infarct microglia was further increased (0.168 ± 0.009) and microglial activation was seen throughout much of the ipsilateral cortex. Astrocytic reactivity as indicated by immunoreactivity for the cytoskeletal protein vimentin was not detected at 1 day but extended approximately 0.5 mm from the edge of the infarct at 3 days. **Conclusion:** In the photothombotic model of stroke, peri-infarct microglial activation develops early and precedes induction of astrocytic reactivity by many hours to days.

ORAL-17-01

EFFICIENT DELIVERY OF SIRNA TO NEURONS USING LAYERED DOUBLE HYDROXIDE NANOPARTICLES

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Purpose: Small interfering RNAs (siRNAs) are capable of targeting and destroying specific mRNAs, making them particularly suited to the treatment of neurodegenerative conditions such as Huntington's Disease. However, the delivery of unprotected siRNAs is ineffective due to their susceptibility to degradation by ubiquitous nucleases. Layered double hydroxide nanoparticles (LDHs) are now emerging as a potential drug delivery system as they exhibit low cytotoxicity and are highly biocompatible. This study aims to develop LDHs as an efficient and safe siRNA delivery system for the central nervous system. **Methods**: Initially, fluorescently tagged dsDNA-cy5-LDH complexes were injected into the lateral ventricles of C57BL/6 mice (n=3) to determine the extent of penetration. Effectiveness of gene targeting was then assessed by injecting siRNA-EGFP-LDH complexes into the ventricles of EGFP expressing mice (n=3). Coronal sections of C57BL/6 mice were processed for fluorescence analysis and EGFP levels were assessed by Western Blotting. **Results**: The fluorescence intensity observed in the brain of the dsDNA-cy5-LDH group was significantly higher than that injected with dsDNA-cy5 alone (Student t test, $p<0.05\%$). The Western Blot results showed that the EGFP protein level in the siRNA-EGFP-LDH group was lower than in the siRNA-EGFP only group (Student t test, $p<0.05\%$). **Conclusion**: Our study demonstrated that intraventricular injection of dsDNA-loaded LDHs resulted in widespread distribution in the forebrain. Injection of siRNA-loaded LDHs into the lateral ventricle resulted in knockdown of the target gene. These studies therefore suggest that LDH particles have great potential as an siRNA delivery system for patients suffering from neurodegenerative disease.

ORAL-17-02

CONSCIOUS, SIMULTANEOUS RECORDINGS OF RODENT VISUAL ELECTROPHYSIOLOGY: IMPROVED CLINICAL TRANSLATABILITY

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PURPOSE: Electroretinogram (ERG) and visually evoked response (VEP) in rats are commonly measured under physiology-altering anaesthetics. We employ conscious, telemetric ERG and VEP recordings to investigate the effect of laboratory anaesthetics on visual functions. METHODS: We implanted Physiotel transmitters (DataSciencesInternational, U.S.A.) in Long-Evans rats (n=9), with the active ERG electrode affixed onto the superior sclera and the active VEP to the visual cortex. ERG and VEP were recorded in conscious animals up to 28 days post-surgery. Electrophysiology under ketamine:xylazine (k:x) or isoflurane were measured in the same cohort at days 7 and 14. All data are expressed as mean (±SEM) and parameters between groups are compared via mixed linear analysis. RESULTS: Conscious ERG returned maximal a-wave (-15±1μV), rod b-wave (39±5μV) and cone b-wave (17±2μV) amplitudes, which were significantly smaller than that under k:x (a-wave -22±4μV; rod b-wave 56±9μV; cone b-wave 24±3μV) but larger than responses under isoflurane (a-wave -10±2μV; rod b-wave 24±5μV; cone b-wave 8±2μV). Isoflurane produced less sensitive a-waves compared to conscious (1917±334 vs 398±126 $m^2.cd^{-2}.s^{-3}$).VEP amplitudes were similar in all conditions, with only P2-N1 amplitude larger in k:x (15±2μV) compared with conscious (12±2μV) and isoflurane (14±2μV). Isoflurane yielded significantly slower VEP (implicit times: P1 25±3ms, N1 53±4ms, P2 81±6ms) than conscious (P1 18±1ms, N1 34±1ms, P2 62±1ms). P2 implicit times were slowed under k:x (72±1ms) compared to conscious. CONCLUSIONS: This is the first study to record wireless ERG and VEP in conscious rats. We show anaesthesia affects both retinal and cortical electrophysiology. This technology can potentially improve translatability of functional assessments from rodent models to humans.

ORAL-17-03

DEEP BRAIN STIMULATION AND CORTICAL ACTIVATION

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Purpose: Deep brain stimulation (DBS) is an evidence-based treatment for Parkinson's disease (PD) and essential tremor (ET), in which small quadripolar electrodes are implanted into the subthalamic nuclei (STN) or ventral intermedial thalami, respectively. The aim of this study was to investigate whether scalp maps resulting from DBS can help identify functionally and differentially-connected subregions of the STN and thalami for optimal placement of electrodes. Methods: DBS was carried out in three PD and three ET patients. Each electrode had 4 contacts, spaced with 1.5mm interelectrode distance (lead model 3389, Medtronic, Meerbusch, Germany). Different combinations of these contacts were activated to stimulate distinct subregions in the STN or thalami. EEG was recorded concomitantly with DBS. Independent component analysis and spectral analysis were applied to estimate the scalp map of the DBS pulses. Results: In both PD and ET patients, the stimulation pulses were pronounced over the motor cortex and frontal areas, however sparse in the parieto-occipital regions. Choosing different DBS contacts resulted in activation of different areas of the cortex, indicating strong ipsilateral subcortico-cortical connectivity. Some combinations of contacts activated only a small area of the cortex while others activated widespread cortical areas. Conclusion: This study provides first evidence that the cortical representation of the DBS pulse may depend on the subcortico-cortical connectivity of distinct narrowly-spaced subregions in the target nuclei. Whether this might be of help to guide electrode localisation and programming can be addressed in larger cohorts by combined clinical, electrophysiological and imaging studies.

ORAL-17-04

A PHYSIOLOGICALLY PLAUSIBLE SPATIOTEMPORAL MODEL OF BOLD ALLOWS DECONVOLUTION OF HEMODYNAMIC AND NEURONAL RESPONSE COMPONENTS

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Purpose: Functional MRI (fMRI) experiments rely on precise characterization of the blood oxygen level dependent (BOLD) signal. As current hardware allows fMRI in the submillimeter range, the need for quantitative modelling of the spatiotemporal properties of this signal becomes pressing. Here, we find that a detailed physiological theory for cortical tissue predicts hemodynamic waves that travel several mm across the cortical surface. This understanding allows a solution to the inverse problem and thus a more precise estimate of the underlying neural activity. We apply this model to high resolution (1.5mm) and super high resolution (0.8mm) fMRI data. **Methods:** A model of spatiotemporal hemodynamics derived from physiology (Aquino et al. PLoS 2012) is used to predict the spatiotemporal hemodynamic response function (stHRF) – the BOLD response to an impulsively local neural drive. The properties of the stHRF were then tested on four subjects. Subjects viewed an evoked visual paradigm, while fMRI was recorded at 1.5 mm or 0.8mm resolution. Spatiotemporal neural activity was estimated by inverting fMRI data using Wiener deconvolution and the stHRF. **Results:** Our predicted hemodynamic waves were validated, traveling 5–10 mm across the cortical surface at an average speed of 4±2 mm/s (S.E.M.) and damped at a average rate 0.8±0.2 /s (S.E.M.). Furthermore, these responses can be separated into a local and a propagating component transitioning at ~ 1 mm. These estimates confirm the prediction of our spatiotemporal model, and the measured features agree with parameter estimates derived from physiology. Deconvolution of these data yields a localized neural activity of ~1 mm that agrees with independent measures of the neuronal point spread function. **Conclusion:** We demonstrate the first successful spatiotemporal deconvolution of the hemodynamic components of BOLD revealing the underlying neural dynamics. Thus demonstrating a method that can be incorporated with existing experiments and models of neural activity.

ORAL-17-05

DEFINING MECHANICAL STATES OF PERISTALSIS USING COMBINED IMPEDANCE/MANOMETRY INTRALUMINAL RECORDING

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Purpose: Utilising measures of gut diameter (video) and intraluminal pressure (manometry) we have defined the mechanical states of the intestinal muscle during neurogenic and myogenic motor activity. Similar analysis of the gut mechanical states *in vivo* is not feasible, as video imaging of the gut cannot be performed. Intraluminal impedance has been used to assess the cross sectional area of the lumen (internal diameter) in human clinical research studies. Therefore, we used a combined manometry/impedance catheter to examine whether impedance could accurately measure changes in diameter, and then when combined with manometry recordings, identify the mechanical states of the muscle. **Methods:** Motor activity of isolated rabbit distal colon were studied in a bath of oxygenated Krebs solution at 37°C. Spatio-temporal maps of changes in diameter were constructed from video recordings and spatio-temporal maps of pressure and impedance were constructed from the measures recorded by a high-resolution impedance/manometry catheter. We developed combined maps of: i) diameter & pressure (DPMaps); ii) diameter & impedance (DImaps); iii) pressure & impedance (PImaps). Correlation between changes in diameter and impedance were assessed with Pearson cross correlation. The calculated mechanical states of the muscle were compared between DPmaps & PImaps. **Results** showed excellent correlation between changes in impedance and diameter ($r = 0.85$). States of active and passive neurogenic activity could be identified and matched to those defined between pressure and diameter. **Conclusion:** These results support the potential application of combined manometry and impedance to measure in humans the mechanical state of gut during normal and abnormal gut motility.

ORAL-17-06

DEVELOPMENT OF VIRAL VECTOR-MEDIATED PHARMACOGENETIC TOOLS TO FACILITATE *IN VIVO* INVESTIGATION OF NUCLEUS INCERTUS / RELAXIN-3 NEURAL NETWORKS

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Purpose: The complex heterogeneity of brain neurons/networks has fostered the use of viral vector-based techniques to facilitate modification and characterisation of specific neuron populations. 'Designer Receptors Exclusively Activated by Designer Drugs' (DREADDs) are modified muscarinic GPCRs that when expressed in neurons can induce either depolarization or hyperpolarization upon activation by the synthetic ligand, clozapine-N-oxide. The nucleus incertus (NI) in the midline tegmentum is a distinct GABAergic nucleus that expresses high levels of the neuropeptide relaxin-3 and is particularly amenable to viral-based manipulations (e.g. Callander GE *et al.*, PLoS One 7, e42300, 2012). Therefore, we are currently developing relaxin-3- and relaxin-3 receptor (RXFP3)- promoter-based viral vectors to drive expression of DREADDs in the NI and populations of its target neurons. **Methods**: The relaxin-3 and RXFP3 promoters have been cloned using the Invitrogen Gateway cloning system. High-titre viral preparations have been produced and are being validated *in vitro* and *in vivo*. **Results**: In studies so far, we have utilised small-scale, viral vector production to demonstrate the viability of *in vitro* transduction and protein expression driven by the relaxin-3 promoter. In future studies, we will express DREADDs in relaxin-3-expressing NI neurons or in RXFP3 receptor-positive neurons present in regions such as the amygdala or medial septum. **Conclusion**: These 'DREADD viral vectors' will allow us to better investigate the role of the ascending NI neural network and RXFP3-targeted neuron populations in the control of arousal/behavioural activation and cognition in response to mild and strong neurogenic stressors such as anxiety and fear conditioning.

ORAL-17-07

TOWARDS DEVELOPMENT OF AAV VECTORS FOR TREATMENT OF LEUKODYSTROPIES

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Background: Acute or chronic demyelination underlies the pathology of leukodystrophies, inherited myelin diseases typically caused by single gene mutations altering function or viability of oligodendroglial or astroglial cells. These disorders are incurable and associated with substantial morbidity and mortality. Recombinant adeno-associated virus (rAAV) vectors have proven to be a safe and versatile tool for gene transfer to the central nervous system. Despite its potential, lack of vectors with cell type selective, glial tropism has precluded gene therapy for leukodystrophies. **Purpose**: Design of rAAV vectors and treatment strategies for gene therapy of leukodystrophies. **Methods**: Examination of AAV vector tropism and spread of novel AAV serotypes expressing GFP controlled by promoters of genes encoding myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP) and chicken beta actin (CBA) in vivo. **Results**: Following intrastriatal injection of 2x109 vg, the novel serotypes AAVrh20, AAVrh39 and AAVcy5 showed significantly better vector spread than mosaic AAV1/2. Despite subtle, serotype specific differences targeting transgene expression to specific cell types depended on the promoter and developmental stage of the animal. In adult mice intrastriatal AAV-CBA-GFP injection resulted in robust neuronal GFP expression, AAV-GFAP-GFP conveyed transgene expression in astrocytes and injection of AAV-MBP-GFP or AAV-MAG-GFP restricted GFP expression to oligodendrocytes. While astrocyte specificity was maintained after neonatal AAV-GFAP-GFP delivery, oligodendrocyte specificity of AAV-MBP-GFP and AAV-MAG-GFP was not, but recurred in animals injected at postnatal day 10. **Conclusion**: In vivo targeted transgene expression depends on serotype, promoter and developmental status at intervention. All require consideration during development of gene therapies targeting leukodystrophies.

ORAL-17-08

MEASURING THE SPATIOTEMPORAL PROFILES OF NEURONAL ACTIVITY AND BOLD IN EARLY VISUAL CORTEX USING HIGH RESOLUTION FMRI

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Background: Crucial aspects of visual scene processing are enacted by neuronal interactions within visual cortex, including lateral interactions within areas and divergent connections along the visual stream. These processes are reflected by short and long range neuronal point spread. Whilst functional Magnetic Resonance Imaging (fMRI) provides, in principle, an ideal opportunity to assess this intra- and inter-areal connectivity, the spatial properties of the BOLD (Blood Oxygen Level Dependent) signal partly reflect neurovascular responses. This includes processes non-separable in space and time. **Methods:** Subjects (n=10) viewed an annular flickering (4Hz) boom-gate stimulus (3.5 degree ecc.) one pixel wide (0.03 degree vis. ang.). Three different moderate contrasts (16% gray, 25% gray 35% M-L isoluminant) on a mid gray (16 candela/m^2) background were used. fMRI was recorded at high resolution (1.5mm) and super high resolution (0.8mm) using 3T MRI system. A detailed spatiotemporal hemodynamic response function (Aquino et al., PLoS Comp. Biol. 2012) allowed us to disambiguate vascular and neuronal contributions to the spatial profile of the BOLD signal. **Results:** We find point spread parallel but not orthogonal to the cortical surface. This spread amounts to 7.5+-0.6 mm in V1, extending to 12 +- 0.8 mm in V2 and 14 +-1.2 mm in V3. A small negative BOLD response occurred 13-20 mm from the primary response unilaterally towards the periphery, exclusively in V1, reflecting inhibitory surround processing in primary visual cortex. These responses were invariant to the use of isochromatic versus isoluminant contrast stimuli (Wade & Rowland, JNsc 2010). Hemodynamic deconvolution reveals the spatial profile of neuronal responses underlying these changes in the BOLD signal, allowing unique insight into the profile of synaptic connectivity within V1 and quantitative estimates of divergence along the visual stream.

ORAL-18-01

ANALYSIS OF PLANNING AND ONLINE CONTROL OF MOVEMENT IN MULTIPLE SCLEROSIS USING A FITTS' LAW RECIPROCAL AIMING PARADIGM

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Purpose: Many assessments of Multiple Sclerosis (MS) related motor symptoms are subjective and do not differentiate between movement planning, execution and accuracy. This study examined these aspects of motor control in MS using a computerised Fitts' law reciprocal aiming task. **Methods:** Twenty two MS participants and 22 matched control participants performed 200 reciprocal movements between two targets. Task difficulty was manipulated as a function of target size and distance. **Results:** MS participants spent longer dwelling in the target before the initiation of the next movement and had a lower peak velocity. These results demonstrated deficits in movement planning. MS participants spent longer in the deceleration phase of movements indicating deficits in the online control of movement. With increasing task difficulty, MS participants showed a disproportionate decrease in peak velocity ($p = .02$, $\eta 2 = .08$) and increase in time spent decelerating ($p = .005$, $\eta 2 = .10$). **Conclusion:** The Fitts' task objectively measures subtle motor symptoms and differentiates deficits in planning, accuracy and online control of movement. The task also taps into wide ranging motor networks. These features make the task ideal for the assessment and rehabilitation of MS related motor symptoms, as well as the measurement of response to therapeutic intervention.

ORAL-18-02

P75NTR MAY BE A BIOMARKER FOR MOTOR NEURON DISEASE PROGRESSION

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Purpose: Biomarkers are urgently required for trials of potential therapies for Motor Neuron Disease (MND), a disease without effective treatments. We have previously shown p75NTR is higher in urine of sporadic MND patients and symptomatic MND mice (SOD1G93A) than in the urine of healthy humans and mice. We now aim to ask if urinary p75NTR is a marker of disease progression and could be used in clinical trials. **Methods:** The age at which p75NTR is upregulated in motor neurons of SOD1G93A mice was quantified by IHC (n=4) and compared to p75NTR levels in urine. Riluzole (140-210 mg/kg/week) trials in SOD1G93A mice are in progress, with p75NTR levels measured in treated and non-treated mice (n=10) across disease progression using a novel ELISA. Urine and neurological data was collected from human MND patients (n=10) and from people living with Parkinson's (n=5), Multiple Sclerosis (n=6) and controls (n=6). **Results:** p75NTR was detectable in urine of healthy SOD1G93A mice (40-60d), and increased until end-stage (145-160d; n=6). Comparatively, little p75NTR was detectable by IHC in motor neurons of SOD1G93A mice before 100d (n=4), suggesting it is not the source of urinary p75NTR. Experiments are underway to analyse p75NTR in urine of SOD1G93A mice treated with riluzole using novel ELISAs. p75NTR levels in urine of people living with MND (n=10) increased with disease severity, as measured by the ALS functional rating scale, and were significantly higher ($p < 0.001$) than in healthy controls (n=6), Parkinson's (n=5) and MS patients (n=6). **Conclusion:** We conclude that urinary p75NTR could be useful as a biomarker for MND to monitor disease progression and hence the effects of potential treatments in trials.

ORAL-18-03

CHRONICALLY ELEVATED STRESS HORMONE ACCELERATES THE ONSET OF MEMORY DECLINE IN HUNTINGTON'S DISEASE MICE

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Purpose: Huntington's disease (HD) is an adult-onset, neurodegenerative disease once regarded as a genetic fate. It is now known that environmental factors can also influence symptom onset (cognitive, psychiatric and motor deficits). However, very few factors have been identified. Recent data suggests that HD mice are more susceptible to acute *stress*. We hypothesized that chronically high stress hormone levels would accelerate symptom onset in HD mice. **Methods:** R6/1 transgenic HD mice and wildtype littermates were treated with corticosterone dissolved in drinking water (25mg/L) or water alone (n=9-14 per group). Treatment started from 6 weeks of age, before the onset of established cognitive (Y-maze) and motor impairments. Additional phenotyping for ethological (nest-building) and sexual (vocalizations to female urine) deficits was also conducted. Behavioural testing occurred from 6-15 weeks of age to monitor symptom onset and progression, after which the brain and adrenal gland were weighed. **Results:** HD mice (CORT and water) showed a decline in nesting scores (from 6 weeks of age) and sexual responses (from 14 weeks). CORT-drinking HD mice developed Y-maze memory impairment earlier than water-drinking HD mice. Other behavioural tests were not affected by CORT in either genotype. Chronic CORT reduced brain and adrenal weights, with a more pronounced reduction in HD mice. **Conclusions:** New behavioural deficits (nest-building and sexual response) have been identified in this HD mouse model. This is also the first evidence that chronic corticosterone can accelerate any aspect of the HD phenotype, suggesting that cognitive function in the HD brain is more vulnerable to stress. Therefore, interventions such as stress management may help delay onset of cognitive deficits in HD individuals.

ORAL-18-04

GLOBAL FATTY ACID COMPOSITION IS ALTERED IN PARKINSON'S DISEASE ANTERIOR CINGULATE CORTEX

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Purpose: We previously demonstrated significant reductions in sphingolipid levels and fatty acyl chain length in Parkinson's disease (PD) grey matter anterior cingulate cortex (ACC). The aim of the present study was to explore whether these changes were sphingolipid-specific. **Methods:** Lipids were extracted from frozen ACC and occipital cortex (OCC) from PD patients (n=9) and age-matched controls (n=10), from the Sydney Brain Bank (supported by Neuroscience Research Australia, University of New South Wales and National Health and Medical Research Council of Australia). Total lipid fatty acid (FA) methyl esters were analysed by gas chromatography. **Results:** In both ACC and OCC 14 FAs were identified. A significant decrease in total FA concentration (pmol/mg tissue) in PD was observed for ACC only (26%), with reductions in 10 individual FAs. FA relative abundance (mol%) was also only altered in ACC (significant changes in 11 FAs). The degree of change between PD and controls (mol%) was greater for sphingolipids than total FA composition, i.e. the mean mol% change for sphingolipids was 4% while for FAs it was 1%. Polyunsaturated FAs were increased in PD (6%) and peroxidation index was also increased (31%), indicating increased predicted susceptibility of PD ACC to peroxidative damage. **Conclusion:** Although changes in total FA composition were similar to sphingolipids in PD ACC, the degree of change in fatty acyl composition was much greater in sphingolipids, indicating that altered sphingolipid metabolism in PD may be particularly important. Altered global ACC FA composition may contribute to increased lipid peroxidation in PD.

ORAL-18-05

REDUCED COPPER, COPPER TRANSPORT PROTEINS AND CUPROPROTEIN SOD1 ACTIVITY IN THE SUBSTANTIA NIGRA IN PARKINSON'S DISEASE

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Purpose: The characteristic motor symptoms of Parkinson's disease (PD) result from relatively selective neuron death within the substantia nigra (SN). Changes in metals are believed to play a key role in cell death mechanisms in this disorder. We recently used synchrotron X-ray micro-and nano-fluorescence technologies to demonstrate a significant decrease in copper (Cu) levels in surviving neurons in the PD SN (p=0.004). Such a reduction suggests changes in copper transport pathways and dysregulation of cuproproteins. **Methods:** We therefore examined the distribution and cellular localisation of Cu transport proteins, and activity of the cuproprotein superoxide dismutase 1 (SOD1), in post-mortem human brains with a pathological diagnosis of PD (n=8), compared with controls (n=8), using inductively coupled plasma-mass spectrometry, western blot, and immunofluorescence. **Results:** We identified a marked reduction in neuronal Cu transport protein 1 (Ctr1) immunoreactivity in the SN in PD. Further, in the PD SN, neuron-associated Ctr1 levels were significantly correlated with Cu levels (p=0.008). In these same PD cases, the pattern of specific SOD1 activity was significantly altered, reflecting regional vulnerability (p=0.028). **Conclusions:** These data suggest that copper pathways and cuproprotein function are reduced in PD, reflecting disease-specific cell vulnerability and could be targeted for treatment.

ORAL-18-06

IDENTIFICATION AND CHARACTERISATION OF A NOVEL GENE ASSOCIATED WITH X-LINKED EARLY ONSET PARKINSONISM

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Purpose: Recent advances in our understanding of Parkinsonian disorders have been driven by the identification of causative mutations in families, where a linkage based approach can be utilised to identify disease associated genes. We have characterised an Australian kindred with three males displaying intellectual disability and early onset Parkinsonism. **Methods:** All available family members were genotyped and linkage analysis was performed. **Results:** A ~17cM shared haplotype was identified at Xq27.3. This region is distinct from the reported *PARK12* locus and achieved the maximum LOD score obtainable for the X chromosome in this family (LOD=0.6). A deletion of ~45Kb was identified within the shared haplotype in all affected brothers. The deletion was predicted to result in the complete loss of a single gene and this was confirmed by PCR analysis. In a second large family with a similar phenotype, we identified a missense mutation in the same gene that segregated with the disease phenotype. The mutation affected a highly conserved region of the protein and was predicted to be damaging/pathological by multiple algorithms. Macroscopic post mortem analysis revealed pallor of the substantia nigra and locus coeruleus. Microscopic analysis revealed loss of pigmented neurons. Abundant Lewy bodies, Lewy neurites and tau immunoreactive tangles were observed within surviving pigmented neurons. **Conclusion:** We have identified a novel gene associated with early onset Parkinsonism. Loss of function is associated with extensive Lewy pathology and dopaminergic neuron loss.

ORAL-18-07

ACUTE EFFECTS OF ROTENONE ON LOCUS COERULEUS AND SUBSTANTIA NIGRA PARS COMPACTA NEURONS: IMPLICATIONS FOR PARKINSON'S DISEASE

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Purpose: While the major hallmark of Parkinson's disease (PD) is relatively selective degeneration of dopaminergic neurons in the Substantia Nigra pars compacta (SNc), there is also substantial loss of noradrenergic Locus Coeruleus (LC) neurons. The loss of these neurons may contribute to non-motor symptoms of PD which often occur earlier in the disease than characteristic motor symptoms. Based on the theory that LC neurons are damaged earlier in the progression of PD, we hypothesize that these neurons are more vulnerable to neurotoxic insult than SNc neurons. **Methods:** Using electrophysiological techniques, we have directly compared the responses of LC and SNc neurons in acute brain slices to rotenone, a mitochondrial complex I inhibitor, widely used to produce animal models of PD. **Results:** Rotenone (0.1-5 μM) produced a dose-dependent ($p<0.05$) decrease in the spontaneous firing of LC and SNc neurons recorded extracellularly, associated with cell membrane hyperpolarisation and a tolbutamide (100 μM)-sensitive outward current in whole cell patch clamp recordings. These effects were largely mediated by ATP-sensitive K^+ (K_{ATP}) channels, the activation of which was greater in SNc neurons than LC neurons ($p<0.01$). When K_{ATP} channels were blocked with tolbutamide, rotenone (1 μM) increased the firing of LC and SNc neurons. This effect which was associated with an inward current, unmasked by intracellular Cs^+, which effectively blocks all K^+ conductances. **Conclusion:** Rotenone activates K_{ATP} channels more strongly in SNc neurons than in the LC, potentially protecting these neurons from detrimental effects of mitochondrial toxins such as rotenone. Thus LC neurons may be more susceptible to neurotoxin-induced damage than SNc neurons.

ORAL-18-08

MAPT GENOTYPE, METHYLATION AND THEIR IMPLICATIONS FOR LATE-ONSET PARKINSON'S DISEASE

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Purpose: Parkinson's disease (PD) is a progressive neurodegenerative disorder for which environmental factors, including diet, have been shown to influence disease risk and have been hypothesised to act via an epigenetic mechanism. The microtubule-associated protein tau (*MAPT*) is a susceptibility gene for late-onset PD where the H1 haplotype is associated with increased risk of disease. This may occur through genotype-specific methylation of the *MAPT* promoter. **Methods:** We determined the level of DNA methylation within the *MAPT* promoter in two lymphocyte DNA cohorts (Queensland PD cohort: 346 PD and 228 non-PD subjects; Memory and Ageing Study: 847 non-PD subjects) and two brain DNA cohorts (Brain bank: 31 PD, 12 non-PD; Cerebellum: 70 non-PD subjects). These were analysed using bisulfite treatment and pyrosequencing. **Results:** In lymphocyte samples *MAPT* genotype and gender were significant predictors of methylation ($p<0.0005$) and higher *MAPT* methylation levels were significantly associated with a later age of PD onset ($p = 0.024$). We observed a significant correlation of blood serum vitamin E levels with lymphocyte *MAPT* methylation ($p = 0.007$). In Brain bank tissue, methylation levels were significantly lower in the putamen of PD patients ($p = 0.001$). In cerebellum, high *MAPT* methylation predicted lower levels of *MAPT* expression ($p = 0.043$). **Conclusion:** This is the first study to demonstrate that differential methylation of *MAPT* is associated with two parameters of PD: disease state and age of onset. Our identification of genotype and micronutrient effects on methylation of the *MAPT* promoter has important implications in understanding the pathogenic mechanism of this gene.

ORAL-19-01

ENTERIC GANGLIOGENESIS DRIVEN BY DIFFERENTIAL CELL ADHESION: CELL BIOLOGICAL AND MATHEMATICAL MODELS

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Purpose: Enteric neural crest cells (ENCCs) assemble into numerous small closely-spaced enteric nervous system (ENS) ganglia while migrating, proliferating and differentiating into neurons and glia/ENCCs within the rapidly growing gut. Ganglionation involves cell aggregation so we described cell adhesion molecules in the ENS, examined ENS cell adhesion experimentally in vitro, and developed a formal model of ganglionation. Methods: Cell adhesion molecules were immunolabelled in developing gut. For cell adhesion experiments, guts were enzymatically dissociated and neural cells selected by FACS with HNK-1 and NCAM antibodies. These cells were allowed to aggregate *in vitro*, and challenged by reagents affecting adhesion molecule function. Mathematical models of aggregation were made using cellular automaton (CA) algorithms encoding differentiation (ENCC to neuron), relative cell-cell adhesive strength (neurons≥ENCC), movement, proliferation (neurons≤ENCC), and gut growth. Results: N-cadherin+ /NCAM+ /Hu+ /Sox10- neurons progressively became centrally placed in enteric ganglia and N-cadherin+ /NCAM- /Hu- /Sox10+ ENCCs were located on the periphery. *In vitro*, ENS cells generated spherical aggregates of uniform size, independent of the conditions but dependent on cadherin and NCAM function. Neurons became centrally placed with peripheral ENCCs. The CA model evolved multiple small, spaced ganglion-like clusters which became relatively stable, with a balance between the number of central neurons and peripheral ENCCs. Conclusion: These results indicate adhesion-driven self-organisation takes part in ganglionation, and are sufficient such that the central/peripheral cellular ganglionic structure emerges. Moreover, the underlying properties of cell movement, proliferation and differentiation that allow the emergence of multiple small, closely spaced stable ENS-like aggregates exist over a broad parameter space, consistent with ENS morphogenesis being a resilient program.

ORAL-19-02

A PATHOGENETIC MODEL FOR TOURETTE SYNDROME

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Purpose:, Tourette Syndrome (TS) is a complex neurodevelopmental disorder affecting up to 1% of school age children with high heritability and association with other relatively common neurodevelopmental disorders. The high heritability of TS holds great promise for enabling identification of the genetic and neuropathological basis of the disorder, however, after decades of international research so very little is known about the molecular architecture of TS. **Methods:,** Literature was reviewed with a particular focus on finding genetic and molecular pathways that overlap between TS and related neurodevelopmental disorder such as Autism Spectrum Disorder (ASD). **Results:,** While the recent successes in gene discovery backed by rapidly advancing genomic technologies have given us new insights into the genetic basis of the disorder, the growing collection of rare and disparate findings have added confusion and complexity to the attempts to translate these findings into neurobiological mechanisms resulting in symptom genesis. However, we identified a previously unrecognised genetic link between TS and a competing series of trans-synaptic complexes (NRXNs, NLGNs, LRRTMs, LRRNs, CBLN2) that links it with Autism Spectrum Disorder (ASD) through neurodevelopmental pathways. We also uncovered a series of closely related neuronal genes located/nested within the introns of genes frequently disrupted in TS. For example, two related neuronal leucine rich repeat transmembrane protein genes, LRRTM3 and LRRN3, are found nested within the introns of genes that are repeatedly disrupted in TS, namely CTNNA3 and IMMP2L, respectively. Members of the LRRTM and LRRN gene families regulate inter-neuronal connectivity and LRRTM3 competes with neuroligins for the trans-synaptic binding of neurexins1 regulating synapse formation and maintenance within the brain. **Conclusion:** We present a pathogenetic model of TS that integrates all five genes so far found to be uniquely disrupted in TS into a single pathogenetic chain of events that has significant clinical and research implications.

ORAL-19-03

SEX SPECIFIC EFFECTS OF PRENATAL STRESS ON MYELINATION IN THE HIPPOCAMPUS, CEREBRAL CORTEX AND CEREBELLUM

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Purpose: Prenatal maternal psychosomatic stress has been associated with many detrimental perinatal outcomes, leading to disruptions in fetal brain development. Previous studies have shown repeated exogenous glucocorticoid administration perturbs fetal brain growth, and that outcomes of prenatal stress are often sexually dimorphic, with males showing higher rates of behavioural disorders in childhood. Our aim was to determine the effect of prenatal stress on late gestation fetal brain development by measuring myelin basic protein (MBP) in the hippocampus, cerebral cortex and cerebellum and assess any sex differences. **Methods**: Stress was induced by exposing pregnant guinea pigs to a strobe light for 2h/day on gestational day 50, 55, 60 and 65 (term 70d). Fetal brains were collected at term for immunohistochemical analysis of a marker of myelination (MBP). **Results**: In those pregnancies exposed to prenatal stress, female fetuses (stress n=6; control n=6) demonstrated higher brain-to-liver ratios ($p<0.01$), indicative of brain sparing. Prenatally stressed male fetuses (stress n=7; control n=8) showed significantly reduced MBP immunostaining in the hippocampus (CA1 $p<0.001$), cerebral cortex ($p<0.05$) and cerebellum ($p<0.001$), indicating compromised brain development. Female fetuses showed no effect of prenatal stress exposure on MBP immunostaining. **Conclusions**: These results suggest that female fetuses employ a neuroprotective growth adaptation in the form of brain sparing in response to prenatal stress. In contrast, male fetuses showed impaired brain development in the form of reduced myelination in response to stress. These data highlight the vulnerability of male fetuses to the effects of prenatal stress and the postnatal outcomes that may ensue.

ORAL-19-04

EMBRYONIC DEVELOPMENT AND MATURATION OF CHOLINERGIC ENTERIC NEURONS

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Purpose: Acetylcholine is an important neurotransmitter used by many different types of neurons in the enteric nervous system (ENS), including excitatory motor neurons and interneurons. However, the development of these cholinergic neurons has not been previously examined as they are difficult to detect in embryonic gut tissue using immunohistochemistry against choline acetyltransferase (ChAT) or other markers of cholinergic neurons. In this study, we used ChAT-Cre;ROSA-YFP mice to investigate the expression of ChAT in the developing murine ENS. Methods: ChAT-Cre;ROSA-YFP mice were generated by mating ChAT-CRE mice with ROSA26-YFP reporter mice. In ChAT-Cre;ROSA-YFP mice, all cells that initiate expression of ChAT express YFP. Immunohistochemistry was performed on embryonic, postnatal day (P)0 and adult gut using anti-GFP antisera to examine the expression of ChAT. Results: The earliest YFP+ cells were detected at embryonic day (E)11.5, when they made up only 5 ± 2.0% of the total number of neurons (identified by Immunoreactivity for the pan-neuronal marker Hu) in the rostral midgut. The number and proportion of YFP+ neurons increased through embryonic development to 18 ± 2% of Hu+ neurons at P0, and 60% in adults. Approximately 20-30% of embryonic YFP+ neurons were also immunoreactive for calbindin; but, there was very little co-expression with nitric oxide synthase. There is a delay between the first expression of pan-neuronal markers and the development of ChAT-expressing neurons, as the most caudal YFP+ cell was always well behind the most caudal enteric neuron in the embryonic gut. Conclusions: ChAT-expressing enteric neurons are present as early as E11.5 in the developing mouse gut, but many neurons do not express ChAT until after birth.

ORAL-19-05

STRIATAL PROJECTION NEURONS ARE GENERATED THROUGH A LATENT PERIOD OF NEUROGENESIS IN THE NEONATAL RODENT BRAIN

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Purpose: Substantial advances have been made in the last decade on our understating of the basic physiology underlying neurogenesis in the postnatal mammalian brain. The bulk of the work in this area has been based in the adult brain. Relatively less is known about the capacity for neurogenesis in specific structures within the neonatal brain. Here we report that the production of medium spiny striatal projection neurons extends into the early neonatal period under normal physiological conditions in the rat brain. **Methods:** This will involve investigating the neuronal phenotype and spatial distribution of cells birthdated with bromo-deoxy-uridine (BrdU) (150mg/kg) at postnatal day 0 (P0), P2, and P5 in the striatum. In another study we utilised replication incompetent retroviral vector overexpressing GFP to determine whether these newly born neurons can innervate their correct targets via ICV injection (1x10^8 cfu/ml). **Results:** Birth-dating of newborn cells with BrdU at P0, P2 and P5 showed a peak neuron production at P0 (1.66±0.5×103), which declined at later time-points (P2 = 0.38±0.14×103 and P5 = 0.28±0.11×103). Additionally, there was a low but stable contribution of interneurons over the same time-period. Importantly, retroviral labeling of new striatal projection neurons with GFP showed long term survival and terminal differentiation with characteristic morphology, including highly elaborated spiny dendrites, and appropriate axonal targeting of the globus pallidus and midbrain. **Conclusion:** This study identifies and characterises a latent period of striatal neurogenesis under normal physiological conditions in the neonatal. This phenomena represents an interesting target for regenerative approaches aimed at restoring striatal circuitry in perinatal pathologies, such as hypoxic and ischemic damage associated with cerebral palsy.

ORAL-19-06

REGENERATION AND PROTECTION IN THE DEAF COCHLEA

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Purpose: The most common cause of deafness is due to the loss of cochlear hair cells and a degeneration of the sensory epithelia. The degeneration of the sensory epithelia leads to a degeneration of the auditory nerves, which are the targets for cochlear implant stimulation. This work aims to prevent the degeneration of the auditory nerves and regenerate the cochlear hair cells. **Methods**: Adenoviral vectors which have been modified to encode for brain-derived neurotrophic factor and neurotrophin-3 or the transcription factor *Atoh1* were injected into the cochlear scala media of deafened guinea pigs (n=5 for each group). Cochleae were examined after 3, 7, 11 or 24 weeks of treatment. **Results**: After a single inoculation of neurotrophin gene therapy there was a significantly greater density of auditory nerves at all time points examined in the region most proximal to the viral injection, when compared to the contralateral cochlea ($p<0.05$). There was also evidence for localised resprouting towards neurotrophin producing cells. The introduction of the transcription factor, *Atoh1*, was shown to cause transduced cells to transdifferentiate towards a hair-cell phenotype when examined after 3 weeks. **Conclusions**: Neurotrophin gene therapy is able to promote an increase in auditory nerve survival for at least 6 months in the deafened guinea pig. Moreover, *Atoh1* gene therapy promotes the regeneration of cochlear hair cells, cells once thought to be lost forever, by causing transdifferentiation of supporting cells towards a hair cell phenotype.

ORAL-19-07

USING ZEBRAFISH TO IDENTIFY FACTORS INVOLVED IN NEUROGENESIS AND BRAIN REGENERATION

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Purpose: We are interested in understanding the underlying mechanisms that regulate neurogenesis and regeneration of the vertebrate brain. We are using zebrafish as a model organism because of their robust regeneration of many tissues including the brain. Results:By using micro-arrays we found that High Mobility Group A genes are up regulated in neural progenitor and stem cells. The HMGA proteins are architectural transcription factors and that can alter chromatin structure and gene expression. We have cloned the HMGA family members in zebrafish and are currently pinpointing their molecular role in stem cell regulation and regeneration. We found that Hmga1 is highly expressed in neural stem and progenitors cells in the developing and mature CNS. Knockdown experiments show that hmga1 play an essential role during embryonic development and brain. Furthermore, Hmga1 expression is significantly upregulated during tissue regeneration and we are now studying its role in this process.

ORAL-19-08

ESTABLISHMENT OF THE OLFACTORY BULB IN EMBRYONIC MICE

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The olfactory system has become a popular model for studying neural regeneration and the underlying mechanisms for developing neural circuits. By utilising transgenic mice (S100-DsRed and OMP-ZsGreen) we have the ability to visualise olfactory neurons (OMP-ZsGreen) and glial cells including olfactory ensheathing cells and astrocytes (S100-DsRed). Currently the mechanism in which olfactory axons find their target within the olfactory bulb is not clearly understood and may be attributed to astrocytes within the olfactory bulb. The formation of the external plexiform layer also remains unclear. During development in E13.5 embryos (N=3) the olfactory bulb houses S100-DsRed positive cells that appear to play a role in the formation of the olfactory bulb. Before the glomeruli are formed these cells do not allow for axons to protrude into the olfactory bulb. When axons do enter the olfactory bulb they do so in location where these cells are minimal. By using several markers it appears that astrocytes may play an important role in this mechanism. In E15 (N=3) embryos these cells are no longer making contact with the olfactory nerve and is in timing for when glomeruli are establishing. The location where these cells resided now appears to be occupied by astrocytes and other cells. In further stages these cells are no longer present. These cells have been identified as astrocytes and play a role in the formation of the olfactory bulb in mice.

ORAL-20-01

MODULATING EFFECT OF ORAL SUCROSE ON β ENDORPHIN AND PAIN PERCEPTION

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Abstract: Modulation of nociceptive response to ad libitum sucrose ingestion (5h) by ventromedial hypothalamus (VMH) has been reported earlier in the same rat. **Purpose:** The role of β endorphin in the pattern of transition from sucrose-fed analgesia to hyperalgesia is not known therefore, we investigated this pattern of transition using microdialysis technique. **Methods:** Adult male wistar rats (n=12) were divided into control and sucrose fed groups. Both control /sucrose fed group of rats were subjected to tail flick test at 0, 0.25, 1, 3,5h (session I-V, by tail flick analgesiometer) and microdialysate samples taken at 30 min interval for 5h in VMH. Samples were assayed for β endorphin using ESI-MS/MS. **Results:** During microdialysis, recovery of beta-endorphin was 11.6041± 6.16% at a flow rate of 1.5 µl/min. Our study indicates that induction of a nociceptive stimulus to the rat tail caused an increase in the levels of beta-endorphin in VMH which was statistically significant at 30 and 120 min in sucrose fed rat as compared to control rats **Conclusion** The results of our study show that β Endorphin plays a role in transition pattern from sucrose-fed analgesia to hyperalgesia. There is an increase in β endorphin level throughout the biphasic nociceptive response in sucrose fed rats which was statistically significant during transition from analgesia to hyperalgesia. Therefore the present study supports the opioidergic basis of initial sucrose fed analgesia while it refutes the decrease in opioid as the basis of late sucrose fed hyperalgesia. **Key words:** Sucrose fed analgesia, Sucrose fed hyperalgesia, Microdialysis.

ORAL-20-02

ENCODING OF OBJECT SOFTNESS BY TACTILE MECHANORECEPTORS IN THE HUMAN FINGER PADS

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Purpose: Humans excel in discriminating the softness of objects through tactile mechanisms alone, but it is not known how cutaneous mechanoreceptors in the finger pads encode compliance and contribute to the subjective estimate of softness. We undertook a neurophysiological investigation of the responses of low-threshold mechanoreceptors in the finger pads to surfaces of differing softness. **Methods:** Unitary recordings were made from 26 SAI, 17 FAI and 9 SAII units via tungsten microelectrodes inserted into the median nerve at the wrist. A servo-controlled stimulator applied ramp-and-hold forces (1, 2, 4 N) at a constant loading and unloading rate (2 N/s) via a flat 2.5 cm-diameter silicone disc over the centre of the finger pad. Nine discs were used, which linearly increased in softness across the range. **Results:** SAI afferents showed the greatest sensitivity to compliance, with a steep monotonic decrease in mean firing rate during the loading and plateau (but not unloading) phases. FA I afferents also showed a linear decrease in firing during the loading but not unloading phase, though the slope was lower. Conversely, SAII afferents showed no change in discharge with object compliance. **Conclusions:** Given their high density in the finger pads and their inverse relationship between firing rate and object compliance during the loading and plateau phases, SAI afferents (together with FAI afferents during the loading phase) are ideally suited to encode object compliance, but the SAII afferents appear to play no role in assessing softness.

ORAL-20-03

GAIN MODULATION OF NEURONAL RESPONSIVENESS BY PERSISTENTLY ACTIVE STATE IN NEOCORTEX

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Cortical persistent depolarization membrane potential state correlates with active behavior state,like awake state. Active behavior state endows the brain with better ability of information processing. However, the underling cellular and synaptic mechanisms are not well known. In this study, we using intracellular recording inVivo to measure spontaneous and sensory evoked synaptic conductance under persistent depolarization membrane potential state and UP-DOWN states which is the typical membrane potential state under deep sleep state (n=32 cells).New results showed that persistent depolarization membrane potential state is a lower conduce state than UP state by caculating total condunctance (n=5,p=0.001), and an excitatory synaptic conduce dominated state by testing synaptic reversal potenial (n=6,P=0.012), comparing with UP state. This synaptic conductance state fundamentally influenced cellular input resistance and membrane potential,thus modulated neuroanl responsiveness. A moderate sensory evoked excitatory and inhibitory synaptic conductance also contributed to stabilize sensory responsiveness and spiking timing in persistent depolarization membrane potential state. Together, this study finds under persistent depolarization membrane potential state, brain optimizes both cellular intrinsic properties and local cortical network to improve sensory coding.

ORAL-20-04

BARRELETTES, BARRELOIDS AND BARRELS: STRUCTURES AND CONNECTIVITY OF THE SOMATOSENSORY PATHWAY REVEALED USING SUPER RESOLUTION TRACTOGRAPHY

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Purpose: We used super-resolution track-density imaging (TDI) to map the major structures and connectivity of the barrel cortex in mice. Sensory innervation follows a stereotypical pattern relaying information from each whisker to the cortex via the brainstem barrelettes, the thalamic barreloids and finally afferents terminate in layer IV of the cortex, referred to as barrels. Our aim was to map these structures and connectivity using super-resolution TDI. **Methods:** Adult C57Bl/6 mice (n=4) were fixed using paraformaldehyde, and 16.4T high-field diffusion weighted images of the ex-vivo brains were acquired at 100µm isotropic resolution. Post-processing methods were applied, including constrained spherical deconvolution in order to resolve crossing fibres and super-resolution TDI using the MRtrix software package, achieving a final isotropic resolution of 20µm. **Results:** The stereotypical pattern of the large whisker barrels in the cortex was clearly defined using whole-brain super-resolution TDI. Localised tractography revealed the topology of the barreloids and barrelettes. Our targeted tracking showed the long-range connectivity of thalamo-cortical afferents, for example seeding the thalamus and targeting the cortex, which recapitulated the projection pattern seen using virus mediated labelling of the thalamo-cortical projections. **Conclusion:** In this study we have shown super-resolution TDI can be used to identify structures and connectivity of the somatosensory pathway in 3D and at a mesoscopic level. Future studies will utilise our current findings to investigate mouse models of neurological disease.

ORAL-20-05

INCREASED EFFICACY OF THE PERIPHERALLY-RESTRICTED KAPPA-OPIOID RECEPTOR AGONIST, ASIMADOLINE, IN CHRONIC VISCERAL HYPERSENSITIVITY

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Purpose: Asimadoline is more effective in diarrhea-predominant IBS (D-IBS) patients with at least moderate abdominal pain at baseline (Mangel et al 2008). The reduction in pain scores and the increase in pain-free days produced by this peripherally-restricted kappa-opioid receptor agonist were enhanced in patients with greater pain at baseline. The underlying mechanism for this effect is not fully elucidated. We hypothesized it may be due to increased expression and function of kappa-opioid receptors (KORs) on colonic afferents. Methods: KOR mRNA and protein expression was assessed in retrogradely-labeled colonic DRG (T10-L1) neurons and compared between healthy mice and those with TNBS induced chronic visceral hypersensitivity (CVH) (Hughes et al., Gut, 2010). Colonic splanchnic high-threshold nociceptor mechanosensory responses were compared in vitro from healthy, inflammatory and CVH mice in the presence and absence of Asimadoline (5-500nM). Results: In CVH mice KOR expression was significantly up-regulated in colonic DRG neurons compared with healthy mice ($P<0.01$, n=8). Asimadoline had no effect on healthy colonic nociceptor mechanosensitivity ($P>0.05$, n=6), but caused dose dependent inhibition of colonic nociceptors during inflammation ($P<0.001$, n=10) and CVH ($P<0.001$, n=10). Conclusion: KORs are expressed in colonic afferents and are up-regulated in CVH. Correspondingly, asimadoline inhibits colonic nociceptors in CVH, suggesting KORs are a silent receptor system that is activated in hypersensitive states. These findings provide a correlate for the increased efficacy of asimadoline in D-IBS patients with at least moderate pain. Supported by Tioga Pharmaceuticals and NHMRC Australia.

ORAL-20-06

WHISKER-MEDIATED VIBRATION DETECTION: NEURAL CODING AND BEHAVIOUR

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Purpose: A key challenge of systems neuroscience is to quantify brain activity underlying sensory perception and behaviour. Here we used the rat whisker sensory system to investigate the neural correlates of vibrotactile detection. The system is well-suited to examining neural coding issues because of its *functional efficiency* and its *structural organization* - in the primary somatosensory cortex each whisker is represented in a cluster of neurons known as barrels. We focused on the receptive mode, where the sensory system detects movements that are generated in the environment. **Method:** Rats (n=4) were trained in a 2-alternative paradigm to detect vibrations at amplitudes of 12.5 and 25µm pseudorandomly presented to their left or right whiskers. Rats had to maintain nose-poke for a variable duration (200 to 400 msec) to trigger the stimulation - a sequence of discrete Gaussian deflections. After detecting the stimulus side, rats indicated their response by orientating to the corresponding spout to receive sucrose reward. Following successful training, rats were implanted with electrodes (either a 16 channel micro-array or 2 tetrodes) for chronic recording from the barrel cortex, whilst they performed the behavioural task. **Results:** Behavioural analysis indicated high levels of efficiency - the mean accuracy of 80% correct and median average sampling durations of 250msec. A time-warped analysis was conducted to compare the activity of barrel cortex neurons when stimulus was presented on contralateral versus ipsilateral side. Results indicated that prior to making a choice, cortical neurons (n=32) reliably coded for receptive tactile information ($p<0.01$ permutation test), as well as coding for correct trials over incorrect ones ($p<0.01$ permutation test). **Conclusion:** Rats maintained high levels of performance across two concurrent detection tasks and cortical neurons correlated with the animal's choice.

ORAL-20-07

AMPULLARY STRUCTURE AND ELECTROSENSORY HYPERACUITY IN SHARKS

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Purpose: Studies of electrosensory prey detection in sharks have led to models of filtering mechanisms in hindbrain cerebellar-like circuits. However, although electroreception involves using spatially distributed sensors to track targets in space, little attention has been paid to spatial information processing. We constructed a realistic model of spatial and temporal information acquisition by a shark's electrosensory system. **Methods:** We previously developed a detailed three-dimensional model of *Squalus acanthias*, including the peripheral electrosensory system. Here we present a finite element model of the sense organs, the ampullae of Lorenzini, with realistic geometrical and electrical properties. We have simulated responses of organs in a virtual environment containing prey-like electric sources, and uniform electric fields resembling motion-induced and other fields encountered in the ocean. **Results:** The canal provides a low-resistance pathway to the apical surface of the sensory epithelium, functionally parallel to a high-resistance pathway through the shark to the basal surface. This causes most of the voltage drop to appear across the receptor epithelium. Restricting current flow to the tip of the receptor cell kinocilium creates a high voltage gradient at that point. The organs are directionally sensitive for both uniform and dipole sources, responding best to uniform and dipole fields parallel to the canal. **Conclusion:** In contrast to recent suggestions that the canal has strong capacitive properties, we have shown that the reported hyperacuity of ampullae may be explicable by a more conventional model in which the canals are "electrical lenses" coupling minute electric fields into receptor currents large enough to modulate transmitter release. Additionally, our model quantifies the organs' directional selectivity, making it possible to map the three-dimensional spatial electrosensory receptive field structure in *Squalus*.

ORAL-20-08

THE EFFECT OF PLACEBO CONDITIONING ON REGIONAL BRAIN BLOOD OXYGEN LEVEL-DEPENDENT SIGNAL CHANGES DURING CAPSAICIN-EVOKED URGE-TO-COUGH

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Purpose: Cough and the urge-to-cough are common symptoms of respiratory disease with few effective treatments. Previous studies have reported that both cough and the associated urge are particularly susceptible to placebo suppression, suggesting a prominent influence of higher brain processing over basic cough pathways. This study investigated the effect of placebo on cortical networks involved in sensory processing of airway irritation evoked by inhalation of capsaicin using functional magnetic resonance imaging (fMRI). **Methods:** During fMRI, healthy, non-smoking participants (n=14) completed a randomised series of trials consisting of either placebo (nasal inhalation of medical air which participants believed was a local anaesthetic) or no-treatment, followed by capsaicin inhalation. Capsaicin doses were individually tailored to cause maximum sensation of airway irritation without coughing. Prior to the fMRI session participants were conditioned to believe the treatment was effective by surreptitiously lowering capsaicin doses following placebo. **Results:** Subjects rated their capsaicin-evoked urge-to-cough significantly lower in placebo compared to control trials ($F[1,13]=47.63$, $p<0.001$). fMRI analysis showed expected activations in cortical regions related to airway sensory processing when subjects received capsaicin. In placebo compared to control trials there was decreased activation bilaterally in primary somatosensory and insula cortices. There was also increased activation in orbitofrontal and prefrontal cortices, similar to reports from placebo analgesia studies. Furthermore, the reduction in ratings during placebo was correlated with activation in these regions. **Conclusion:** This study provides further evidence that higher brain networks modulate responses to airway irritation. It also confirms that placebo administration activates endogenous inhibitory networks and decreases activation in regions involved with processing incoming sensory signals from the airways.