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ORAL-01-01

MULTISENSORY INTEGRATION OF VISUAL AND AUDITORY RATE INFORMATION IN OLDER ADULTS

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Purpose The percept of conflicting auditory and visual temporal rate information varies from partial cue integration to cue segregation depending on the difference in temporal rate information in each modality (Roach et al, Proc Roy Soc B, 2006). Irrelevant auditory rate information influences the temporal rate percept of a visual stimulus and vice versa, with the relative strength of influence depending on the precision of uni-modal rate estimates. We aimed to determine whether normal ageing alters these processes. **Methods** Ten younger (aged 22-32) and nine older (aged 60-75 years) adults participated. Observers performed a rate discrimination task for each modality separately, and also when conflicting rate information was presented in the other sensory modality. Rate discrimination performance was measured under conditions of equated auditory and visual rate discrimination threshold for each observer. **Results** Older adults had poorer unimodal auditory modulation depth thresholds ($p=0.04$), hence to balance rate discrimination performance between modalities required greater auditory amplitude modulation ($p=0.01$). For the multimodal task: when the discrepancy between the visual and auditory rate was small, cues were partially integrated. Under conditions of equated unimodal sensitivity, the degree of cue integration did not differ between older and younger adults. **Conclusions** Normal ageing does not impact on the flexible processes used by the brain to determine whether to integrate or segregate auditory and visual temporal rate information. Ageing may result in differences in the combination of temporal auditory and visual cues in natural situations due to age related differences in the relative discriminability of temporal information from vision and audition

ORAL-01-03

THE EFFECT OF CONTRALATERAL STIMULATION ON COCHLEAR AMPLIFIER PERFORMANCE

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PURPOSE: Medial olivocochlear (MOC) efferent neurons in the auditory system can be activated by sound delivered contralaterally, causing suppression of outer hair cell (OHC) electromotility (reverse transduction) and micromechanics in the ipsilateral ear which underlies the 'cochlear amplifier'. This modulation is called 'contralateral suppression' (CS) and has a timescale of ms-secs. The effect on OHC function can be observed as a reduction in the amplitude of the Distortion Product Otoacoustic Emissions (DPOAE) of the ipsilateral ear. CS contributes to protection against noise-induced hearing loss and anti-masking which allows for attentive hearing in noisy environments. Here we describe a new model for investigation of the regulation of the 'cochlear amplifier' by CS, matching the rapid adaptation profile of CS to the dynamics of transient noise loading on the cochlea. **METHODS:** The immediate responses of quadratic (f1-f2) DPOAEs after 20 seconds of ipsilateral sound (band limited) with, or without, contralateral stimulation were analysed in C129/Bl6J mice. These experiments were conducted on mice under ketamine / xylazine / acepromazine anaesthesia and in accordance with University of New South Wales Animal Care and Ethics Committee approval. **RESULTS:** Our data ($n=9$) demonstrate that contralateral acoustic stimulation can abate noise-induced suppression of the cochlear amplifier. Ipsilateral noise alone (80 dB) was found to cause a decrease in the quadratic DPOAE of 9.7 ± 1.59 dB from an amplitude of 20.8 ± 1.00 dB. CS (96 dB) given concurrently with the ipsilateral noise produced on average 3.1 ± 0.80 dB less noise-induced reduction in 'cochlear amplifier' adaptation ($P = 0.005$ paired t-test) with the DPOAE recorded at cessation of the noise having been reduced by 6.6 ± 1.26 dB. **CONCLUSION:** This supports the proposed role of CS in enabling rapid modulation of sound transduction in response to the dynamic acoustic environment. This could prove to be a valuable model for studying fast cochlea dynamics and MOC activity.

ORAL-01-02

WHAT FACTORS DETERMINE THE PERCEIVED COMPLEXITY OF COMMONPLACE SOUNDS?

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Purpose: A powerful functional test of hearing is of normal speech in background noise. However, speech-in-noise tests are confounded by factors including the listener's linguistic abilities. We have been developing a sounds-in-noise test using commonplace environmental sounds. Here we determine what spectral properties of these sounds correlate with people's perceptions of their complexity. **Methods:** 11 subjects rated complexity (and familiarity and pleasantness) of 161 sounds on a 7-point Likert scale. Ratings were mean-normalised as 2 subjects scale shifted ratings to lower values than the other 9 subjects. We applied different complexity measures: (a) Harmonics-to-Noise Ratio (HNR) measuring the amount of power which is harmonic in the signal, (b) Fractal Dimension (FD) estimates which indicate the heterogeneity or roughness of the shape, (c) Wiener entropy (or Spectrum Flatness Measure, SFM) which measures the uniformity of the signal within the power spectrum, and (d) Spectral Structure Variability or Index (SSV or SSI) which measures how much the SFM varies over time. **Results:** Three measures – HNR and two FD measures – produced significant correlations to human ratings of sound complexity. However, even at best, the correlations accounted for $< 5\%$ of the variance. This was significantly improved by combinations of factors. To overcome the dimensionality problem of using multiple measures, we applied Principal Components Analysis (PCA) which identified two factors that accounted for $> 70\%$ of the variance; adding in a third factor accounted for $> 86\%$. **Conclusions:** No single statistical measure perfectly correlated with subjective measures for environmental sound complexity, whereas combinations of measures and their relative differences showed more significant correlations. The combinations of complexity measures varied between types of sounds, e.g., artificial sounds versus human/animal sounds.

ORAL-01-04

PRIMARY AUDITORY CORTEX OF ANAESTHETIZED MARMOSETS: NEURONAL SENSITIVITY TO INTERAURAL LEVEL DIFFERENCES FOR NATURAL VOCALIZATIONS

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Purpose: Interaural level differences (ILD) allow determination of the origin of high-frequency sounds along the azimuth dimension. Binaural comparisons of ILD occur first in the brainstem, but are created de novo at many higher levels. Thus the extent to which cells in the primary auditory cortex (A1) can use this cue to determine the direction from which natural sounds originate requires further study. **Methods:** We recorded from single and multi-units in A1 of two marmoset monkeys, anaesthetized with sufentanil ($6\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$) and N_2O (70% in O_2). Auditory stimuli were three marmoset vocalisations (Ock, Tsik and Twitter), delivered to both ears with ILDs ranging from +20dB (favouring contralateral ear), through +10dB, 0dB, -10dB to -20dB (favouring ipsilateral). The average binaural levels tested included 30, 50 or 70dB SPL. **Results:** The majority (21/23) of units responded to at least one of the 3 calls. 14 units preferred calls favouring contralateral ear, 5 preferred the ipsilateral ear, and 2 were non-selective for ILD. Neuronal selectivity depended on both intensity and calls: while many showed greatest selectivity at 70dB, a substantial proportion ($> 40\%$) showed better ILD tuning at 30 or 50dB for at least one call. These results can be explained by static non-linearity where responses to louder stimuli saturate, or even super-saturate, which in turn suggest that gain normalization mechanisms are involved. **Conclusion:** Even though ILD selectivity of individual units is stimulus- and intensity-dependent, the population activity in A1 contains ILD information for all three calls over a broad range of sound intensities. This information can then be "read-out" downstream for sound location to be represented in a call- and loudness-invariant way.

ORAL-01-05

VISUAL AND AUDITORY PERCEPTION DEFICITS SUGGEST ALTERED NEURAL INTEGRATION FUNCTION IN PSYCHOSIS

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Background Sensory dysfunction in schizophrenia underlies complex cognitive and affective symptoms of the disorder, and evidence from psychophysical paradigms suggests that this dysfunction results from impairment in integrating low-level neural signals into complex cortical representations. However, while this integration deficit is assumed to be specific to schizophrenia, it is unclear whether this is the case, since previous research has not tested broader patient populations. In addition, it is not known whether observed deficits generalise across sensory modalities, since all evidence to date is drawn from visual psychophysical studies. **Methods** The present study assessed a broad sample of psychiatric patients on psychophysical measures testing visual contrast detection, visual motion integration, auditory tone detection, and auditory tone integration. We tested 269 participants, including 104 participants with a schizophrenia spectrum disorder, 38 with bipolar affective disorder, 32 with depression, 33 with other psychiatric conditions, and 62 healthy control participants. **Results** Across diagnostic groups and sensory modalities, patients with psychosis were impaired on tasks requiring sensory integration, and unimpaired in simple visual contrast and tone detection. Impairment in sensory integration was correlated with the severity of positive psychotic symptoms. **Conclusions** Our results demonstrate that impaired functional connectivity is not specific to schizophrenia, as has previously been assumed. Instead, sensory deficits are closely related to the presence of psychotic symptoms independent of diagnosis. In addition, our results show for the first time that integrative sensory processing is impaired in modalities other than vision, consistent with hypotheses that propose a generalised deficit of neural integration in psychotic disorders.

ORAL-01-07

COCHLEAR GENE EXPRESSION ANALYSIS IN RESPONSE TO NOISE STRESS IN MICE

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PURPOSE: Excessive noise exposure and the associated hearing loss is an increasing global problem in modern society. Shifts in hearing sensitivity after noise overstimulation will lead to either a temporary (TTS), or permanent, threshold shift (PTS). Many cochlear structures are affected by noise overstimulation, but changes in gene expression after PTS-inducing noise have also been identified. Understanding the possible transcriptional responses underlying TTS has not previously been investigated at a genome level. We report the regulation of cochlear gene expression in C57BL/6J mice in response to TTS-inducing noise. **METHODS:** Mice (n=24) were anaesthetised with a ketamine/xylazine/acepromazine cocktail in accordance with University of New South Wales' Animal Care and Ethics Committee approval. Mice exposed to noise (86 dB or 95 dB, 4 – 32 kHz) for 30 min, had their evoked auditory brainstem responses (ABR) measured to broadband clicks, or 16 kHz tonepips, before and after the noise exposure, to quantify the TTS. Cochlea RNA extraction was performed on tissue 1, 2, 4, 8 and 24 hours after the noise exposure. cDNA templates were hybridised to the Affymetrix® mouse gene array 1.1ST, and gene expression analysis was performed using GenePattern software. **RESULTS:** The level of TTS produced by 86 dB broadband noise was ~ 12 dB, which recovered with 96 hours. The 95 dB noise produced greater threshold shifts (~ 40 dB), and longer recovery time, but fully resolved within two weeks. A number of genes were found to be up-regulated across all five times with TTS (p<0.001; minimum 2-fold up-regulation). Maximum up-regulation was observed at 4 hours after 86 dB noise (almost 20-fold). Generally, up-regulation began two hours after noise exposure. **CONCLUSION:** In this study, we have determined a range of genes whose increased cochlear expression in response to TTS-level noise likely reflects molecular responses that contribute to adaptation to acoustic overstimulation and may confer protection from noise-induced hearing loss.

ORAL-01-06

SPATIAL SELECTIVITY OF RESPONSES OF CAUDOLATERAL AUDITORY CORTEX FIELDS

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Purpose: It has been hypothesized that the caudal auditory cortical belt fields are the origin of a "where" cortical pathway processing the location of a sound source, but this hypothesis has not been rigorously tested. **Method:** We recorded from caudal auditory belt areas in two marmosets anaesthetised with sufentanil (6µg.kg⁻¹.hr⁻¹) / N₂O (70% in O₂). At each recording site, we studied spatial selectivity of neurons by examining responses to interaural level differences (ILDs), a major cue to azimuthal location, with four stimuli: a pure tone at the characteristic frequency (CF) of the cells at that site and three monkey vocalizations. The monkey vocalizations had similar spectral characteristics but different temporal features. All stimuli were presented with ILDs ranging from 20 dB louder in one ear to 20 dB louder in the other ear, in 2.5 dB ILD steps, with levels in the two ears varied symmetrically around one of three average binaural levels of 30, 50, and 70 dB SPL. Each stimulus condition was presented 20 times and peri-stimulus spike counts across repetitions averaged to assess spatial selectivity. **Results:** Most sites responded to pure tones (17/17) and to monkey vocalizations (15/17). However, only a small number showed spatial selectivity for tones (7/17) while the majority (14/15) exhibited strong spatial selectivity for the complex sounds. Further, at each site, neuronal spatial selectivity was discriminative for particular calls only, even though the spectral characteristics of the complex sounds were similar. **Conclusion:** Neurons of caudal auditory areas are spatially selective. The selectivity is strongest for complex stimuli but depends on the temporal features and identity of the stimuli rather than their frequency content.

ORAL-01-08

CELL-BASED NEUROTROPHIN DELIVERY FOR AUDITORY NEURON SURVIVAL IN DEAFNESS

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Purpose: The cochlear implant (bionic ear) provides auditory cues to patients with a profound sensorineural hearing loss by electrically stimulating the auditory neurons of the cochlea. However, auditory neurons degenerate in deafness, which may limit the efficacy of the cochlear implant. Cell-based therapies are a potential clinically viable method for delivering neurotrophins to the deaf cochlea to prevent auditory neuron degeneration in deafness, and we have previously shown significant survival effects for up to four weeks post-implantation using such techniques. This study investigated auditory neuron survival following (a) cell-based neurotrophin treatment in conjunction with chronic electrical stimulation from a cochlear implant, and (b) long-term cell-based neurotrophin delivery. **Methods:** Primary adult rat fibroblasts were nucleofected to express brain-derived neurotrophic factor (BDNF-Fbs), and were encased in alginate capsules. Guinea pigs (n≥5 per group) with an ototoxically induced, bilateral, profound hearing loss were implanted unilaterally with encapsulated BDNF-Fbs plus a stimulating electrode array for one month; or bilaterally with encapsulated BDNF-Fbs and empty capsules for one, three or six months. **Results:** The implantation of encapsulated BDNF-Fbs into the scala tympani of the deaf guinea pig cochlea significantly enhanced auditory neuron survival in comparison to empty (control) capsules; moreover, the survival effects of this cell-based neurotrophin treatment persisted for at least six months (p<0.01). Enhanced auditory neuron survival was also observed following combined cell-based BDNF treatment and chronic electrical stimulation from a cochlear implant (p<0.01). **Conclusion:** Cell-based neurotrophin treatment is a potential clinically viable method for preserving auditory neuron survival in deafness. Enhanced auditory neuron survival may allow for enhanced perceptual outcomes in cochlear implant patients.

ORAL-02-01

POSTURAL CONTROL IN YOUNG FEMALE FMR1 PREMUTATION CARRIERS

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Background: Fragile X syndrome-premutation (FX-PM) status is caused by a CGG-repeat expansion (55-200 repeats) on the FMR1 gene and is associated with an increased risk for a late-onset tremor and ataxia syndrome (FXTAS). Currently, the extent to which subtle balance and cognitive difficulties indicate at-risk profiles in young females is unknown. **Aim:** Here we investigate the inter-relationships between postural control, cognition and attention in females with the FX-PM. **Method:** 28 female FX-PM carriers (22-55 years old) and 28 age- and intelligence-matched controls were recruited for this study. All participants provided blood for CGG-repeat analysis, completed visuospatial tasks and self-reported on attentional problems. Measures of proprioception and postural sway from the Physiological Profile Assessment (PPA) were also completed. To vary attentional resources allocated to the postural control task, body displacement was measured under conditions of eyes open or closed, when standing on floor or foam, and with or without a concurrent dual task (i.e., verbal fluency). **Results:** Our data shows that performance on all simple postural control tasks was not perturbed in the female FX-PM; however, postural steadiness decreased significantly (i.e. greater body displacement) during the most difficult postural task (i.e. dual task interference when standing on foam). Importantly, while correlations showed potential interactive influences between postural control, proprioceptive deficits, motor planning/sequencing and visuospatial abilities only in the female FX-PM, no such association was observed for self-reported attentional problems. **Conclusion:** We report novel interrelationships between previously reported difficulties in motor planning, visuospatial processing and postural stability in the female FX-PM. Moreover, our data showing attentional interference on neuromotor networks is indicative of reduced information processing capacity during postural control and supportive of cerebellar involvement in the female FX-PM.

ORAL-02-03

USING OPTOGENETIC STIMULATION TO UNDERSTAND THE FUNCTION OF C3 NEURONS

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Purpose: The sympathetic vasomotor actions of adrenergic C1 neurons of the rostral ventrolateral medulla oblongata have been extensively characterised. In contrast the function of adrenergic C3 neurons of the dorsomedial medulla oblongata is not understood, although these neurons also densely project to sympathetic preganglionic regions regulating cardiovascular function. **Methods:** Experiments were performed in P21 male Sprague Dawley rats (n=4). Lentiviral vectors, expressing the light-activated cationic channel channelrhodopsin-2 (ChR2) under the control of a *phox2* promoter, were injected into both C1 and C3 regions. Two weeks after injections, the working heart-brainstem preparation (WHBP) was used to record perfusion pressure (PP), thoracic sympathetic nerve activity (tSNA), heart rate (HR) and phrenic nerve activity (PNA) during photostimulation of C1 or C3 neurons. **Results:** Photostimulation of ChR2-expressing C3 neurons caused a consistent increase in HR (+26±13 beats/min) and a mild increase in tSNA (+0.46±0.11 µV) and PP (+1.8±0.6 mmHg) in the WHBP, with no effect on PNA. Photostimulation of ChR2-expressing C1 neurons caused a smaller increase in HR (+13±4 beats/min) compared to C3 photostimulation, a larger increase in tSNA (+0.84±0.06 µV) and PP (+2.9±0.1 mmHg), and also no effect on PNA. Laser pulse triggered waveform averaging of low frequency (1 Hz) photostimulation demonstrated a significantly longer delay between stimulus and response when photostimulating C3 neurons compared to C1 neurons. **Conclusion:** Similarly to C1 neurons, C3 neurons are sympatho-excitatory in the WHBP, although the two groups display distinct response characteristics.

ORAL-02-02

CONSISTENT INTER-INDIVIDUAL DIFFERENCES IN THE CARDIOVASCULAR RESPONSES TO SUSTAINED MUSCLE PAIN IN HUMAN SUBJECTS

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Purpose: We recently showed that the cardiovascular responses to tonic muscle pain, induced by intramuscular infusion of hypertonic saline over 60 mins, are characterized by sustained increases in muscle sympathetic nerve activity (MSNA), blood pressure (BP), and heart rate (HR) in some subjects but sustained decreases in others (Fazalbhoy et al., 2012). Given that MSNA is constant on a day-to-day basis, we tested the hypothesis that inter-individual differences are invariable and the same responses to muscle pain are evoked in the same subjects studied on different days. **Methods:** MSNA was recorded via tungsten microelectrodes inserted into the peroneal nerve in 6 healthy subjects on two occasions, separated by at least 2 weeks. Tonic pain was induced for ~60 minutes by intramuscular infusion of hypertonic saline (7%) into the ipsilateral tibialis anterior muscle. Pain was sustained at a tolerable level (5-6/10 on a visual analogue scale). **Results:** Subjects who showed increases (or decreases) in MSNA, BP, and HR on one day showed the same pattern on the next. **Conclusions:** We conclude that the cardiovascular responses to long-lasting pain are reproducible in a given individual, which may explain to why some people develop high blood pressure when experiencing chronic pain while others do not. **Ref:** Fazalbhoy A, Birznies I & Macefield VG (2012) Exp Physiol 97: 1084-1092.

ORAL-02-04

METALLO-COMPLEXES AS THERAPEUTICS IN MOUSE MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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Purpose: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons. Symptoms include progressive paralysis and death 3-5 years after diagnosis. Riluzole, the only approved ALS therapeutic has marginal clinical efficacy. The metallo-complex Cu^{II}(atsm) has previously been shown to have neuroprotective activity and has been used as a PET imaging agent in humans. The purpose of this study was to investigate the therapeutic efficacy of Cu^{II}(atsm) in the SOD1G37R mouse model of ALS. **Methods:** SOD1G37R transgenic mice and non-transgenic littermates were orally gavaged daily using a vehicle solution supplemented with Cu^{II}(atsm) or riluzole from the pre-symptom age of 4 weeks or post-symptom onset until disease end-stage. Cu^{II}(atsm) was administered at 0, 10, 30 or 60 mg/kg body weight for pre-symptom treatments and 0 or 30mg/kg for post-symptom. Riluzole was administered at 20mg/kg. Locomotor function of the mice was assessed and survival data collected. In each treatment group, n>9. **Results:** Treatment with Cu^{II}(atsm) commencing pre-symptom onset improved the survival and locomotor function of SOD1G37R mice in a dose-dependent manner, with the highest dose (60 mg/kg) improving survival by 26% (P<0.001). Post-symptom onset treatment with Cu^{II}(atsm) increased survival by 11% (P=0.04). The therapeutic activity of Cu^{II}(atsm) was significantly greater than that of riluzole. **Conclusion:** Cu^{II}(atsm) improved locomotor function and increased survival of SOD1G37R mice and showed greater efficacy than riluzole, the only approved ALS therapeutic. Cu^{II}(atsm) represents, to date, the most successful pre-clinical drug for the treatment of ALS and is an excellent candidate for further pre-clinical characterisation and progression into clinical trials.

ORAL-02-05

CENTRAL PATHWAYS FOR SWEATING IN HUMANS

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Purpose: To identify and compare the brain regions activated during sweating events caused by heat or by mental stress. **Method:** Functional magnetic resonance imaging was used to identify brain regions activated during spontaneous sweating events, measured by electrodermal responses in human participants. Sweating was induced by whole-body heating (n=11) or by mild mental stress (Stroop task, n=11). Sweating events occurred randomly during those periods. Separate images were acquired for the brainstem (1.8x1.8x2.6mm voxels) and the cerebrum (3.6x3.6x4mm voxels). **Results:** Regional brain activations (voxel inclusion $z > 2.3$, cluster corrected threshold $p < 0.05$) at the time of spontaneous sweating events during both stimuli included bilateral insula and cingulate cortices, periaqueductal grey matter, and rostral ventral medulla. The same brainstem sites were linked to sweating events during both thermal and mental stress. **Conclusion:** Common neural pathways appear to mediate sweating of either cause.

ORAL-02-06

USE OF ILLICIT STIMULANT DRUGS IS ASSOCIATED WITH LONG TERM CHANGES IN HUMAN MOTOR CIRCUITRY

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Purpose: Illicit use of stimulant drugs such as methamphetamine, ecstasy, and cocaine is a major problem in Australia and throughout the world. The aim of the current study was to investigate the long-term effect of illicit stimulant use on human motor cortex and corticospinal circuitry. We hypothesized that individuals with a history of illicit stimulant use would exhibit altered corticospinal excitability and intracortical inhibition within motor cortex. **Methods:** The study involved 26 abstinent stimulant users (28±7 yrs) and two groups of control subjects: 9 cannabis users (23±7 yrs) and 17 non-drug users (25±7 yrs). Each subject completed a routine urine drug screen, drug history questionnaire, neuropsychological assessment, and single- and paired-pulse transcranial magnetic stimulation (TMS) over motor cortex. The EMG response evoked by the stimulation (motor evoked potential or MEP) was recorded from the contralateral first dorsal interosseus. **Results:** At a given stimulus intensity, MEP latency and size were significantly greater in abstinent stimulant users than in non-drug users during relaxation and weak index finger abduction ($P < 0.045$). Stimulant users also exhibited significantly greater muscle activity during performance of a given task ($P = 0.004$) and tended to exhibit a longer cortical silent period duration ($P = 0.06$). However, resting motor threshold and the response to paired-pulse TMS were unaffected. **Conclusion:** The results suggest that abstinent stimulant users exhibit long-term changes in the excitability of motor cortical and corticospinal circuitry and muscle activity during movement. These changes may partly underlie anecdotal and objective reports of movement dysfunction in chronic stimulant users.

ORAL-02-07

ALDOSTERONE-SENSITIVE NEURONS WITHIN THE SOLITARY TRACT NUCLEUS (NTS) RECEIVE DIRECT PRIMARY AFFERENT INPUT

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Purpose: A group of neurons within the CNS are sensitive to the mineralocorticoid aldosterone by virtue of expressing 11 β -hydroxysteroid dehydrogenase (HSD2) that oxidises competing glucocorticoids. The largest population and most of these putative aldosterone sensitive neurons have cell bodies within the solitary tract nucleus (NTS). These HSD2 neurons project to the ventral bed nucleus of the stria terminalis (BNST) and various regions of the pons. Although previous studies suggest broad interactions between visceral and hormonal information at the NTS, little is known about this specialised group of NTS neurons (e.g. 2nd order or higher). Here, we examined interactions between solitary tract (ST) afferents and NTS neurons projecting to the central nucleus of the BNST (NTS-BNST). **Methods:** To identify NTS-BNST projection neurons, fluorescent retrograde tracers were injected into BNST. Not less than 10 days later we recorded from tracer filled NTS-BNST neurons in 250 μ M horizontal slices. **Results:** Graded intensity ST shocks evoked postsynaptic currents (PSC) whose responses became more complex with increasing shock intensity indicating multiple convergent inputs. Analysis of ST-PSC amplitude, synaptic jitter and failure rates identified three populations of neurons. Some NTS-BNST neurons (n=4) received low jitter ST-EPSCs identifying them as 2nd order neurons with direct primary afferent input. An equal proportion of NTS-BNST neurons (n=4) were classed higher order exhibiting multiple high jitter EPSCs and IPSCs with frequent failures. Surprisingly a number of neurons were found to receive no ST-driven inputs (10 of 18); suggesting their role may be unrelated to processing primary afferent information per se. **Conclusion:** The identified neuron classes raise interesting questions about differential integration of visceral information with aldosterone sensitivity and the BNST in complex behaviours.

ORAL-02-08

PREFRONTAL CORTEX MEDIATES RESPIRATORY ACTIVATION IN RESPONSE TO BRIEF ALERTING STIMULI AND STRESS

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Purpose: Respiratory arousal is one of the earliest signs of autonomic activation in response to alerting stimuli. It has a lower threshold of activation than traditionally used cardiovascular indices and is more sensitive to rapid or subtle stimuli; however, neuronal structures that are involved in respiratory arousal are unknown. One of the likely candidates for this process is prefrontal cortex (PFC). **Methods:** In order to assess potential contribution of PFC to respiratory arousal, we performed respiratory recording by whole-body plethysmography in six Wistar rats implanted with brain cannulas targeting the PFC. On different days they received intra-PFC microinjections of either GABA_A agonist muscimol (200nmol in 200nl) or saline. After 40-minute baseline recording, rats were presented with six brief acoustic stimuli of increasing intensity (40-90dB white noise 500msec long), followed by 30-second light stimulus (2000lux) and 15 minutes of restraint stress. **Results:** Acoustic stimuli produced brief increases in respiratory rate (RR) and tidal volume (T_V) proportional to stimulus intensity, with significant linear trends between stimulus intensity and both RR ($p < 0.001$) and T_V ($p < 0.001$). Muscimol significantly inhibited latency of respiratory responses to higher intensity stimuli (80dB and 90dB, $p = 0.028$ and 0.047 respectively), but did not affect the actual change in RR or T_V . Presentation of light stimulus significantly increased RR ($p = 0.001$) with muscimol significantly inhibiting this response ($p = 0.023$). Finally, restraint stress significantly increased RR, which was evident during the first, second and third 5-minute intervals of restraint (all $p \leq 0.003$). Respiratory response to restraint during the third 5-minute interval was significantly inhibited by muscimol ($p = 0.040$). **Conclusion:** PFC is one of the key neuronal structures that mediates respiratory activation in response to both brief and prolonged stimuli and stressors.

ORAL-03-01

AAV-MEDIATED OVEREXPRESSION OF CANNABINOID RECEPTOR INTERACTING PROTEIN CRIP1A DECREASES SEIZURE ACTIVITY IN MICE

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Background: Glutamatergic CB1 receptors in glutamatergic but not in GABAergic hippocampal neurons provides endogenous protection against kainic acid (KA)-induced seizures. The effects of signaling through CB1 in distinct neuronal populations might be caused by molecular differences in the constituents in the pool of CB1 interacting proteins. Cannabinoid receptor interacting protein (Crip1a) is a novel cytosolic CB1 binding partner with unknown function. Crip1a is down-regulated in the human epileptic hippocampus indicating an anticonvulsive role for this protein. **Purpose:** Here we investigated the hippocampal expression domains of endogenous Crip1a and addressed the effects of Crip1a overexpression in the KA seizure model. **Methods:** Specific antibodies was used to reveal expression of Crip1a in the mouse hippocampus. Somatic Crip1a gene transfer was achieved by delivery of neurotropic adeno-associated virus (AAV). The severity of KA-induced (30 mg/kg; i.p.) acute epileptiform seizures was monitored for two hrs. Stimulation of [35S]GTPγS binding in hippocampal homogenates of AAV-CRIP1a mice (n = 8) and controls (n = 7) was determined by various concentrations of the CB1 receptor agonist HU-210. **Results:** Endogenous Crip1a immunoreactivity was abundantly detected in the projections of hippocampal neurons. Despite its pre-synaptic localization Crip1a did not robustly co-localize with CB1. AAV-Crip1a-mediated transgene expression in the hippocampus was increased two-fold compared to controls, and matched endogenous Crip1a expression domains. AAV-Crip1a animals showed protection against KA-induced seizures and reduced the mortality. Overexpression of CRIP1a in hippocampal neurons resulted in significantly enhanced cannabinoid-induced G protein activation. High-resolution immunolabeling in naive mice revealed HU210 treatment did not change the proportion of either overlapping or physically separated CB1R/CRIP1a profiles. However, the proportion of contacting profiles significantly increased after CB1R activation. **Conclusion:** Crip1a facilitates CB1 signaling in pyramidal hippocampal neurons and acts as a safeguard against the adverse effects of excessive excitatory network activity.

ORAL-03-02

HUMAN AUXILIARY SUBUNITS KCTD12 AND KCTD16 DIFFERENTIALLY MODULATE GABA(B) RECEPTOR FUNCTION

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Purpose: GABA(B) receptors are implicated in many brain diseases including addiction and epilepsy. Recently KCTD12 and KCTD16 were identified as GABA(B) receptor auxiliary subunits. These auxiliary subunits' expression varies between brain regions and may account for the functional heterogeneity of GABA(B) receptors. Here we characterise the impact of human KCTD12 and KCTD16 on GABA(B) receptor kinetics and pharmacology. **Methods:** We developed a robotic *Xenopus* oocyte two-electrode voltage clamp assay using GIRK current as the reporter of GABA(B) receptor activation. GABA and baclofen dose-response relationships were constructed for different KCTD co-expressions. EC₅₀, slope and receptor kinetics were analysed. The impact on the positive allosteric modulator CGP7930 was also tested. **Results:** During a minute of 100μM GABA application, co-expression of KCTD12 with GABA(B) receptor accelerated 20-80% rise time from 2.77s to 1.27s, and receptor desensitization was increased from 3.9% to 54.7% (compared with GABA(B) receptor expression only, n>7 oocytes). In contrast, KCTD16 co-expression did not modulate receptor kinetics. Neither KCTD12 nor KCTD16 altered the EC₅₀ or Hill slope of GABA or baclofen dose-response curves (n>10 oocytes at each agonist concentration). However, both KCTD12 and KCTD16 enhanced CGP7930 potentiation on EC₂₀ GABA from 11.5% to 20.3% and 24.4% respectively (n>33 oocytes). **Conclusion:** Our *in vitro* data showed that KCTD12 and KCTD16 differentially impact GABA(B) receptor kinetics and pharmacology suggesting that they may partly explain the functional heterogeneity of the GABA(B) receptor. Furthermore, this differential pharmacology may be exploited to selectively modulate subclasses of GABA(B) receptors.

ORAL-03-03

HUMAN CA_v2.3 (R-TYPE) CALCIUM CHANNELS ARE INHIBITED BY ANALGESIC α-CONOTOXIN VC1.1 VIA A VOLTAGE-INDEPENDENT PATHWAY INVOLVING C-SRC TYROSINE KINASE

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Background and purpose. Ca_v2.1 (P/Q-type), Ca_v2.2 (N-type) and Ca_v2.3 (R-type) calcium channels contribute to synaptic transmission in the nervous system and are modulated via pathways involving G protein-coupled receptors. α-Conopeptide Vc1.1 from *Conus victoriae* has been identified as a selective inhibitor of Ca_v2.2 channels acting via GABA_B receptors. We investigated the modulation of stably expressed human Ca_v2.1 and Ca_v2.3 channels by baclofen, GABA, Vc1.1 and cyclised Vc1.1 in HEK-293 cells transiently expressing human GABA_B receptors. **Methods and Results.** Depolarization-activated whole-cell barium currents (I_{ba}) through Ca_v2.1 or Ca_v2.3 channels were inhibited by baclofen or GABA (in all cases, maximum inhibition was <50% and IC₅₀ values were ~400 nM). Vc1.1 had no effect on Ca_v2.1 channels (n≥20) but potently inhibited Ca_v2.3 channels (n≥30) in a concentration-dependent manner and cyclised Vc1.1 had an IC₅₀ = 300 pM. Depolarizing paired pulses, in which a 20 ms pre-pulse to +80 mV served to relieve any voltage-dependent component of a G protein-mediated inhibition, revealed that ~90% of the baclofen-inhibited I_{ba} fraction was voltage-dependent in cells expressing Ca_v2.1 channels, whereas, in cells expressing Ca_v2.3 channels, the effect of baclofen or Vc1.1 was solely mediated by a voltage-independent pathway. The effect of Vc1.1 was pertussis toxin-sensitive (n = 6) and could be abolished by intracellular application of the phosphorylated, selective pp60c-src tyrosine kinase (c-src) inhibitor peptide (n = 7) or by co-expression of a dominant negative mutant R²⁹⁵/F⁵²⁷ c-src protein (n = 6). Conversely, wild-type c-src overexpression significantly increased the Vc1.1-induced inhibition of Ca_v2.3 channels (n=7). **Conclusion.** These results identify GABA_B-coupled Ca_v2.3 channels as target for α-conopeptide Vc1.1 and demonstrate the key role of the voltage-independent pathway in this process.

ORAL-03-04

CANNABINOID RECEPTOR INTERACTING PROTEIN (CRIP1a) MODULATES CB1 RECEPTOR-MEDIATED DOWNSTREAM SIGNALLING EVENTS IN NEUROBLASTOMA X GLIOMA CELLS

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Purpose: The CB1 cannabinoid receptor is the most abundant GPCR in the CNS, playing critical roles in regulating many physiological processes such as neurogenesis, synaptic plasticity, learning and memory. CB1 receptors preferentially couple to Gi/o proteins to modulate multiple downstream signalling events including inhibition of adenylate cyclase and alteration of voltage-gated calcium channels. The aim of the present study was to investigate effects of cannabinoid receptor interacting protein (CRIP1a) on intracellular levels of cAMP and Ca²⁺ in the NG108-15 cell line. **Methods:** Western blotting was used to measure CRIP1a protein knockdown following siRNA treatment. Intracellular cAMP levels were determined using an AlphaScreen cAMP kit. Calcium imaging studies were conducted using the calcium sensitive dye, fura-2/AM. **Results:** CRIP1a knockdown was observed in cells treated with 20 nM CRIP1a-siRNA 24 and 48 hours post-transfection. The cannabinoid agonist WIN55,212-2 reduced forskolin (10 μM)-stimulated level of cAMP in a concentration-dependent manner in untreated and mm-siRNA treated NG108-15 cells, but not in CRIP1a knockdown cells (n=4-5). The CB1 inverse agonist AM251 had no significant effect on forskolin-stimulated level of cAMP but dose-dependently reduced effects of 100 nM WIN55,212-2 in untreated, mm-siRNA treated and CRIP1a knockdown cells (n=3-5). Forskolin at 10, 30 and 100 μM increased the basal level of Ca²⁺. WIN55,212-2 at 10 μM increased Ca²⁺ level but the response was not statistically significant (n=3-5). CRIP1a knockdown had no significant effect on Ca²⁺ signal to WIN55,212-2 or forskolin, but significantly reduced KCl-induced Ca²⁺ influx (n=6-8, p<0.05). **Conclusion:** Our results showed that reducing the expression of CRIP1a protein altered the CB1 receptor-mediated downstream signalling events.

ORAL-03-05

N-ARACHIDONOYL DOPAMINE MOBILIZES CALCIUM VIA HUMAN CB1 AND CB2 RECEPTORS WITHOUT INHIBITING ADENYLYL CYCLASE: EVIDENCE OF BIASED AGONISM

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Purpose: Responses to cannabinoids are primarily mediated by CB1 and CB2 receptors, which couple preferentially to G_q/G₁₂ G-proteins. Restricted coupling to G_s and G_i-type G-proteins has been reported for CB1 receptors, although no agonist acts exclusively at one G-protein subtype. Here, we explored the CB receptor signalling of N-arachidonoyl dopamine (NADA), an endogenous CB1 agonist. **Methods:** CHO or HEK293 cells expressing hCB1 or hCB2 receptors or AtT20 cells expressing rCB1 receptors were grown in 96 well microplates. Intracellular calcium [Ca]_i and membrane potential were measured using proprietary fluorescent dyes (Molecular Devices) using a Flexstation microplate reader, adenylyl cyclase (AC) activity was also measured using an alphascreen assay and immunofluorescent surface receptor quantification was performed. Results are mean±s.e.m. of n=3-5 determinations. **Results:** NADA did not modulate forskolin-stimulated hyperpolarization of CHO-hCB1 or CHO-hCB2 cells, or affect forskolin-stimulated AC activity in HEK293 cells. NADA did not stimulate GIRK-mediated hyperpolarization of AtT20-rCB1 cells. However, NADA elevated [Ca]_i in CHO-hCB1 cells (nominal pEC₅₀ 4.8±0.2, maximum increase in fluorescence 159±24%). NADA elevated [Ca]_i in CHO-hCB2 over a similar concentration range to a maximum of 155±9%. The elevations of [Ca]_i by NADA were antagonized by AM251 (300nM) in CHO-hCB1 and AM630 (300nM) in CHO-hCB2. NADA did not affect [Ca]_i in untransfected CHO cells. NADA did not significantly affect [³H]-SR141716A binding to hCB1 but inhibited SR141716A-induced hCB1 upregulation in HEK293 cells, implying interaction with a distinct binding site on the receptor. **Conclusion:** NADA appears to be an endogenous, biased agonist at hCB1 and hCB2, with a signalling profile distinct from any previously identified CB receptor agonist.

ORAL-03-07

EXAMINATION OF VOLTAGE-ACTIVATED CURRENTS ELICITED BY EXTERNAL ELECTRICAL STIMULATION

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Purpose: Despite the widespread use of electrical stimulation for artificially activating neural tissue, the exact mechanism of how this approach works is not entirely understood. We sought to examine this mechanism by comparing external electrical stimulation with a variety of internal stimulation paradigms in single neurons. **Methods:** Whole cell patch-clamp electrophysiology was utilised in voltage-clamp mode to examine the flow of current under a voltage step protocol in mouse retinal neurons. External electrical stimulation was delivered using a subretinal, 50µm platinum electrode at various amplitudes, superimposed on voltage steps and the resulting ionic currents recorded. These data were then compared to various internal stimulation protocols. **Results:** Current/voltage relationships and pharmacology showed that both Na⁺ and K⁺ voltage-gated ion channels could be activated by external electrical stimulation (n=8). Comparison of internal vs external electrical stimulation confirmed that external stimulation likely collapses the membrane potential briefly by creating a local area of negative charge, causing activation of these v-gated channels. The degree of depolarisation required increased as stimulus pulse width decreased, to a point where no response could be elicited. **Conclusion:** Although the change in charge immediately surrounding the target neuron can be measured directly, it was previously unknown how voltage-gated ion channels experience this unnatural stimulus. We have provided a protocol that can be used to determine how external electrical stimulation activates voltage-gated channels. These data can be used in the development of neuronal prostheses to fine-tune stimulation paradigms to suit activation characteristics of target neurons.

ORAL-03-06

LIQUID STATE COMPUTATION IN CHOLINERGICALLY MODULATED CORTICAL NETWORKS

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We used simulations of biophysically realistic cortical neurons to investigate the dynamics of cortical networks under control conditions and when modulated by acetylcholine. With this model, we investigated both the temporal and spatial cohesion of coordinated cortical activity, as well as the computational capabilities of these networks. The networks we used were composed of 5 layers (with layers 2 and 3 pooled) of neurons. The individual neurons were Hodgkin-Huxley type single compartmental neuron models, with the exception of layer 5 neurons, in which we explicitly simulated the apical dendrite. We simulated both pyramids and several types of interneurons. Both the intrinsic excitability and the connections via chemical synapses and gap junctions were modeled after the experimental literature. All simulations were conducted in NEURON. We observed a shift from slow to fast (gamma) oscillations when simulating the effects of acetylcholine. The gamma oscillations showed a phase reversal between the deep and superficial cortical layers, as experimentally predicted. We furthermore observed a dependence of computational capabilities on the neuromodulatory state of the network.

ORAL-03-08

PATIENT AUTOANTIBODIES REVEAL THE ROLE OF MUSCLE SPECIFIC KINASE IN MAINTAINING THE MATURE NEUROMUSCULAR JUNCTION

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A subset of myasthenia gravis patients have autoantibodies against Muscle Specific Kinase (MuSK), a protein essential for assembling acetylcholine receptors (AChRs) at the developing muscle fibre membrane. To investigate the role of MuSK at the mature neuromuscular junction (NMJ) we injected mice with patient autoantibodies that deplete MuSK from the endplate and studied the time course of both structural and functional changes. C57B6/J mice, receiving daily injections of anti-MuSK patient IgG, lost weight from day 10 and demonstrated whole-body weakness at day 14. Electromyography showed synaptic impairment from day 6 in the gastrocnemius muscle and from day 10 in the hemidiaphragm muscle. During the 15 day injection series confocal microscopy revealed linear declines in the area and density of postsynaptic AChRs in the five muscles examined: tibialis anterior, diaphragm, sternomastoid, omohyoid and masseter (in each muscle 3-5% / day). Recordings from the diaphragm muscle demonstrated matching declines in the amplitudes of the spontaneous miniature endplate potentials, nerve-evoked endplate potentials and in the safety factor for neuromuscular transmission (all declined at 3% / day). A compensatory presynaptic mechanism became impaired from day 10, suggesting an ongoing role for MuSK in the homeostasis of the mature neuromuscular junction. Together these results provide evidence that progressive and gradual loss of acetylcholine receptors from postsynaptic clusters is sufficient to cause synaptic failure and muscle weakness in this mouse model of anti-MuSK myasthenia gravis. It also highlights the role of MuSK throughout life in targeting and maintaining AChRs at the postsynaptic membrane of the NMJ.

ORAL-04-01

INTER-GENERATIONAL SEX-SPECIFIC TRANSMISSION OF AN ANXIETY PHENOTYPE DUE TO SIRE COCAINE EXPOSURE

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Purpose: Given that relapse to cocaine abuse correlates with elevated levels of anxiety, and environmental information can be inherited, we used behavioral tasks and molecular biology to investigate an anxiety phenotype in F1 offspring due to sire (F0) cocaine exposure.

Methods: Male Sprague Dawley rats received 60 days of cocaine (.25 mg/infusion) self-administration (N=6) or yoked saline injections (N=6) before being mated with naïve females. At P60, F1 anxiety-like behaviors were measured with Novelty Induced Hypophagia (NIH, N=31-25) and Defensive Burying (DB, N=10-14) tasks. Depressive-like behaviors were measured with Forced Swim Task (FST, N=8). Serum corticosterone was analyzed using an ELISA. Differential gene expression in the amygdala, a brain region implicated in anxiety and cocaine addiction phenotypes, was investigated using a 2-color hybridization Agilent gene expression array (N=8). Global gene expression analysis is ongoing and select targets will be explored with RT-PCR. **Results:** A sub-threshold dose of cocaine (2.5mg/kg i.p.) caused increased anxiety as measured by latency to feed in the NIH task in naïve rats, without altering FST behaviors (N=18). In NIH, male but not female F1 cocaine-sired animals displayed increased latency to feed relative to their saline-sired littermates. In DB, F1 females spent more time burying than their male littermates, but the task revealed no effect of sire cocaine exposure. There was an effect of sex, but not sire, on corticosterone levels. **Conclusions:** Acute, sub-threshold, cocaine exposure can elicit an anxiety-like, but not depressive-like, behavioral phenotype in naïve rats. F1 male offspring of cocaine-experienced sires display an anxiety-like phenotype, while the F1 female offspring appear unaffected. These results suggest a sex-specific, inter-generational transmission of an anxiety phenotype due to sire cocaine experience.

ORAL-04-02

MODULATION OF FEEDING BY CHRONIC VIRAL EXPRESSION OF A RELAXIN-3 PEPTIDE AGONIST IN RAT HYPOTHALAMUS

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PURPOSE: The neuropeptide, relaxin-3, has previously been demonstrated to modulate feeding through acute studies using relaxin-3 and/or a selective peptide agonist (R3/I5) to activate its receptor, relaxin family peptide receptor 3 (RXFP3). This study uses recombinant adeno-associated viruses (rAAV) to produce R3/I5 chronically, within the RXFP3-rich hypothalamus of rats and assess the behavioural and biochemical impact. **METHODS:** 2 cohorts of male Sprague Dawley rats were infused bilaterally into the hypothalamus with either rAAV expressing R3/I5 (rAAV1/2-R3/I5) or control GFP (rAAV1/2-GFP) (n=8/12 and 8/8). In cohort 1 feeding and bodyweight was monitored daily for 7 weeks and viral targeting was assessed using immunohistochemistry. In cohort 2, after a 3 month rAAV1/2-R3/I5 expression period, the target region of hypothalamus was dissected, mRNA extracted, and the expression level of a number of target genes was assessed using qPCR. **RESULTS:** In cohort 1, chronic expression of rAAV1/2-R3/I5 in hypothalamic neurons led to an increase in daily food intake (~5.18g) and bodyweight gain (~23%), compared to control animals over the 7 weeks. Cohort 2 confirmed this increased daily food intake. Hypothalamic gene expression analysis showed high expression of transgene mRNA after 3 months, with no down-regulation of receptor, RXFP3, mRNA. We observed a ~50% down-regulation of oxytocin mRNA in R3/I5 rats compared to control (P<0.01), and no difference in expression between groups of major hypothalamic feeding peptides, NPY, POMC or AgRP. **CONCLUSION:** Chronic rAAV1/2-R3/I5 mediated RXFP3 activation in the hypothalamus leads to sustained altered feeding in rats which may be mediated by a novel mechanism involving oxytocin down-regulation.

ORAL-04-03

INTERACTIONS BETWEEN GNRH, KISSPEPTIN AND GnIH NEURONS IN THE EQUINE HYPOTHALAMUS

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Purpose Kisspeptin and gonadotrophin-inhibitory hormone (GnIH) are well established as regulators of gonadotrophin-releasing hormone (GnRH) secretion in mammals. However the full extent of the interactions between these three neuropeptides is still unknown. This pilot study aimed to clarify the interactions of kisspeptin, GnIH and GnRH neurons in the equine hypothalamus. **Method** Fluorescence immunohistochemistry was used to map kisspeptin, GnIH, and GnRH neurons across the hypothalamus of cycling mares (n=3), and the interactions between the cell bodies and the fibres of the three neuronal populations characterised. Cross reactivity of the antibodies was tested by pre-adsorption controls. **Results** Preadsorption controls showed that the antibodies were all specific. Scattered GnRH immunoreactive(ir) neurons were identified in the periventricular region, and the arcuate nucleus (ARC), with both kisspeptin and GnIH-ir fibres making contact with GnRH-ir cell bodies. Similarly, populations of kisspeptin and GnIH-ir cell bodies (in the ARC/ventromedial hypothalamus (VMH), and paraventricular nucleus (PVN) respectively) received inputs from GnRH-ir fibres. GnRH-ir fibres were found in close apposition to kisspeptin-ir fibres in the median eminence (ME), but not with GnIH-ir fibres. Within the kisspeptin-ir VMH population, a subpopulation of GnIH-ir neurons was identified. A dense population of fibres immunoreactive for both kisspeptin and GnIH was identified throughout the PVN, VMH, and within the ARC / ME. This co-localisation was not true for all immunoreactive fibres. **Conclusion** This research strongly suggests a direct relationship between kisspeptin and GnIH neurons, and has identified a potential shared pathway of kisspeptin and GnIH in their regulation of GnRH neuronal activity in the mare.

ORAL-04-04

ENTEROCHROMAFFIN CELLS RELEASE 5-HT WITH SYNAPTIC KINETICS

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Purpose Enterochromaffin (EC) cells are enteroendocrine cells located in the mucosal layer throughout the gastrointestinal tract and produce ~95% of the body's serotonin (5-hydroxytryptamine or 5-HT). This circulating 5-HT is vital for a multitude of bodily functions including enteric motility, bone mass, liver regeneration and haemostasis. Dysfunctional 5-HT release from EC cells has been implicated in human health disorders including irritable bowel syndrome, Crohn's disease and osteoporosis. Understanding how 5-HT is released from EC cells and the mechanisms that control this release is therefore important in terms of both health and disease. In spite of their importance however, no study has yet examined the mechanisms controlling 5-HT release from single primary EC cells. **Results** This study presents for the first time a rapid method of isolating and purifying (~99% 5-HT-positive) guinea pig and human EC cells in primary culture. We used carbon fibre amperometry to measure 5-HT release from single EC cells and find that they release ~60-100 times less 5-HT per vesicle fusion event compared to other endocrine cells and that the kinetics of 5-HT release resembles that occurring in synapses. Thus 5-HT may be released from EC cells via an extremely fast "kiss and run" type of exocytosis not previously observed in endocrine cells. We also identify a range of endogenous factors including acetylcholine, glucose and 5-HT itself which stimulate calcium entry and 5-HT release from EC cells. **Conclusion** These findings are the first study demonstrating the basic properties underlying stimulus-secretion coupling in EC cells. This includes a mode of exocytosis unique amongst all reported endocrine cells and suggests a novel paradigm in neurotransmitter release studies.

ORAL-04-05

LONG TERM ALTERATIONS IN GENE EXPRESSION IN LIMBIC REGIONS AND ADRENAL TYROSINE HYDROXYLASE ACTIVITY INDUCED BY A NEONATAL IMMUNE CHALLENGE ARE ASSOCIATED WITH AN ANXIETY-LIKE PHENOTYPE

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Purpose: Neonatal immune activation, by administration of lipopolysaccharide (LPS) on postnatal days (PNDs) 3 and 5 in rats, results in persistent changes to the HPA axis and increased anxiety-like behaviours in adulthood. We have recently reported neonatal LPS exposure to induce an immediate and sustained increase in tyrosine hydroxylase (TH) activity in the adrenal medulla. In adulthood, neonatally treated rats exhibited increased autonomic arousal indicated by increased respiratory responses to mild sensory stimuli, as evidenced by whole-body plethysmography. In this study, we examined the long term effects of neonatal LPS challenge on TH activity and expression of brain factors, implicated in modulation of the stress response and anxiety states. **Methods:** Wistar rats were treated with either LPS (0.05 mg/kg, i.p.) or saline on PNDs 3 and 5 (n=6 per group). **Results:** Neonatal administration of LPS resulted in a significant increase in adrenal TH activation in adolescence (PND50) and adulthood (PND85). Increased circulating corticosterone levels were evident in the same LPS-treated animals. In addition, neonatal LPS treatment resulted in dysregulation of CRH receptor type 1, CRH binding protein, GABA-A receptor $\alpha 2$ subunit and glucocorticoid receptor mRNA levels in limbic regions (i.e. prefrontal cortex, hippocampus, hypothalamus). **Conclusions:** These physiological changes were associated with increased anxiety-like behaviours, suggesting that neonatal LPS challenge produces a persistent change in the HPA axis and ANS functioning, contributing to an anxiety-like phenotype.

ORAL-04-07

LACTATION-INDUCED CHANGES IN PHOSPHO-STAT5 AND PHOSPHO-ERK SIGNALLING IN TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS

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Purpose: The concentration of circulating prolactin is regulated by the Tuberoinfundibular Dopaminergic (TIDA) neurons of the hypothalamus. TIDA neurons inhibit the release of pituitary prolactin by secreting dopamine into the median eminence. Prolactin negatively feeds back on TIDA neurons and activates both Signal Transducers and Activator of Transcription (STAT)5 and Extracellular signal Related Kinases (ERK) signalling pathways. During lactation the actions of the TIDA neurons change in order to accommodate an increase in circulating prolactin. **Methods:** Phospho-STAT5 and phospho-ERK prolactin responses were evaluated in both virgin and lactating mice to investigate changes in affinity and efficacy of these pathways. Bromocriptine treated mice received an intraperitoneal injection of prolactin, ranging from 0-10 mg/kg-1. After 20 min, mice were anaesthetised with 3mg/kg-1 of sodium pentobarbitone and underwent cardiac perfusion using 4% paraformaldehyde. Hypothalamic sections were prepared and immunohistochemistry used to dual label phospho-STAT5 or phospho-ERK and tyrosine hydroxylase (TH) (a marker of dopaminergic neurons). Dual labelled cells in the dorsomedial arcuate nucleus were counted as a percentage of the total TH labelled cells. **Results:** Lactating mice showed diminished responses for both phospho-STAT5 and phospho-ERK across the prolactin concentration range. Phospho-STAT5 responses were significantly reduced in lactating animals at treatments of 1.0-3.0mg/kg-1 ($p < 0.05$). Significant phospho-ERK signalling was only observed in virgin mice when treated with 10 mg/kg-1 prolactin whilst lactating animals showed a minimal response. **Conclusions:** Decreases in the efficacy and affinity of phospho-STAT5 and phospho-ERK signalling indicate a marked alteration in prolactin signalling in TIDA neurons during lactation.

ORAL-04-06

OREXIN NEURONS ARE HYPORESPONSIVE TO STRESS FOLLOWING MATERNAL SEPARATION

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Purpose: Early life stress (ELS) has been shown to predispose animals to anxiety- and depression-like behavior in adulthood. Recent evidence suggests that repeated stress in adulthood results in suppressed activity of the orexin system. Here, we examined the effects of maternal separation (MS), a common rodent model of ELS, on the expression of anxiety- and depression-related behaviour and orexin cell activation following re-exposure to stress in adulthood. We also investigated whether these effects can be reversed by daily voluntary exercise. **Methods:** Male rat pups (n=72) were removed from dams for 0 or 3hrs on postnatal days (PND) 2-14. A subset of MS animals (n=6) was allowed access to exercise wheels for 1h/day from PND40-73. On PND75, animals were exposed to restraint and then tested for 10mins in the open-field apparatus. Following restraint, rats were perfused and brains were dual-labelled for Fos-protein and orexin. Blood was also collected to assess serum corticosterone levels. **Results:** MS animals exhibited decreased exploratory behaviour in the open field apparatus and elevated corticosterone levels, compared to controls. This was associated with a decrease in the percentage of Fos-positive orexin cells in the perifornical area of the hypothalamus and a decrease in total orexin cells in the lateral hypothalamus. Exercise reversed the behavioural effects of MS. **Conclusions:** ELS resulted in hypoactivity of the orexin system in response to adult stress and was accompanied by suppressed mood-related behaviours. These findings are consistent with suppressed activity of the orexin system in animal models of depression and in patients with depressive disorders.

ORAL-04-08

LEPTIN SIGNALLING IN THE DORSAL VAGAL COMPLEX AND VENTROLATERAL MEDULLA WITH AGE AND HIGH-FAT DIET INDUCED OBESITY

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Purpose: The brainstem is a known contributor to central energy regulation and responds to leptin. Currently, the leptin signalling response to high-fat diet induced obesity in the brainstem is poorly understood. In this study we examined key leptin signalling markers in the Dorsal Vagal Complex (DVC) and Ventrolateral Medulla (VLM) of mice during mid-term (8 weeks) and long-term (20 weeks) high-fat diet induced obesity. **Methods:** C57Bl/6 male mice aged 12 weeks were fed either a high-fat (HF) or a low-fat control diet (LF), then examined after 8 or 20 weeks of feeding. Leptin signalling markers in the brainstem were examined by Western blot (n=3-8) following the intracerebroventricular injection of leptin or saline. **Results:** In the DVC, central leptin injection significantly increased pSTAT3 levels in the 8 week LF group ($p = 0.021$), but not in the HF group. However, leptin administration did not increase pSTAT3 levels in either 20 week LF or HF group. In the VLM, pSTAT3 was significantly increased in the 8 week HF group compared with the LF group ($p = 0.038$). pAMPK in the VLM decreased ($p = 0.023$), while POMC in the DVC increased ($p = 0.049$), in response to leptin injection in the 20 week LF group, but not in the 8 week LF group. **Conclusion:** Both high-fat diet and age impede leptin-pSTAT3 signalling in the DVC of the brainstem. Mid-term high-fat diet can activate pSTAT3 in the VLM. Age associated leptin-pSTAT3 deficiency in the brainstem may be compensated for by decreased pAMPK signalling and increased POMC expression in response to central leptin.

ORAL 5 – NEURAL ENGINEERING/BIONICS

Sponsored by The Finkel Foundation

ORAL-05-01

RECREATING VISION WITH A RETINAL PROSTHESIS

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Visual information in the environment is encoded in the trains of action potentials produced by retinal ganglion cells (RGCs). In order to re-create vision in patients with severe retinal degeneration, a 'perfect' prosthesis would re-create these patterns of action potentials as closely as possible. Our data demonstrate that at the level of single RGCs, this is now possible. Whole cell patch clamp recordings were made from individual cat alpha RGCs in vitro. Visual stimuli including spots, gratings and videos were presented to the retina using the microscope's own optics spanning 12 x 16 degrees of visual angle. RGC responses were recorded and the precise timing of each spike during the visual stimulus programmed into the stimulator as biphasic current pulses (100us cathodic/400us anodic). A 200x200um diamond electrode was positioned next to the cell and stimulated with a train of biphasic pulses replicating the recorded spike times. Information theoretic analyses were carried out to determine how well the action potentials evoked by electrical stimulation reproduced the information contained in the light responses. Our data demonstrate that nearly all of the information available in the light response could be reproduced in this manner for the majority of cells we recorded. However, sustained epochs of very high frequency stimulation sometimes introduced distortions: in some cells suppressing activity and in others causing excessive spontaneous activity. In summary, electrical stimulation of the retina is indeed capable of conveying the visual information encoded by cat alpha RGCs.

ORAL-05-02

A BIDOMAIN APPROACH TO PREDICT THE ACTIVATION MAP OF THE RETINA IN VISUAL PROSTHESES

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Purpose: Visual neuroprosthetic devices restore lost vision to the patients suffering from retinitis pigmentosa by directly stimulating the remaining neurons in the retina with extracellular electrodes. Having the knowledge of the activation map of the retina as a result of extracellular electrical stimulation is crucially important in designing optimal current waveshapes of the electrode array. We developed an analytic model that can predict the required threshold and subsequently the activation map for epiretinal stimulation. **Methods:** We have derived equations for longitudinal mode of stimulation for a neurite -plus-thin-extracellular-sheath. Using bidomain approach we have derived an analytic expression for the spatio-temporal behaviour of the membrane potential for a fibre bundle stimulated by an ultrafine electrode. We have illustrated the validity of the equations using Finite Element Method (FEM) simulation. **Results:** The error of the analytic expression is less than 7% compared to the FEM simulation results. Furthermore, the threshold values along the fibre bundle predicted by the model for Alpha ganglion cells of rabbit retinal ganglion cells stimulated by ultrafine microelectrodes matches the experimental data available in the literature. **Conclusions:** The fact that the model matches the experimental data confirms that the derived analytic expressions are reliable and can be used to design optimal stimulation waveshapes for an electrode array which has the potential to enhance the performance of visual neuroprosthetic devices.

ORAL-05-03

IMPEDANCE VARIATION AND STIMULATION EFFICACY OF CHRONIC CORTICAL IMPLANTS IN RAT MOTOR CORTEX

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Purpose: Implanting a device into the living brain causes a cascade of reactions from neurons and immune processes, which ultimately impact on the ability of the electrodes to stimulate effectively the tissue over the long term, and may be reflected in changes in the impedance of the electrodes. In present study we have examined electrode impedance and stimulation efficacy for electrode arrays implanted in rat motor cortex. **Methods:** Under aseptic condition five adult rats were implanted with a 4-electrode array in motor cortex. Anaesthesia was induced with Halothane at 5% in O₂ and thereafter maintained at 1.5%-2.5% during surgery. Heart rate was monitored continuously and withdrawal reflexes checked regularly to ensure adequate anaesthetic level is maintained. Body temperature was kept at 37.5°C. Electrode impedance and stimulation threshold to produce observable motor output was determined at regular intervals throughout the period of implantation. **Results:** Typically, impedance of the electrodes increased following implantation and showed considerable variability over the next 6 weeks, and then stabilized, in most cases to levels similar to those immediately after implantation. Stimulation usually caused a significant reduction in electrode impedance. Threshold to induce motor output, e.g. whisker movement, was generally between 75-100µA though occasionally it could be as low as 50µA; these values are consistent with the lower current density generated by the electrodes designed to have a larger-than-normal stimulating surface area. No systematic change in stimulation thresholds occurred over the duration of implantation. **Conclusions:** 1) the observed large variation in electrode impedance during the early phase of device implantation may reflect the reaction of brain tissue to the implant and formation of an encapsulating glia scar around the electrodes; 2) the functional effects of the device do not appear to be blocked by factors that affect electrode impedance during the course of implantation.

ORAL-05-04

SPATIOTEMPORAL INTERACTIONS IN THE VISUAL CORTEX USING PAIRED ELECTRICAL STIMULATION OF THE RETINA

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Purpose: Retinal prostheses aim to provide functional vision using spatiotemporal patterns of electrical stimulation. In this study we used a paired pulse paradigm to investigate the effect of time, distance and current between pairs of electrical stimuli in the retina on multi-unit spiking activity in the visual cortex. **Methods:** A retinal prosthesis was implanted in the suprachoroidal space of seven cats. Retinal electrodes were stimulated using pulse pairs where the current of the first pulse and the delay between the two pulses varied (0-1.5mA; 20-500ms) while the current of the second pulse was kept constant. Pulses were presented on the same electrode (n=16) or on electrodes separated by 1 (n=17) or 2mm (n=10). Spiking activity was recorded from the visual cortex using a 60-channel array. Interactions were quantified on each cortical recording site by comparing spike counts following the second pulse in the paradigm with that evoked by presentation of the second pulse alone. **Results:** Both delay and current were found to affect spiking. Irrespective of electrode separation, for delays less than 100ms, the presence of the first pulse suppressed spiking activity following the second pulse compared to activity with the second pulse alone. This effect was observed even when the first pulse was presented at sub-threshold levels. Interactions between two stimuli were minimal for delays larger than 100ms. **Conclusion:** Spatiotemporal interactions occur for up to 100ms and extend over several millimeters of the retina. Results of such interactions should be carefully taken into account when developing complex spatiotemporal patterns of electrical stimulation for retinal prostheses.

ORAL 5 – NEURAL ENGINEERING/BIONICS

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ORAL-05-05

DOPED CONDUCTING POLYMER COATED NEURAL RECORDING ELECTRODES

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Purpose: Neural implants are being used to treat a range of disorders including chronic pain, Parkinson's disease and epilepsy. Current electrode materials are not ideal, with variable performance, low signal to noise ratio (SNR) and poor chronic stability. Conducting polymer coated electrodes improve *in vivo* performance, significantly reducing impedance and resulting in larger SNR. However, to date, no systematic comparison of materials has been undertaken. **Methods:** Neural recording electrodes (n=192) were coated with polypyrrole (Ppy) or poly-3,4-ethylenedioxythiophene (PEDOT) with either poly(styrene sulfonate) (PSS), dodecylbenzene sulfonate (DBS), chondroitin sulfate (CS), para-toluene sulfonate (pTS) or sulfate (SO₄) dopants at varying thicknesses. They were characterised by optical microscopy, cyclic voltammetry, impedance spectroscopy and electrophysiological recording to white noise bursts when implanted into a rat model inferior colliculus. **Results:** All polymer coatings had lower impedance and larger charge densities than uncoated electrodes, resulting in larger SNR and allowing smaller electrodes to be used for neural stimulation. Doped PEDOT had lower electrochemical and electrophysiological variation than doped Ppy coatings. PEDOT-pTS also showed no evidence of fouling, indicating good biostability after acute implantation [1]. **Conclusions:** A method for comparing electrode materials has been successfully implemented, and a large number of doped conducting polymers tested. PEDOT-pTS displayed remarkable non-fouling properties with a high SNR and large charge density. 1. Harris, A.R., et al., Conducting polymer coated neural recording electrodes. *Journal of Neural Engineering*, Accepted.

ORAL-05-07

PROMOTING ENGRAFTMENT OF TRANSPLANTED NEURAL STEM CELLS/PROGENITORS USING BIOFUNCTIONALISED ELECTROSPUN SCAFFOLDS

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With the brain's limited capacity for repair, new and innovative approaches are required to promote regeneration. While neural transplantation for a number of neural disease/injuries have been demonstrated, major limitations in the field include poor cell survival and integration. This, in part, is due to the non-conductive environment of the adult brain, failing to provide adequate chemical and physical support for new neurons. Here we examine the capacity of fibrous poly ϵ -caprolactone (PCL) scaffolds, biofunctionalised with immobilised glial cell-derived neurotrophic factor (GDNF), to influence primary cortical neural stem cells/progenitors *in vitro* and enhance integration of these cells following transplantation into the brain. Immobilisation of GDNF was confirmed prior to *in vitro* culturing and at 28 days after implantation into the brain, demonstrating long-term delivery of the protein. *In vitro*, we demonstrate that PCL with immobilised GDNF (iGDNF) significantly enhances cell viability and neural stem cell/progenitor proliferation compared to conventional 2-dimensional cultureware. Upon implantation, PCL scaffolds including iGDNF enhanced the survival, proliferation, migration, and neurite growth of transplanted cortical cells, whilst suppressing inflammatory reactive astroglia. These findings have implications for the development of improved cell transplantation therapies for the treatment of neural injuries.

ORAL-05-06

SUPRACHOROIDAL ELECTRICAL STIMULATION: ASSESSING EFFICACY OF REPETITIVE ELECTRICAL STIMULATION

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Pulse train stimulation has been proposed as a method of stimulation for visual prostheses, as it can potentially replicate spike trains generated by the retinal ganglion cells. However, it is not clear how spikes generated by such stimulation are encoded in the higher visual centres. In the current work, we investigated the nature of responses in the visual cortex to repetitive electrical stimulation of the retina. Electrode arrays (84 platinum discs) were acutely implanted in the suprachoroidal space of normally sighted feline eyes (n=10). Cathodal first constant biphasic current pulses (500ms burst - 500 μ s/phase and 25 μ s interphase gap) were presented at rates of 1- 20 Hz on individual electrodes (n=15). Responses to electrical stimulation were recorded using multichannel arrays in the primary visual cortex (V1). Multiunit responses (n=480) were quantified by the rate transfer function and we evaluated the maximum firing rate, Best Repetition Rate (BRR) and high cut-off rate (at 50%maximum firing rate). The repetition rate transfer function showed a characteristic band pass filter shape with the best repetition rate at 9.27 ± 0.95 (mean \pm SD) Hz with a cut-off frequency at 11.67 ± 0.91 Hz. The capacity of V1 neurons to follow repetitive stimulation up to 20 Hz reflects their ability to encode temporally complex signals. These results suggest that the rate of repetitive stimulation should be kept below 12 Hz for efficient transfer of visual information using suprachoroidal visual prostheses.

ORAL-05-08

EPIRETINAL STIMULATION USING ULTRANANOCRYSTALLINE DIAMOND ELECTRODES

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Purpose: Nitrogen incorporated ultrananocrystalline diamond (N-UNCD), owing to its excellent biocompatibility and conductive properties has shown to be a promising material as a stimulating electrode. The aim of this study is to test if N-UNCD electrodes could be successful for use in an epiretinal prosthesis for restoring vision. **Methods:** Electrode arrays containing 120x120 μ m N-UNCD electrodes (n=24) were fabricated on a polycrystalline diamond substrate. A pars plana vitrectomy was performed in two normally-sighted anaesthetised cats. Arrays were attached to the inner surface of the retina using a retinal tack. Impedances were measured both *in saline* before implantation and *in vivo*. Multiunit responses were recorded from the visual cortex in response to epiretinal stimulation using a 60-channel array (Blackrock Microsystems, UT). **Results:** Impedances 24 hours post-implantation (23.2 ± 4.1 kohm, *Mean \pm SEM*) were not found to be significantly different to those measured *in vitro* (16.2 ± 3 kohm). Epiretinal stimulation led to localised activation of the visual cortex. Lowest cortical thresholds ranged between 29.5-136.4 μ C/cm² for single electrode stimulation, between 7.2-99.3 μ C/cm² for three electrodes simultaneously stimulated and between 18.3-26.1 μ C/cm² for 12 electrodes simultaneously stimulated. In all cases charge densities required to evoke cortical responses were well within the electrochemical safety limit for diamond electrodes. **Conclusion:** N-UNCD electrodes can be useful for an epiretinal prosthesis with some electrodes requiring exceptionally low charge levels to activate the visual cortex. Variability in cortical thresholds may be attributed to distances between stimulating electrodes and the retina.

ORAL-06-01

HDAC6 MUTATIONS RESCUE HUMAN TAU-INDUCED MICROTUBULE DEFECTS IN *DROSOPHILA*Xiong Y.¹, Zhao K.¹, Wu J.X.¹, Jin S.² and Zhang Y.Q.¹¹Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China. ²College of Life Sciences, Hubei University, Wuhan, Hubei 430062, China.

Tau is a neuronal microtubule-associated protein (MAP) that binds to and stabilizes microtubules (MTs). However, in Alzheimer's disease (AD), frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), and other tauopathies, tau is hyperphosphorylated and aggregated into straight or paired helical filaments (PHFs). Microtubules are disrupted in the brains of patients and animal models of tauopathies. Furthermore, administration of the MT-stabilizing agents paclitaxel and epothilone D to human tau-expressing mice results in improved MT density and axonal integrity, as well as enhanced cognitive performance. However, paclitaxel has poor blood-brain barrier (BBB) permeability and thus is unsuitable for clinical treatment of brain diseases. Epothilone D is BBB-permeable; however, as a general microtubule stabilizer and genotoxic agent, it may have side effects such as neuropathy and neutropenia. **Purpose and Methods:** in this study, we aim to find new strategies for mitigating tau toxicity by identifying interacting genes that can suppress tau-induced MT defects. Ectopic expression of wild type and V337M mutant human tau in *Drosophila* muscle cells resulted in prominent MT defects. **Results:** from a pre-selected genetic screen, we found that a null mutation in *HDAC6* which encodes histone deacetylase 6 rescued tau-induced MT defects. The rescue of these tau-induced MT defects by the *HDAC6* mutation was further verified by treatment with tubacin, an inhibitor of the tubulin-specific deacetylase activity of HDAC6. **Conclusion:** this study thus identifies HDAC6 as a novel potential drug target for the treatment of AD and related tauopathies.

ORAL-06-02

REGIOSPECIFIC LOSS OF ESSENTIAL SPHINGOLIPIDS IN THE EARLY STAGES OF ALZHEIMER'S DISEASEKain N.¹, Couttas T.A.¹, Garner B.² and Don A.S.¹¹University of New South Wales. ²University of Wollongong.

Purpose: A major risk factor for Alzheimer's disease (AD) is the E4 allele of the brain lipid transporter ApoE. This protein mediates sphingolipid transport, a family of lipids that play important roles in signal transduction and cell recognition in neuronal membranes. Growing evidence suggests altered sphingolipid metabolism in the brains of AD patients. We investigated whether alterations to sphingolipids occur early in AD pathogenesis. **Methods:** We used liquid chromatography tandem mass spectrometry (LC-MS/MS) to analyse sphingolipid metabolites in 6 brain regions: hippocampus, where initial AD pathology occurs, inferior temporal and superior frontal cortex grey and white matter, and cerebellum. Tissue samples from 34 subjects were divided into four groups based on neurofibrillary tangle (NFT) pathology: Braak stages I/II (n=8) corresponding to preclinical pathological changes, III/IV (n=7) at which Mild Cognitive Impairment occurs, V/VI patients with full blown AD (n=10), and age-matched controls with no NFT pathology (n=9). Eight groups of lipids within the sphingolipid pathway comprising 104 individual metabolites were quantified and heat maps were used to visualise the data. **Results:** The neuroprotective factor sphingosine 1-phosphate (S1P) decreased in line with development of NFT pathology. The loss of S1P was statistically significant in Braak stages III/IV and V/VI for hippocampus and temporal cortex (P < 0.05). In addition, the abundant myelin lipid galactosylceramide was significantly decreased in the hippocampus (P < 0.05) of pre-clinical (Braak I/II) subjects. **Conclusion:** AD progression and its associated cognitive decline are accompanied by an early decline in the neuroprotective factor S1P and the myelin lipid galactosylceramide. Future studies will investigate whether loss of these lipids contributes to hippocampal neurodegeneration in the early stages of AD.

ORAL-06-03

BIOMETAL HOMEOSTASIS IN CHILDHOOD NEURODEGENERATIVE DISEASEGrubman A.¹, Kanninen K.^{1,2}, Duncan C.¹, Lidgerwood G.¹, Choo X.Y.¹, Caragounis A.¹, Moujalled D.¹, Liddell J.¹, Crouch P.J.¹ and White A.R.¹¹Department of Pathology, The University of Melbourne, Parkville, Australia. ²University of Eastern Finland, Kuopio, Finland.

Purpose: Deregulation of biometal homeostasis is an important hallmark of neurodegenerative disorders including Alzheimer's, Parkinson's and motor neuron diseases. Metal modulating compounds have demonstrated success as therapeutics in numerous animal models of neurodegeneration, but their mechanism of action remains poorly understood. Neuronal ceroid lipofuscinoses (NCLs) are a group of fatal childhood neurodegenerative diseases for which there is no treatment or cure. Given the pathological similarities of NCLs to other neurodegenerative disorders, this study investigated the novel concept that alteration of biometal functions is a driving disease feature in natural sheep and mouse models of NCLs. **Methods:** Biometal concentrations in affected and peripheral tissues were determined using ICP-MS analyses (n=6 animals per group). Alterations to metal trafficking pathways were investigated using western blotting, immunofluorescence and immunoprecipitation techniques. *In vitro* effects of metal-delivering complexes were investigated in primary neurons and astrocytes harvested from control or NCL-affected mice, using viability, neurite outgrowth and ELISA assays (n=3). **Results:** Significant region-specific biometal accumulation and deregulation of metal trafficking pathways was observed prior to the onset of detectable disease symptoms. This study identified direct interactions between the disease-associated protein, CLN6, and a down-regulated ER-resident zinc transporter, Zip7, which may contribute to impaired biometal homeostasis in NCLs. Moreover, the metal complex, Zn(atm), restored zinc-dependent functions, and exerted neuroprotective and anti-inflammatory effects on neurons and astrocytes harvested from CLN6 mice. **Conclusion:** This study was the first to demonstrate impaired metal homeostasis in NCLs. The protective and metal modulating effects of Zn(atm) treatment *in vitro* suggest Zn(atm) may be an ideal candidate for NCL therapeutic trials.

ORAL-06-04

SYNAPTIC PROTEOME IN ALZHEIMER'S DISEASE

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Purpose: Synaptic dysfunction occurs early in Alzheimer's disease (AD) and is recognized as a primary pathologic target for AD treatment. Synapse degeneration or dysfunction contributes to clinical signs of dementia through altered neuronal communication and the degree of synaptic loss strongly correlates with cognitive impairment. The exact molecular mechanisms underlying synaptic degeneration are still relatively unclear; therefore, identifying abnormally expressed synaptic proteins in AD brain will help to elucidate those mechanisms and can lead to the identification of therapeutic targets that might slow AD progression. **Method:** In the present study, synaptosomal fractions from post-mortem human brain tissue of AD (n = 6) and control (n = 6) were compared using 2D-differential in gel electrophoresis. Within each diseased state, two vulnerable areas (hippocampus and temporal cortex) were compared with two relatively spared areas (motor and occipital cortices). Consequently, Proteins exhibiting significant changes in their expression were identified (≥20% change, Newman-Keuls P-value <0.05) using either MALDI-TOF or ESI-QTOF mass spectrometry. These results are being followed up using the Multiple Reaction Monitoring (MRM). MRM is a very powerful and sensitive mass spectrometry technique suitable for quantitative analysis. **Results:** Total of 28 different synaptic proteins exhibited greater than two-fold differences between expression in AD and normal subjects. These proteins are involved in regulating cellular functions including energy metabolism, signal transduction, vesicle transport, structural and antioxidant function. **Conclusion:** By using two comparative proteomics techniques, gel-based and gel-free, this study showed that synaptic proteins in human AD brain are significantly different from those in control brain.

ORAL-06-05

RCAN1 OVER-EXPRESSION RESULTS IN IRON ACCUMULATION AND INCREASED OXIDATIVE STRESS IN THE MOUSE BRAIN

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Purpose: Maintenance of iron homeostasis in the brain may be crucial for delaying neurodegenerative processes. Iron is an essential survival factor; but also has the potential to cause the formation of free radicals that are detrimental for cell viability. Alzheimer's disease (AD) patients have elevated brain tissue iron and both AD and Down syndrome (DS) sufferers show elevated levels of oxidative stress. RCAN1 (Regulator of calcineurin 1) protein is elevated in both disorders so we wished to investigate the consequences of excess RCAN1 on iron accumulation and oxidative load. **Methods:** We quantified iron and APP (amyloid precursor protein) levels in mice over-expressing RCAN1 and tested whether the chronic over-expression of RCAN1 affects survival of cells when exposed to oxidative stress. **Results:** RCAN1-Tg mice showed a significant ($p < 0.05$; $n = 3$) increase in iron levels in the hippocampus [$8.24 \pm 0.774 \mu\text{g/g}$ versus $5.12 \pm 0.557 \mu\text{g/g}$ in the WT] and increased APP expression ($p < 0.05$; $n = 3$). When PC12 cells constitutively over-expressing RCAN1 were subjected to varying concentrations of H_2O_2 , the RCAN1 over-expressors exhibited decreased viability ($p < 0.002$). Primary neurones derived from RCAN1-Tg mice also showed a significant ($p < 0.001$; $n = 3$) decrease in cell viability after treatment with H_2O_2 . In preliminary experiments, PC12 cells constitutively over-expressing RCAN1 exhibited a reduced total antioxidant capacity (CUPRAC assay) when compared with wild-type PC12 cells, suggesting that constitutive RCAN1 over-expression impedes the cells' capacity to deal with oxidative stress. **Conclusions:** When over-expressed at levels similar to that in DS and AD, RCAN1 may exacerbate iron accumulation and oxidative load.

ORAL-06-07

ESTREN ATTENUATES BETA AMYLOID-INDUCED LESIONS OF CORTICAL CHOLINERGIC PROJECTIONS IN VIVO

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Purpose: Estren (4-estren-3 α , 17 β -diol) is a selective non-classical estrogen like signaling activator with neuroprotective effects *in vitro*. In this study, we have tested the effect of estren treatment on $\text{A}\beta_{1-42}$ -induced cholinergic cell and fiber loss using an *in vivo* animal model for Alzheimer's disease (AD). **Methods:** Adult female wild-type mice were ovariectomized. Two weeks later $0.5 \mu\text{l}$ of $300 \text{ nM } \text{A}\beta_{1-42}$ was microinjected into the right substantia innominata-nucleus basalis magnocellularis complex (SI-NBM) and 1 h later followed by sc administration of 0.3, 3 or 30 ng/g bw estren in 0.1 ml ethyl-oleate or vehicle alone. Twelve days after $\text{A}\beta_{1-42}$ injection, animals were transcardially perfused with 4 % paraformaldehyde, the brains removed and coronal sections cut. The cholinergic fiber loss was visualized by acetylcholinesterase (AChE) histochemistry and the death of cholinergic neurons identified by cholin-acetyltransferase (ChAT) immunohistochemistry. **Results:** Animals that received unilateral $\text{A}\beta_{1-42}$ injection into the SI-NBM showed ~30 % decrease in ChAT-immunoreactive cell bodies in the SI-NBM and AChE-stained fibers in the somatosensory cortex of the lesioned hemisphere. A single injection of estren 1 h after $\text{A}\beta_{1-42}$ administration did not have an effect on cholinergic cell loss in the SI-NBM, but it restored the ipsilateral cholinergic fiber density in the somatosensory cortex in a dose-dependent manner. The most effective cholinergic fiber restoration was observed with 30 ng/g estren treatment ($p < 0.01$; $n = 6$), while the 0.3 ng/g estren administration had no significant effect ($n = 6$). **Conclusion:** These findings indicate that estren might hold potential as a neuroprotective agent and also as an important molecular target for the treatment of AD.

ORAL-06-06

SORTILIN INTERACTS WITH AMYLOID PRECURSOR PROTEIN AND REGULATES ITS LYSOSOMAL TRAFFICKING AND PROCESSING

Yang X.F.¹, Virassamy B.², Vijayaraj S.L.², Lim Y.¹, Saadipour K.², Wang Y.J.³, Zhong J.H.¹, Morales C.R.⁴ and Zhou X.F.¹

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The processing of Amyloid precursor protein (APP) is multifaceted, including protein sorting, transport, internalization and sequential proteolysis. However, the exact mechanism of APP intracellular trafficking and distribution remains unclear. In this study, we used several experimental approaches, including co-transfection, co-localization, co-immunoprecipitation and sucrose gradient fractionation to determine the protein-protein interaction between sortilin and APP as well as the effect of the interaction on APP trafficking in co-transfected human embryonic kidney (HEK) 293 cells and neurons of sortilin knockout mice. We identified for the first time that sortilin can directly interact with APP at both head-head and tail-tail regions, where the sortilin FLVHRY motif (residues 788-793) and APP NPTYKFFE motif (residues 759-766) appear to be crucial for the interaction between sortilin and APP. An increase of APP in lipid rafts and a decrease of APP in late endosomes and lysosomes were found in the cortical neurons of sortilin knockout mice. Moreover, the deletion of the FLVHRY and the dileucine (residues 823-831) motifs were associated with a decrease of APP lysosomal targeting in co-transfected HEK293 cells. Lack of the FLVHRY motif also resulted in an increase of APP distribution in lipid rafts, which was consistent with the results from experiments in sortilin knockout mice. We also found that overexpression of sortilin-FLVHRY deletion constructs augmented the production of cellular beta-amyloid. Our data suggest that sortilin is implicated in APP trafficking and processing, which might govern APP into the non-amyloidogenic pathway.

ORAL-06-08

GUINEA PIGS AND ZEBRAFISH FOR INVESTIGATION OF ALZHEIMER'S DISEASE GENETICS

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Purpose: Mice and rats are members of the rodent family *Muridae* in which genes orthologous to the human genes implicated in Alzheimers disease (AD) appear to be evolving rapidly. Could other small animal models be more appropriate for investigating the genetics of AD? We sought to investigate AD gene sequence and expression/regulation conservation in the rodent *Cavia porcellus* (guinea pig, family *Caviidae*) and the zebrafish as alternatives. **Methods:** We analysed protein coding sequence conservation, and particular examples of splicing isoforms and expression regulation (by qPCR and ELISA) for AD gene orthologues in guinea pigs and zebrafish. **Results:** In all the above rodents the genes/proteins APP, PSEN2, BACE1, ADAM10, APOE, IDE and MAPT show similar levels of amino acid residue identity to human orthologues. However, PSEN1 shows higher identity in guinea pigs (96%) than mice (93%). Unlike mice, adult guinea pig brains express both 3R and 4R isoforms of MAPT. Guinea pigs express the PS2V isoform of PSEN2 that is absent in both mice and rats. Zebrafish possess an alternative transcript splice form (PS1IV) related in structure and function to PS2V. Formation of PS2V/PS1IV is induced by chemical mimicry of hypoxia in guinea pigs ($p < 0.05$) and zebrafish ($p < 0.05$). The control of PS2V/PS1IV formation by HMGA1 has been conserved during 450 million years of divergent evolution. A diet rich in cholesterol also upregulates PS2V formation ($p < 0.01$) and BACE1 mRNA levels ($p < 0.01$) in guinea pigs. It downregulates ADAM10 mRNA levels ($p < 0.001$) and causes increased frontal cortex and CSF A β 40 levels ($p < 0.05$) as in humans. **Conclusions:** For some analyses, guinea pigs and zebrafish represent superior models of AD gene/protein behaviour relative to mice and rats.

ORAL-07-01

THE EFFECTS OF HIGH FAT AND SUGAR DIETS ON COGNITION IN THE RAT

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Purpose: The long term intake of high energy diets in both humans and rodents is detrimental to physical, as well as cognitive health however research on the short-term impact of these diets is almost non-existent. This study investigated whether a short-term high fat or high sugar diet could affect cognition and if so, whether any diet-induced cognitive impairments were comparable to that seen with a high fat and sugar diet.

Methods: Male Sprague Dawley rats (n=8 per group) were randomly assigned to either regular chow diet (RD), cafeteria diet (chow plus cakes, biscuits, lard) supplemented with 10% sucrose solution (CAFS+), RD supplemented with 10% sucrose solution (S+), or a cafeteria diet without sucrose (CAFS-). All rats were assessed on the object and place tasks after five, 11 and 20 days on the diets to examine perirhinal-dependent (object) and hippocampal-dependent (place) recognition memory. Blood was sampled for hormones. **Results:** The CAFS+, S+ and CAFS- rats showed similar impairments in their ability to identify a novel place from five to 21 days (exploration ratios 0.49 to 0.55) compared to controls (0.68±0.02, n=8) but were able to identify a novel object. CAFS- and CAFS+ diets increased plasma leptin and insulin concentrations (P<0.05) which both displayed moderate negative relationships with exploration ratios on the final place task. **Conclusion:** High fat and/or sugar diets selectively impair hippocampal-dependent recognition memory prior to the emergence of weight differences and the development of metabolic alterations linked to obesity. This leaves open the possibility that diet-induced cognitive impairments could contribute to obesity by interfering with hippocampal-dependent higher-order learning and memory processes.

ORAL-07-02

SUBOCCIPITAL INTRACRANIAL ERP RECORDINGS FOR VISUAL OBJECTS: ELECTROPHYSIOLOGICAL EVIDENCE FOR SEPARATE SPECIALIZED AREAS

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Patients with medically intractable epilepsy and implanted with intracranial electrodes for clinical seizure localization were recruited for ERP studies during their implant hospitalization. ERPs were extracted via the EEG component of the continuous clinical video-EEG recording used to capture and characterize the patients seizures leading up to epilepsy surgery. Several patients had occipital electrode placements, including intraparenchymal, mesial, lateral and inferior locations, allowing for the mapping of near-field recordings of responses to visual stimuli, including visual objects such as faces, face parts, hands, and objects of special (or expert) interest to the patient, as well as simple pattern stimuli. Of particular interest were large, focal ERPs to visual objects recorded from the vicinity of the fusiform gyrus, with field patterns indicating adjacent but distinct response sources that were separable based on their near field voltage topography. The implications of these results for competing theories of visual object recognition will be discussed.

ORAL-07-03

FRONTAL LOBE CONTRIBUTIONS TO COGNITIVE FLEXIBILITY AND CONTROL

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Purpose: The cognitive flexibility to select appropriate rules in a changing environment is essential for survival and is assumed to depend on the integrity of prefrontal cortex, however the neural substrate and mechanisms underlying these processes are still unclear. In complementary lesion-behavioural and single-cell recording studies we have investigated the neural basis of cognitive flexibility in adapting to rule changes. **Methods:** We trained 18 monkeys to perform an analog of the Wisconsin Card Sorting Test (WCST). The WCST is routinely used in neuropsychological assessment of patients with frontal cortex damage or mental diseases to assess abstract reasoning and cognitive flexibility. In 15 monkeys, we studied the effects of circumscribed bilateral lesions in different regions of prefrontal and medial frontal cortex on their ability to perform WCST. We also recorded single cell activity from the same cortical regions to study the underlying neuronal mechanisms. **Results:** We found that lesions within dorsolateral prefrontal cortex, orbitofrontal cortex or anterior cingulate cortex impaired the cognitive flexibility in adapting to rule changes indicating that these cortical areas play crucial and complementary roles in organizing cognitive flexibility in WCST. However, monkeys with lesions in posterior cingulate cortex or dorsomedial prefrontal cortex showed no impairment in performing WCST. **Conclusion:** WCST is a multifaceted test that demands basic perceptual and motor processes in addition to the coordination of multiple cognitive processes. Our findings indicate that dissociable processes of rule-guided behaviour depend on distinct medial and prefrontal regions. Deficiencies in these processes might underlie the behavioural manifestation of patients with brain damage or mental diseases.

ORAL-07-04

OPTOGENETIC ANALYSIS OF THE CEREBELLAR CIRCUITS INVOLVED IN ZEBRAFISH MOTOR LEARNING

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Purpose: The patterns of cerebellar activity that are necessary and sufficient to effectively regulate motor programs remain an active area of research. The zebrafish model organism has similar cerebellar circuitry compared to mammalian models, however also offers a number of advantages in imaging and genetic manipulations. **Methods:** We have used two assays developed in the lab in combination with optogenetic tools to investigate motor learning in larval zebrafish. In a viscosity-based assay, larvae learn to change swimming behaviours to recover bout distance in higher viscosity media. In a classical conditioning assay, immobilised larvae learn to associate an innocuous tone with a tail shock stimulus. **Results:** We have generated transgenic zebrafish lines for the expression of optogenetic tools in the cerebellum, including GCaMP5, ArchT-YFP and eNpHR3.0-mCherry. Using these tools, we show that the ability to recover normal swim distance over 5 minutes in 5x viscosity media is decreased when the cerebellum is inhibited by activation of ArchT (n=11), but not by activation of eNpHR3.0 (n = 4). We also show that larval zebrafish undergo classical conditioning after 15 pairings of a 250Hz tone with a co-terminating 100 msec, 9V shock (n = 12). Using the calcium indicator GCaMP5, we have made recordings of activity in cerebellar neurons during these pairings and found stimulus-related signals in both Purkinje and eurydendroid cell types. **Conclusion:** Larval zebrafish offer a number of advantages for the study of cerebellar activity in motor learning that are ideally utilized by combining behavioural assays with the use of optogenetics. This lays the groundwork for detailed analysis of the patterns of cerebellar activity underlying motor learning.

ORAL-07-05

RXFP3 DELETION ENHANCES ETHANOL-INDUCED ATAXIA AND REDUCES ETHANOL PREFERENCE FOLLOWING FORCED SWIM STRESS IN C57BL/6J MICE

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Purpose: Relaxin-3/RXFP3 signalling can modulate ethanol consumption and seeking in rats. While RXFP3-deficient mice show normal baseline ethanol consumption, the current study investigated the impact of RXFP3 deletion on ethanol sensitivity and stress-related drinking. **Methods:** Ethanol-induced motor impairment was measured on the accelerating rotarod in RXFP3 null (KO) mice and wild-type (WT) littermates. Mice (n=50) received training, 2 baseline trials and were then injected with ethanol (2.0 g/kg i.p.). Latency-to-fall from the rotarod was recorded at post-injection intervals. In the stress study, mice (n=48) were single-housed and provided 5% v/v ethanol and water solutions for 2 weeks. Ethanol concentration was subsequently increased to 10% v/v for 3 weeks. Male (n=9WT/20KO) and female (n=9WT/10KO) mice were restrained for 30 min/day for 7 consecutive days. After 1-week recovery, mice were exposed to 5 min of forced-swim stress on 2 consecutive days. Ethanol preference, intake (g/kg) and total fluid intake (ml/kg) were measured. **Results:** Ataxia: RXFP3-KO mice were more ataxic than WT littermates at 45 (p=0.004) and 60 min (p=0.003) post-ethanol injection. Stress-related drinking: Male RXFP3-KO mice exhibited reduced ethanol preference (p=0.031) on forced-swim stress days compared to WT littermates, with no change in total fluid intake (p>0.05). However, this effect was not observed in females (main effect of genotype, p=0.972; treatment*genotype interaction, p=0.965). **Conclusion:** RXFP3-KO mice display normal baseline ethanol consumption, but our data suggest RXFP3 signalling alters ethanol sensitivity and contributes to the maintenance of ethanol preference during stress in male C57BL/6J mice.

ORAL-07-07

MICRORNA REGULATION OF OLFACTORY LEARNING AND MEMORY IN HONEYBEES

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Purpose: In the current study we aimed to identify genes and their regulatory microRNAs (miRNA) associated with olfactory learning and memory in the honeybee brain. Associative learning of odours was used to explore differential brain gene expression between learning and non-learning bees. **Methods:** We used an experimental paradigm known as proboscis extension reflex (PER). Groups of bees (n=10) were trained to respond to an olfactory stimulus by extending their tongue to receive a sugar reward. Immediately after conditioning, the brains were dissected and RNA extracted for microarray and qPCR analyses. MicroRNA (miRNA) targets were predicted using bioinformatic tools. *In situ* hybridization was used to localise miRNAs in the honeybee brain. Functional validation of miRNAs was conducted by feeding bees with miRNA inhibitors followed by evaluation of learning performance using the PER assay. **Results:** A total of 53 genes were found to be differentially expressed in learning versus non-learning bees. 81% of these genes were down-regulated in learning bees and the negative correlation between their expression levels and the number of miRNA target sites indicates that miRNAs are important for olfactory learning and memory in honeybees. miR-210, miR-928 and miR-932 were found to be expressed mainly in the mushroom bodies. RNA interference of miR-928 and miR-932 decreased olfactory memory retention in honeybees. **Conclusion:** miRNAs seem to be critical in regulating synapse development during olfactory learning and memory in insects. Understanding the mechanisms by which these molecules act promises a shift in our understanding of brain plasticity mechanisms, including the functional role of miRNAs in learning and memory.

ORAL-07-06

COCAINE SELF-ADMINISTRATION DURING ADOLESCENCE LEADS TO DIMINISHED PRIMED- BUT EXAGGERATED CUE-INDUCED REINSTATEMENT COMPARED TO ADULTHOOD

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Purpose: Adolescence is a developmental period associated with a greater likelihood of addiction to drugs than any other age. Therefore, we examined potential differences in the acquisition, extinction and reinstatement of cocaine-seeking behaviour between adolescent versus adult rats using intravenous-self administration (IVSA). **Methods:** Postnatal day (P)34 (±1) and P69 (±1) rats were trained to lever press for cocaine (3mg/kg/infusion) over 10-14 days. Following stable self-administration, rats received daily lever extinction sessions, where lever pressing had no consequence. In experiment 1, primed reinstatement was assessed by i.p. cocaine injection (10mg/kg). In experiment 2, cocaine infusions were coupled with a light CS. After lever extinction, animals underwent a single CS extinction session, consisting 120 CS-alone presentations with levers retracted. Controls remained in their home cages. Next day, all were tested for cue-induced reinstatement. **Results:** There was no effect of age on acquisition or extinction of lever presses in either experiment (ps > .05). Experiment 1 showed significant reinstatement of lever responses upon injection of cocaine compared to saline (p < .005), with adult rats displaying significantly higher cocaine-induced reinstatement than adolescents (p < .05). In experiment 2, both ages showed significant CS-induced reinstatement, which was reduced by CS extinction (ps < .05). In contrast to experiment 1, adolescent rats showed significantly higher CS-induced reinstatement than adults (p < .05). **Conclusion:** While adolescent drug abuse may lead to lower relapse upon drug-exposure compared to adults, it enhances vulnerability to relapse when exposed to drug-associated cues even following extinction. Such powerful effect of cues following adolescent drug abuse potentially explains the greater likelihood of addiction at this age.

ORAL-07-08

MEASURING THE LEVEL OF CONSCIOUSNESS BASED ON THE AMOUNT OF INTEGRATED INFORMATION

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Purpose: According to the integrated information theory of consciousness, the amount of integration information, which is defined within the framework of information theory, is correlated with the level of consciousness. The theory has been indirectly supported by recent clinical observations and experimental evidences showing that various forms of loss of consciousness such as anesthesia, dreamless sleep, and vegetative state results from disintegration of information in the brain. Thus, measuring the exact amount of integrated information based on the real neuronal data would be tremendously important in clinics, and it would be also theoretically very interesting as it may solve the mystery of consciousness. However, integrated information in any realistic organisms has been extremely difficult to compute due to several computational and experimental challenges. **Methods:** Here, we propose a novel practical measure to quantify the integrated information. We applied our new measure to the electrocorticogram (ECoG) data simultaneously recorded with 128 electrodes in monkeys (n=4, 12 experiments) before and after injection of anesthetic drugs to test whether the amount of integrated information actually drops when consciousness is lost. **Results:** We found that when the monkey lost consciousness, the integrated information in 8-24Hz sharply dropped as the theory predicted (FDR q=0.05). **Conclusion:** Our results imply that the low-frequency activity across the brain might serve to integrate information, the degree of which might be correlated with the level of consciousness.

ORAL-08-01

TRAUMATIC BRAIN INJURY: CHARACTERISATION OF BEHAVIOURAL AND ELECTROPHYSIOLOGICAL DEFICITS 24 HOURS POST-INJURY

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Purpose: Traumatic brain injury (TBI) occurs when an external force damages the brain, and is often associated with complex patterns of brain abnormalities that lead to deficits in cognitive and motor function. Despite convincing human data indicating that changes in sensory processing may contribute significantly to these deficits, there are few animal models of sensory processing post-TBI. Here we report TBI-induced changes in neuronal firing properties in rat sensory barrel cortex (receiving whisker input), using in vivo extracellular recording techniques and coordinated whisker motion to drive activity. **Methods:** TBI (n=8) was induced under anaesthesia (1.5% halothane) using the weight-drop impact acceleration method, with sham controls receiving surgery only (n=6). Sensorimotor function was assessed and after surgery. At 24hrs post-trauma extracellular recordings were obtained from barrel cortex in anaesthetised animals (1.5-2.5% halothane in O₂), using both simple trapezoidal and complex naturalistic whisker motion to stimulate responses. **Results:** Significant behavioural deficits were observed in TBI animals in the motor tasks. Characterisation of neuronal output in these animals revealed a depth-dependent suppression of excitatory responses: there were reduced responses in supragranular layers through to input layer IV but not infragranular layers, in response to simple and complex whisker stimuli in a manner consistent with a shockwave following impact. In contrast, increased spontaneous firing rate was recorded in TBI animals in layers IV and V. **Conclusion:** These changes in sensory processing are precursors and triggers to TBI-induced sensory cortical plasticity. They provide a basis for modelling of the shock wave propagation through the brain's soft tissues to determine how variations in impact velocity produce different extents of behavioural deficits in the immediate hours following trauma.

ORAL-08-02

MODULATING AQUAPORIN-4 CHANNELS: EFFECTS ON CEREBRAL OEDEMA FOLLOWING BRAIN INJURY

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Purpose: While a number of secondary injury factors have been identified in traumatic brain injury, cerebral oedema is widely accepted as a potentially fatal complication for which current treatments remain inadequate. Aquaporin (AQP) channels have been identified as a novel target to modulate post-traumatic oedema. The present study used novel AQP4 modulators to characterize the role of AQP4 channels on cerebral oedema in cold lesion-induced CNS injury, widely considered to be an exclusive vasogenic oedema model. **Methods:** Male Sprague-Dawley rats (250 - 300 g) were subject to cortical cold lesion injury and administered either AQP4 agonist (AqpF026; 0.2 mg/kg iv), AQP4 antagonist (AqpB013; 0.8 mg/kg iv) or equal volume saline vehicle 30 minutes post-injury (n=7/group). Brain oedema was maximal at 24h post injury, which was the timepoint used to evaluate outcomes including oedema, BBB permeability and cellular architecture. A subgroup of animals that were neither injured nor treated were included as sham controls. **Results:** Neither the AQP4 agonist nor the AQP4 antagonist reduced oedema at 24 h following cold lesion-induced brain injury. However, the agonist resulted in reduced albumin leakage when compared with the antagonist, to the extent that agonist treated animals were not significantly different from sham animals. **Conclusion:** The non-efficacy of the AQP4 modulators on brain oedema suggests that oedema in cold lesion-induced cortical injury does not involve AQP4 channels. We propose that with open tight junctions, which is typical of cold lesion injury, AQP4 channels may potentially play a minor role in water regulation. Nonetheless, the AQP4 agonist reduced albumin entry, suggesting a potential interplay between albumin and AQP4 activity.

ORAL-08-03

TYPE-1 INTERFERON SIGNALLING PLAYS A KEY ROLE IN THE EXACERBATION OF NEURO-INFLAMMATION FOLLOWING TRAUMATIC BRAIN INJURY

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Purpose: Traumatic brain injury (TBI) represents the major cause of death in young individuals in industrialised countries. TBI triggers acute neuro-inflammation, which exacerbates primary brain damage. We propose that type-1 interferons (IFNs), which signal through the type-1 IFN receptor (IFNAR), are a major driver of the neuro-inflammation in TBI. Previously, we have shown that IFNAR1^{-/-} mice are protected after TBI, and that the administration of an IFNAR1 blocking monoclonal antibody (MAR1) elicits neuro-protection in WT mice following TBI. **Methods:** TBI was induced using a computer-controlled impactor with a 2mm diameter tip delivering an injury above the right parietal cortex of 8-week-old male C57BL/6J wild type (WT) and IFNAR1^{-/-} mice. Brains were excised 2, 4, 6 and 24 hours after TBI for qPCR and immunohistochemistry. **Results:** IFN α mRNA levels were increased 5-fold in WT mice compared to IFNAR1^{-/-} mice 2h after TBI (p<0.05, n=3). In addition, pSTAT3, a downstream mediator of type-1 IFN signalling, was highly expressed in astrocytes of WT, but not IFNAR1^{-/-} mice 6h after TBI (n=3). Surprisingly, immunohistochemistry identified increased Mac-1 (a marker of activated microglia and macrophages) positive cells in IFNAR1^{-/-} mice 24 hours after TBI (n=3). This suggests that abrogation of type-1 IFN signalling alters the response of both resident and peripheral immune cells to TBI. **Conclusion:** This study has revealed type-1 IFN signalling to be a critical factor in shaping the neuro-inflammatory environment following TBI, confirming its crucial role in secondary injury progression.

ORAL-08-04

DEVELOPMENTALLY-LINKED PROTEOMIC CHANGES OF THE SPINAL CORD OF THE *MONODELPHIS DOMESTICA*: RESPONSE TO SPINAL INJURY

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Aim: Spinal cord (SC) injury in the adult results in permanent loss of function as the mature central nervous system (CNS) is generally incapable of neuronal re-growth through the injury site in contrast to the immature CNS, which is capable of considerable neuronal repair. This is partly attributed to the difference in CNS environment as it transitions in development from a permissive to a non-permissive state. The aim of this project is to identify proteins that change during this transition and in response to injury using proteomics, immunoblotting and immunocytochemistry when suitable antibodies are available. **Methods:** SC injury was performed on newborn *Monodelphis* at either P7 or P28 at thoracic level (T)10 under isoflurane anaesthetic as previous studies showed that newborn pups injured in first two weeks of life recover morphologically and functionally but older pups do not (Wheaton et al., 2011). SC segments were divided into upper (rostral to lesion) and lower (caudal to lesion) and collected either 1 day or 7 days post-injury together with samples from age-matched control littermates. Proteomic profiling was performed by isoelectric separation followed by molecular weight separation on pooled samples (n=20 for P7-injured, n=4 for P28-injured). Silver stained gels were analysed by densitometry and protein bands that showed differences (± 0.5) compared to controls (Noor et al., 2011) were subject to mass spectrometry. **Results:** *Monodelphis* SC develops significantly between the ages tested. Mass spectrometry identified 43 unique protein bands in the upper SC and 52 proteins in the lower SC that were developmentally regulated and 124 proteins in the upper SC and 56 proteins in the lower SC that changed in response to injury. So far two proteins (14-3-3 epsilon and ubiquitin) were further validated using western blot, immunocytochemistry and RT-qPCR. **Conclusion:** Several proteins were affected by SC injury in an age-dependent manner. RT-qPCR of ubiquitin and 14-3-3 epsilon showed that regulation occurs at both gene and protein levels. **References:** Noor et al (2011), PLoS One 6(11):e27465. Wheaton et al (2011), PLoS One 6(11): e26826.

ORAL-08-05

MOTOR FUNCTION RECOVERY IN SPINAL CORD INJURED RATS AFTER NANOPARTICLES IMPLANTATION AND ELECTROMAGNETIC FIELD EXPOSURE

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Purpose: The aim of the present study was to observe the effect of magnetic nanoparticle (NP) implantation with magnetic field exposure on the motor function recovery after complete spinal cord transection (SCI). **Methods:** In male Wistar rats complete transection of spinal cord was done at T13 level and divided in 4 groups, 1) SCI, 2) MF, 3) NP and 4) NP+MF. 24 hr after injury, the rats of 2nd and 4th groups were exposed to magnetic field (50 Hz, 17.96µT, 2h/d for 5 wks), whereas, in 3rd and 4th groups, 25 µg/ml superparamagnetic ironoxide NPs solution embedded in 3% agarose gel was implanted at the site of transection. Post-injury locomotor recovery was assessed by BBB locomotor score every week till the 5weeks. At the end of study, rats were sacrificed, spinal cord isolated and processed for histological analysis. **Results:** The BBB score decreased significantly post-injury in all the groups. There was gradual recovery in all the groups but was significant in NP+MF groups in comparison to other groups. Further in the SCI and NP groups at the lesion epicenter, either dense collagenous connective tissue with sieve like structure or a single cystic cavity surrounded by thick layer of collagen was observed in Trichrome stained sections suggesting formation of scar. Though in the MF group, the collagen was rather diffuse and less dense, in NP+MF group it was negligible indicating absence of scar formation. The epicenter in the NP+MF group mostly had cysts, leucocytes, macrophages and glial cells. Few viable neurons were also evident in this group at a distance of 1mm from the epicenter. The total tissue damage volume was also significantly less. **Conclusion:** These observations suggest that NP implantation and MF exposure promotes locomotor recovery and decrease lesion volume and scar formation in SCI rats.

ORAL-08-07

ISOPROTERENOL OR NOREPINEPHRINE TREATMENT MODULATES NEUROGENESIS AFTER AN ENDOTHELIN-1 HIPPOCAMPAL STROKE MODEL IN ADULT MOUSE

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Purpose: The hippocampus is vulnerable in various disease states, including stroke, but pools of activatable hippocampal stem/precursor cells represent novel therapeutic targets to repopulate damaged regions. We hypothesise that activating latent stem cells with norepinephrine following stroke will enhance neurogenesis, which may critically contribute towards improved behavioural outcomes. **Methods:** To model stroke, adult female C57Bl/6 mice received a unilateral intrahippocampal injection of vasoconstrictor Endothelin-1. Stem/precursor cell activation was evaluated using the neurosphere assay. To examine activation *in vivo*, starting 7d post-stroke, animals received daily intraperitoneal injections for 7d of either selective beta-adrenergic receptor-agonist isoproterenol, previously shown to activate hippocampal precursors, or saline, followed by BrdU. Animals were sacrificed 1d or 14d after the final injection (14d or 28d post-stroke), and immunohistochemistry and cell counts conducted. **Results:** Whilst there was a reduction in doublecortin-positive neuron density in the stroke hemisphere of saline-treated animals to 65% and 43% of control hemisphere levels at 14d and 28d respectively (n=6-8/group, p<0.05), we found no associated reduction in precursor cell numbers as stroke and control hemispheres produced similar neurosphere numbers (n=36). Moreover, the stem/precursor cell population was preserved post-stroke because large neurospheres could be generated with norepinephrine-treatment (n=18). While there was no change in BrdU-positive cell density in isoproterenol-treated animals at either timepoint, there was a significant elevation of doublecortin-positive cell density (149%) in the stroke hemisphere compared to saline-treated controls (p<0.05) at 28d, but not 14d. **Conclusion:** This study shows that following hippocampal stroke resident stem/progenitor cells are retained and appear to respond to norepinephrine as evidenced by increased doublecortin-positive cell density. Future experiments will examine the effect of this increased neuronal density on hippocampal function.

ORAL-08-06

DOES OEDEMA DEVELOPMENT CONTRIBUTE TO RAISED INTRATHECAL PRESSURE FOLLOWING TRAUMATIC SPINAL CORD INJURY?

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Purpose: It is well established that blood spinal cord barrier (BSCB) disruption occurs following severe spinal cord injury (SCI), resulting in the development of vasogenic oedema. Such oedema is known to contribute to further tissue damage and ultimately greater functional deficits. Furthermore, increased oedema is thought to result in raised intrathecal pressure, subsequently causing reduced blood flow and further tissue death. However, few studies have clearly demonstrated such an association. Accordingly, this study aims to determine to what extent increased oedema contributes to raised intrathecal pressure following SCI, through the assessment of BSCB permeability, oedema and intrathecal pressure following a balloon compression model of SCI. **Methods:** Adult male New Zealand white rabbits were anesthetized and SCI was induced. Subgroups of animals were assessed for BSCB permeability (Evan's Blue, n=10), oedema (wet weight/dry weight, n=16), intrathecal pressure (n=15) and histological analysis (n=20). **Results:** BSCB permeability was significantly increased at the injury epicentre in all injury groups compared to shams (p<0.001). Spinal cord oedema at the injury epicentre was significantly increased post-injury at all time points (p<0.001). At 3 days post-SCI oedema was maximal and extended rostrocaudally. Significantly increased intrathecal pressure postinjury was observed for the entire 5 hr monitoring period (p<0.01). **Conclusion:** This study demonstrates that BSCB permeability and oedema are associated with increased intrathecal pressure. Whilst initial increases in intrathecal pressure were associated with increased blood volume caused by mechanical disruption, later increases coincided with maximal oedema development, suggestive that oedema indeed contributes to raised intrathecal pressure following SCI. As such, reducing intrathecal pressure following SCI represents a target for future therapeutic interventions.

ORAL-08-08

CELLULAR CHANGES FOLLOWING FOCAL ISCHEMIA OF THE INFANT AND ADULT NONHUMAN PRIMATE PRIMARY VISUAL CORTEX

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Background: Understanding the intrinsic proliferative responses after brain injury, especially in nonhuman primates (NHP), is an important step in elucidating the mechanisms underlying neuroplasticity, repair and regeneration. In this study, we investigated the cell-proliferative response triggered using a novel model of focal ischemia in neonatal (PD14; n=3) and adult (n=3) marmoset monkeys. **Methods:** Endothelin-1 (ET-1) was injected (0.3µL; 1µg/µL) over 6-9 injection sites surrounding calcarine branch of the posterior cerebral artery. Animals were subsequently pulsed with the thymidine analogues (TA) 5-Iodo-2'-deoxyuridine (IdU; 57.5mg/kg; days 1-3 post-injury) and 5-chloro-2'-deoxyuridine (CldU; 42.75mg/kg; days 7-9 post-injury) and transcardially perfused after 3-weeks. Proliferative responses were investigated using histological and immunohistochemical procedures. **Results:** Analysis of Nissl-stained sections revealed that the ischemic zone consistently encompassed only operculum V1. IdU/CldU immunohistochemistry revealed larger populations of CldU+ versus IdU+ cells present at both ages indicating greater cell-proliferation rates during the second week post-injury compared to controls, with highest density proximal to the lesion core. The wide-spread distribution patterns of IdU/CldU+ cells in neonates suggests that cells may have been generated distal to the injury site, subsequently migrating into the penumbra versus the focal distribution in adults indicating local proliferative responses. At both ages, IdU/CldU+ cells in the penumbra were largely Olig2+ in neonates (indicating oligodendroglia/neuronal lineage potentials) and GFAP+ in adults. Small population of CldU+/Sox2+ cells were present in the neonatal and adult penumbra confirming the generation of neuronal progenitors one week post-injury. **Conclusion:** Our results demonstrate that cellular proliferation and potential neurogenesis occurs in response to cortical ischemia in both neonatal and adult marmosets. Understanding the survivability and fates of these cells, especially in NHP models may provide translational, clinically relevant insights into the capacity for repair in humans following brain injury.

ORAL-09-01

14-3-3ZETA MAINTAINS PHOSPHORYLATION OF NDEL1 TO CONTROL NEURAL MIGRATIONSaleh E.O.^{1,2}, Ramshaw H.¹, Xu X.¹, Lopez A.F.¹ and Schwarz Q.¹¹Center for Cancer Biology, SA Pathology, Adelaide, South Australia.²School of Medicine, The University of Adelaide, Adelaide, South Australia.

Neuropsychiatric disorders such as schizophrenia are likely caused by a large number of genes with a small effect. Primarily they have complex traits believed to arise from multiple deficiencies within connected biological networks controlling neuronal migration, axonal pathfinding and synapse formation. Our lab have recently shown that 14-3-3zeta mouse mutants display deficits reminiscent to schizophrenic patients such as severe capacity to learn and remember, hyperactivity and disrupted sensorimotor gating. Developmental abnormalities of the hippocampus were also observed which rose from aberrant neuronal migration and synapse formation. I have shown that phosphorylated Ndel1 levels are reduced in the 14-3-3zeta mouse mutants and thus propose that 14-3-3zeta binds CDK5 phosphorylated Ndel1 to promote interaction with LIS1 and thereby promote neuronal migration. To test this model I have developed an in vitro migration assay with neural stem cells that recapitulates the in vivo finding. This provides an ideal model system to dissect the molecular mechanism in the neural migration pathway. Consistent with my finding that Ndel1 is aberrantly phosphorylated in 14-3-3zeta mutants, the in vitro migration assay revealed severe migration defects in the 14-3-3ζ deficient neurons compared to wild-type. These findings impersonate the LIS1 and NDEL1 deficient neurons which displayed severe and mild migration defects, respectively and thereby supports our proposed model. Furthermore, I have also found that the nuclear-centrosome coupling during migration is perturbed in vitro. This suggests that defects in coupling may contribute to migration defects in the 14-3-3zeta mutants. Thus, we concluded that 14-3-3zeta do indeed play a role in neuronal migration by sustaining NDEL1 phosphorylation.

ORAL-09-03

ADULT HIPPOCAMPAL NEUROGENESIS, RHO KINASE INHIBITION AND ENHANCEMENT OF NEURONAL SURVIVAL

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Adult neurogenesis occurs throughout life; however the majority of these new neurons do not survive. Enhancing survival of these new neurons will increase the likelihood that these neurons could return function following injury. Inhibition of Rho kinase is known to increase neurite outgrowth and regeneration. Previous work in our lab has demonstrated a role for Rho kinase inhibition and survival of new born neurons from the sub-ventricular zone. **Purpose:** to determine the role of Rho kinase inhibition on hippocampal neurogenesis. **Methods:** Rho kinase inhibitor, Y27632 (20 μM and 100 μM), and proliferative marker EdU were infused in the lateral ventricle via a cannula connected to a mini-osmotic pump on the back of the mouse for 7 days (n=5 per condition). Animals were perfused 8d or 35d following surgery. Immunohistochemistry for NeuN, GFAP, Dcx and EdU were performed. **Results:** Doublecortin and EdU positive cell numbers were not significantly different following 8 days, suggesting no effect on neuroblast generation or proliferation. Following infusion of 100 μM of Y27632, the number of newborn NeuN+EdU neurons at 35 days in the granular cell layer of the dentate gyrus of the ipsilateral side of the infusion did not display a significant difference, however there was an increase on the contralateral side, suggesting a dose effect. Infusion of a lower dose (20 μM) Y27632 resulted in an increase in NeuN+EdU cells in the granular cell layer of the ipsilateral side following 35 days. Furthermore, examination of the rostral migratory stream revealed larger areas of DAPI and GFAP along with higher numbers of EdU+ cells. **Conclusion:** Rho kinase inhibition enhances the survival of new born neurons in the dentate gyrus with a specific dosage effect. These results suggest that inhibition of Rho kinase following injury could be beneficial for increasing the survival of new neurons that may aid recovery.

ORAL-09-02

PHENOTYPICALLY DISTINCT NEURAL PRECURSOR CELL POPULATIONS GENERATE PROGENY THAT HAVE DISCRETE TRANSCRIPTIONAL PROFILES IN THE ADULT HIPPOCAMPUS

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Purpose: Studies from our laboratory have uncovered the presence of two discrete populations of quiescent neural precursor cells that can be activated by either neuronal depolarisation (high K⁺) or neurotransmitter norepinephrine (NE). In this study, we sought to determine the phenotypic identity of these distinct precursor populations and investigated the molecular properties of their progeny. **Methods:** We used the neurosphere assay together with specific transgenic mouse lines and flow cytometry to identify and isolate NE- and K⁺- responsive stem and precursor cell populations. Using next-generation sequencing we conducted transcriptome profiling of the neural precursor cell progeny. **Results:** Stimulation of Hes5-GFP-positive cells with NE resulted in a significant increase in the neurosphere activity (NE: 216.9±42.5%, n=4, p<0.05) with appearance of very large neurospheres (>200 μm in diameter) suggesting stem cell activation. In contrast, K⁺ response was indirect and required the presence of Hes5-GFP-negative cells. Interestingly, stimulation of Nestin-GFP-positive cells with either NE or K⁺ led to a significant increase in the neurosphere activity (n=9, p<0.05). Furthermore, we conducted a screen using monoclonal antibodies to 87 characterised cell surface antigens and found a further enrichment for NE- versus K⁺-responsive populations. Transcriptome analysis revealed 442 genes that are expressed at significantly different levels between the progeny derived from NE- versus K⁺-activated stem cells (n=4 biological replicates, p<0.05) and have identified specific ion channels, neurotransmitter receptors and transcription factors that may constitute 'signature' molecular profile for each of the populations. **Conclusion:** Collectively, these results suggest that phenotypically distinct populations of neural precursor cells generate molecularly discrete progeny that may differentially contribute towards various hippocampal-dependent functions.

ORAL-09-04

NEURON-SPECIFIC ALTERNATIVE SPLICING IS A MECHANISM FOR INCREASING THE DIVERSITY OF BRAIN WIRING PROTEINS

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Purpose: To determine whether the two alternative isoforms of the *Drosophila Dscam2* gene are expressed in a neuron-specific manner. Down syndrome cell adhesion molecule 2 (Dscam2) is a functionally conserved transmembrane protein expressed on the surface of neurons. Dscam2 mediates self (homophilic) binding between two opposing membranes; this binding event induces repulsion. During *Drosophila* visual system development, two different neurons, L1 and L2, require Dscam2 homophilic repulsion for forming boundaries between repeated structures in the brain and for specifying photoreceptor synapses. However, these two neurons physically contact each other within the same nerve fibre. Why then, are L1 and L2 not repelled from each other? Given that *Dscam2* encodes two alternative isoforms that have unique binding specificities, we hypothesise that L1 and L2 express distinct Dscam2 isoforms. **Methods:** We engineered the endogenous *Dscam2* locus to express the Gal4 transcription factor in an isoform-dependent manner. Cells expressing the different isoforms were visualised by crossing these strains to responder lines harbouring UAS-fluorescent protein transgenes. L1 and L2 were identified based on their distinct axonal morphologies in the fly visual system. **Results:** We found exclusive isoform expression in these two neurons; L1 cells express Dscam2 isoform B (L1 vs L2; n=97 vs 0), whereas L2 cells express isoform A (L1 vs L2; n=0 vs 85). **Conclusion:** These data suggest that neuron-specific alternative splicing is a mechanism for increasing the diversity of cell recognition molecules in the brain. Regulation of functionally distinct alternative isoforms may allow for broadly expressed wiring proteins to act specifically in subsets of neurons.

ORAL-09-05

NPAS4 REGULATES THE EXPRESSION OF SOX1 IN AN EARLY NEURAL PROGENITOR CELL POPULATION DURING NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Purpose: In the adult organism, the activity-dependent transcription factor Npas4 plays an important role in several key aspects of neurobiology including inhibitory synapse formation, neuroprotection and memory. However, little is known regarding the role of Npas4 during neurodevelopment. In this study, we used neural differentiation of embryonic stem cells (ESCs) as a model to investigate the expression and function of Npas4 during early development. **Methods:** Noggin-induced differentiation of human ESCs and differentiation of mouse ESCs as an adherent monolayer were used as *in vitro* models of early embryonic development. **Results:** We found that while Npas4 is not expressed by undifferentiated ESCs, it becomes transiently up regulated during neural differentiation of both mouse and human ESCs (n=3) at a stage of differentiation that is characterised by proliferation of neural progenitor cells (NPCs). Knock down of Npas4 expression in mouse ESCs undergoing neural differentiation (n>3) resulted in a decrease in the percentage of cells expressing the neuroectoderm marker Sox1, though expression of Nestin, a marker of more mature NPCs, was not affected. **Conclusion:** Our findings suggest that Npas4 acts upstream of Sox1 during neural differentiation and further suggest a potential role for Npas4 in aspects of NPC maintenance and/or differentiation. Here we provide the first evidence that Npas4 is expressed during development by a population of early NPCs and that it may have a developmental role that is unrelated to its function in the adult brain.

ORAL-09-07

ENRICHMENT ACCELERATES PNN FORMATION IN THE HIPPOCAMPUS

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PURPOSE: Environmental enrichment (EE) has been shown to improve sensory, motor, and cognitive performance, as well as accelerate the timing of 'critical periods'. The closure of these developmental epochs has been associated with the formation of perineuronal nets (PNNs) around parvalbumin positive (PV+) inhibitory interneurons. Although previous work has revealed that PNNs first appear postnatally within the hippocampus, the manner in which they form within this important brain area (critical for memory formation) is not known. Further, how environmental factors affect the formation of these structures within the hippocampus has yet to be determined. **METHODS:** Brains from P10, P15 and P21 C57BL6J pups raised in either enriched (EE) or standard environments (SE) from birth, were sectioned and processed for WFA (a lectin that binds to PNNs) and/or parvalbumin (PV+) antibody labeling within the hippocampus. Images were acquired using confocal fluorescence microscopy, and the number and location of both PNNs and PV+ cells were assessed using Neurolucida. **RESULTS:** Preliminary comparison of dorsal hippocampus has revealed that the number of both PNNs as well as PV+ cells are significantly greater in EE (n=3) compared to SE (n=4) raised pups at P10 (PNN: EE (mean + sem): 23.66667 ± 6.437736; SE: 3.25 ± 1.600781; p = 0.008; and PV+: EE: 16 ± 2.081666; SE: 1.25 ± 0.629153; p < 0.001). **CONCLUSION:** Our preliminary analysis suggests that EE accelerates both the formation of PNNs as well as the expression of PV within the hippocampus. This is consistent with the precocious maturation of inhibitory networks associated with the closing of critical periods observed within cortical and subcortical areas in enriched animals.

ORAL-09-06

EXAMINING THE ROLE OF STIM PROTEINS IN THE DEVELOPING NERVOUS SYSTEM

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The regulation of cytosolic calcium levels is essential for neuronal function. A key source of calcium within neurons is the endoplasmic reticulum (ER), the regulation of which, is poorly understood. The stromal interaction molecules (STIM1 and STIM2) are calcium-sensing proteins located in the ER membrane. STIM proteins interact with Orai proteins on the plasma membrane to initiate calcium influx and refill depleted intracellular calcium stores within the ER. We have demonstrated that STIM1 mediated store-operated calcium entry (SOCE) is necessary for growth cone chemotactic responses to the extracellular guidance cues BDNF and Sema-3a¹. We now show that turning towards Netrin-1 also requires STIM1 expression ($-6.2 \pm 1.5^\circ$; $p < 0.0001$). Chemorepulsion from a microgradient of Sema-3a can be rescued by cAMP activation ($-12.18 \pm 4.36^\circ$) or cGMP activation ($-6.0 \pm 1.8^\circ$) thereby demonstrating that STIM1 interacts with cyclic nucleotides as well as orchestrating SOCE within neurons. Given that STIM1 is required for normal growth cone function, we asked whether STIM2 functions in a similar manner. STIM2 has been described as the "neuronal isoform" of the STIM family and there has been much debate over the role of STIM2 in SOCE regulation. Within the embryonic and adult nervous system, there is ubiquitous distribution of STIM1. STIM2 expression however, is restricted to radial glia, and expression levels steadily decline during postnatal development. Our data demonstrate that STIM1 and STIM2 play discrete cellular-specific roles within the developing nervous system. 1. Mitchell et al., 2012, J. Neurochem, 122: 1155-1166.

ORAL-09-08

THE ROLE OF INNERVATION IN DEVELOPMENT OF LIMB MUSCLE PROGENITORS

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The relationship between nerve and muscle is important both embryonically and throughout postnatal life. We used the rat as an animal model to investigate the effect of chemical denervation *in utero* on the development of muscle progenitor cells expressing the paired homeobox genes Pax3 and Pax7 - which are early markers of the myogenic lineage. In normal embryos, immunohistochemical examination showed that Pax3^{ve} progenitors were first seen in the hindlimb at embryonic day (E) 12.5, followed by Pax7^{ve} progenitors one day later at E13.5. Limb innervation was first observed at the same time as entry of Pax7^{ve} progenitors, with nerve and Pax7^{ve} progenitors closely co-localised. *In utero* denervation was achieved through intraperitoneal injection of β -bungarotoxin into embryos at either E15.5 or E16.5, followed by immunohistochemical examination of extensor digitorum longus (EDL) and tibialis anterior (TA) muscles either 24h or 48h later (n=6 for all groups). Following 24h of denervation, we saw a significant decrease in the number of Pax7^{ve} progenitors in denervated muscles compared to controls ($P < 0.05$). Concurrently, there was a significant increase in the number of cells expressing myogenin, a marker of muscle differentiation ($P < 0.05$) in both muscles. Increased apoptosis of Pax7^{ve} progenitors as assessed by active caspase-3 labeling was seen in the EDL alone ($P < 0.05$). Quantitative PCR corroborated these findings, with many genes associated with differentiation and apoptosis being upregulated, and genes associated with proliferation and cell cycle regulation being downregulated following denervation. From these results, we conclude that differentiation and survival of Pax7^{ve} myogenic progenitors are critically regulated by developmental interactions with the muscle nerve.

ORAL-10-01

REDUCED DORSAL HORN NEURON ACTIVATION FOLLOWING INTRA-COLONIC ICILIN ADMINISTRATION

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Purpose: Nociceptive signalling via colonic afferents is an important target in the management of visceral pain. Icilin, an agonist of TRPM8, reduces the mechanosensitivity of colonic nociceptive afferent endings *in vitro*. (1) How this peripheral inhibition correlates with altered signalling in the spinal cord remains to be shown. We aimed to determine if intra-colonic icilin altered the responsiveness of dorsal horn (DH) neurons to noxious colorectal distension (CRD). **Methods:** Fluorescent retrograde tracer was injected into the colon of mice seven days before CRD (80mmHg). Two minutes prior to CRD, mice received an enema of icilin (5µM in 0.1M PBS; N=3) or no enema (N=3). CRD was followed by perfuse fixation, removal of thoracolumbar (T10-L1) and lumbosacral (L6-S1) spinal cord and processing for immunohistochemical detection of the neuronal activation marker, phosphorylated MAP kinase ERK 1/2 (pERK). The number of pERK-immunoreactive (IR) DH neurons was averaged from 20 sections/spinal segment/animal and compared using unpaired student t-tests. **Results:** Following noxious CRD, pERK-IR neurons were present in the superficial DH laminae I, III, and deeper lamina V of the TL spinal cord and laminae I-III and lamina V in the LS spinal cord. Pre-treatment with icilin resulted in a significant reduction in the average number of pERK-IR DH neurons in spinal segments T11-T12 ($p=0.0027$), T13-L1 ($p=0.0214$) and L6-S1 ($p=0.016$). There was no significant change in T9-T10. **Conclusion:** Peripheral administration of the TRPM8 agonist icilin significantly reduces the number of DH neurons activated by noxious CRD, correlating with the action of icilin on colonic nociceptor peripheral endings. These data indicates that reducing colonic nociceptor mechanosensitivity in the periphery correspondingly modulates nociceptive signalling into the spinal cord. (1) Pain (2011)152:1459-68.

ORAL-10-02

EXPRESSION OF THE GHRELIN RECEPTOR GENE IN NEURONS OF THE MEDULLA OBLONGATA OF THE RAT

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Reports of the distribution of neurons that express the ghrelin receptor (GHSR) in the medulla oblongata are ambiguous. We have used a sensitive non-radioactive method to investigate GHSR mRNA distribution in rat medulla (n=12) by *in situ* hybridisation. Strong expression of the GHSR gene was confirmed in neurons of the facial nucleus (FacN, 7), the dorsal vagal complex (DVC) and the semi-compact (but not the compact) nucleus ambiguus (AmbSC and AmbC). In addition, expression of GHSR was found in other regions, where it had not been described before. GHSR-positive neurons were observed in the gustatory rostral nucleus tractus solitarius (rNTS) and in areas involved in vestibulo-ocular processing (such as the medial vestibular nucleus and the nucleus abducens). GHSR expression was also noted in ventral areas associated with cardio-respiratory control, including the gigantocellular reticular nucleus, the lateral paragigantocellular nucleus, the rostral and caudal ventrolateral medulla, the (pre)-Bötzing complex and the rostral and caudal ventrolateral respiratory group. However, GHSR-positive neurons in ventrolateral areas did not express markers for cardiovascular presympathetic vasomotor neurons, respiratory propriobulbar rhythmogenic neurons or sensory interneurons. GHSR-positive cells intermingled with catecholamine neurons in the dorsal vagal complex but these populations did not overlap. Thus, the ghrelin receptor occurs in the medulla oblongata in i) second order sensory neurons processing gustatory, vestibulo-ocular and visceral sensation, ii) cholinergic somatomotor neurons of the FacN and autonomic preganglionic neurons of the DMNX and AmbSC, iii) cardiovascular neurons in the DVC, Gi and LPGi, iv) neurons of as yet unknown function in the ventrolateral medulla.

ORAL-10-03

NATURAL STIMULI EVOKE CO-ORDINATED ACTIVATION OF SYMPATHETIC, CARDIAC, RESPIRATORY AND MOTOR OUTPUTS AFTER DISINHIBITION OF NEURONS IN THE INFERIOR AND SUPERIOR COLLICULI

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The superior (SC) and inferior (IC) colliculi are involved in the behavioural and cardiovascular responses to threatening stimuli. Previously, we observed that disinhibition of neurons in this area evoked highly synchronised bursts of sympathetic and respiratory activity. **Purpose:** We examined whether the response also generated somatomotor activity. Furthermore we tested whether naturalistic stimuli, following disinhibition of the colliculus, could generate the coordinated sympathetic, respiratory and motor response. **Methods:** In urethane-anaesthetised, pancuronium paralysed and artificially ventilated rats, microinjections of picrotoxin (100 pmol/ 50 nl) into ventral sites of the SC and IC evoked the same pattern of response observed in our previous work. **Results:** Abrupt sound (n=7) or bright light (n=5) stimuli generated coordinated bursts of activity in sympathetic (Δ sSNA 309 ± 30 vs. 217 ± 38), respiratory (Δ PNA 60 ± 18 vs. 43 ± 15), somatomotor activity (Δ sciatic 17 ± 4 vs. 15 ± 3) and blood pressure (Δ BP 30 ± 4 vs. 31 ± 8). Simultaneous stimulation of more than one type of sensory input evoked an enhanced response. Naturalistic stimuli had no effect when injections of picrotoxin were made into sites 0.5 mm away from these 'hot spots' in the colliculi. **Conclusion:** Neurons in the SC and IC can generate co-ordinated behavioural responses, cardiovascular and respiratory changes that are appropriate for a defensive behaviour triggered by a sudden threatening stimulus. Activation of sensory inputs evokes an alerting response only after blockade of inhibitory input into a discrete region of the colliculus.

ORAL-10-04

BLOCKADE OF EPITHELIAL SODIUM CHANNELS (ENAC) REDUCES PRESSURE-INDUCED PERISTALSIS IN GUINEA PIG INTESTINE

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Purpose: Mechanical stimulation of the gastrointestinal epithelium causes release of 5-hydroxytryptophan (5-HT), which we have shown is reduced in the presence of non-selective acid-sensing ion channel (ASIC) blockers amiloride and benzamil, but not the ENaC blocker triamterene. Luminal 5-HT is known to modulate enteric reflexes including peristalsis, therefore, the aim of this study was to investigate the effects of ASIC and ENaC blockers on pressure-initiated peristalsis. **Methods:** Short (5cm) segments of ileum from guinea pigs (554±65g) were cannulated and the oral end was connected to a reservoir used to increase intraluminal pressure. Amiloride (300µM) and benzamil (100µM), at concentrations sufficient to also block ENaC, or triamterene (30µM) were added to the lumen. The pressure at which four successive peristaltic contractions occurred (peristaltic threshold) and their time course (e.g., interval between contractions) were recorded with a pressure transducer (anal) and compared with a paired t-test. **Results:** Amiloride (n=7) had no significant effect on peristaltic threshold (control: 19 ± 2 mmHg versus amiloride: 24 ± 4 mmHg) or amplitude (14.4 ± 1.3 mmHg versus 13.5 ± 0.8 mmHg), but significantly increased the interval (18.9 ± 2.0 s versus 22.0 ± 2.1 s, $P < 0.05$). Similarly, benzamil (n=6) did not significantly alter threshold (21 ± 1 mmHg versus 26 ± 3 mmHg) or amplitude (20.7 ± 1.4 mmHg versus 20.2 ± 1.2 mmHg), but increased the interval (17.3 ± 2.1 s versus 28.0 ± 4.9 s, $P < 0.05$). Triamterene (n=5) likewise increased the interval (12.2 ± 1.2 s versus 14.7 ± 1.9 s, $P < 0.05$); however, it also significantly increased amplitude (12.0 ± 1.3 mmHg versus 13.4 ± 1.2 mmHg, $P < 0.05$) and raised peristaltic threshold (25 ± 3 mmHg versus 33 ± 3 mmHg, $P < 0.05$). **Conclusion:** Blockade of ENaCs in the ileal lumen caused an increased peristaltic threshold and an increased interval between contractions. Any subtle influence of ASIC specific blockade on peristaltic parameters may have been masked by the large changes seen during ENaC blockade.

ORAL-10-05

ACTIVITY OF ENTERIC MOTOR NEURONS DEDUCED FROM THEIR MECHANICAL CONSEQUENCES IN ISOLATED RABBIT COLON

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Purpose: The activity of excitatory and inhibitory enteric motor neurons in intact segments of intestine during motor activity has not yet been recorded. We have developed a strategy to deduce the activation of the enteric motor neurons from the biomechanics of the intestine. **Methods:** Motor activity was studied in isolated segments of distal colon taken from rabbits, placed in a bath of oxygenated Krebs solution at 37°C. We constructed combined maps of diameter and pressure changes (DPMs) and developed tools to extract from these composite maps, biomechanical parameters. The analysis was based on the assumption that active contractions can only be generated by pacemaker activity (myogenic activity) or by activity of enteric excitatory motor neurons. Similarly active relaxation can only be generated by the activity of enteric inhibitory neurons. **Results:** Distension of distal colon elicited myogenic and neurogenic motor patterns (1). Plotting diameters versus corresponding intraluminal pressures generated orbits with linear segments that corresponded to six states of isotonic, auxotonic and isometric contractions and relaxations. Passive dilations and contractions and quiescent states could be identified. Spatiotemporal maps of biomechanical states of the intestinal wall were plotted resulting in a tapestry of motor patterns. These maps were simplified by plotting mechanical states of the intestine that result solely from active contractions and relaxations. **Conclusions:** Mapping mechanical states of the intestine permits quantitative analysis of neural peristaltic contractions and rhythmic myogenic activity. **References:** (1) Dinning PG, *et al.* Am J Physiol Gastrointest Liver Physiol. 2012; 303: G83-G92.

ORAL-10-07

A COMPUTATIONAL MODEL OF INTRINSIC SENSORY NEURONS OF THE GUT

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Purpose: Intrinsic sensory neurons (ISNs) of the enteric nervous system respond to stimuli such as muscle contractions, distortion of the mucosa and chemical changes in the lumen. ISNs form a recurrent network that probably drives many intestinal motor patterns and reflexes. ISNs express a large number of voltage and calcium gated ion channels. However, it remains unclear how the interactions between the different ionic currents can produce both normal and pathological behaviours. **Methods:** We constructed a detailed computer model of ISNs which includes voltage-gated sodium and potassium channels, an N-type calcium channel, a big conductance potassium (BK) channel, a calcium dependent non-specific cation channel (CAN), intermediate conductance potassium (IK) channel, hyperpolarisation activated cation (I_h) channels and internal calcium dynamics. The model was based on data from the literature and our electrophysiological studies. **Results:** The model reproduced the physiological observations of firing in response to multiple current pulses (250pA 10ms duration at 50Hz, n=8) or prolonged depolarising current pulses (50-350pA for 500ms), and responses to prolonged hyperpolarising current pulses. A sensitivity analysis for each conductance showed that I_h, IK, CAN and BK had the largest influence on the number of action potentials observed during a prolonged depolarisation. The model also predicts that changes to the voltage of activation for I_h has a large influence on the number of action potentials, but that changes to the time constant of activation for I_h have a minor effect. **Conclusions:** Our model identifies how interactions between different ionic currents can influence the excitability of ISNs and indicates an important role for I_h in disease states.

ORAL-10-06

A HUMAN MODEL FOR UNDERSTANDING SMALL BOWEL MOTILITY

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Background: At the human ileocolonic junction the small bowel empties into the caecum in a controlled, pulsatile manner. However, the relative inaccessibility of the region means that it remains the least studied region of the entire human digestive tract. In vivo manometric and myoelectric studies provide some descriptive information, but ethical constraints limit the intervention that helps to determine the myogenic and neurogenic input. **Aim:** To devise an in vitro, human small bowel experimental model utilising the ileocolonic junction removed from patients undergoing right hemicolectomies. **Methods:** Motor patterns of the terminal ileum were recorded with a high-resolution fibre optic manometry catheter (sensor spaced at 1cm intervals). Recordings were either intraluminal (n=4) or via clips attached extraluminally to the ileal serosa at close proximity (10mm spacing, n=5) and connected to individual sensors on the fibre-optic catheter. Data is collected over 1 hour. For intraluminal recording concurrent video recordings were made of changes in gut diameter. **Results:** Repetitive contractions are seen throughout the experiments in both intraluminal and extraluminal experiments, measuring 5.2 +/- 0.7 cycles per minute (N=9). Phasic contractions were present and consistent throughout the hour-long recording. In 1 intraluminal experiment clear propagating activity was recorded by video and the corresponding diameter maps matched the propagating activity recorded by the fibre-optic catheter. **Discussion:** Both cyclic and propagating activity can be captured from our small bowel recordings. Fibre-optic technology can be used to capture motility patterns of isolated human small intestine.

ORAL-10-08

ATAxin-2 INTERACTS WITH FUS AND INTERMEDIATE-LENGTH POLYGLUTAMINE EXPANSIONS ENHANCE FUS-RELATED PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the degeneration and death of motor neurons. Over 30 mutations have been described in the fused in sarcoma (FUS) gene, causing approximately 5% of FALS and 1% of SALS. The mechanisms underlying neurodegeneration are not fully understood. Ataxin-2 is a polyglutamine protein which normally contains 22 repeats, recently ataxin-2 with intermediate length repeats (27-33) was shown to increase the risk of ALS. **Purpose:** To investigate the role of intermediate ataxin-2 with FUS pathology, and to elucidate the effect of the intermediate-length CAG repeats on NSC-34 neuronal cell line, expressing wild type and mutant FUS proteins. **Methods:** NSC-34 cell line was transiently co-transfected with wildtype or mutant FUS proteins, and ataxin-2 Q22/Q31 constructs. Activation of ER stress and fragmentation of the Golgi apparatus were assayed by immunocytochemistry. Distribution of FUS and ataxin was examined by immunohistochemistry in post-mortem spinal cord tissues from sporadic ALS, mutant FUS-linked familial ALS, and control patients without any neurological disorders, and immunoprecipitation was performed to detect FUS and ataxin 2 interaction. **Results:** We show that ataxin-2 with an ALS-linked intermediate length repeat (Q31) is a potent modifier of FUS pathology in cellular disease models. Translocation of FUS to the cytoplasm and ER stress were significantly enhanced by co-expression of mutant FUS with ataxin-2 Q31. Ataxin-2 also co-localized with FUS in sporadic and FUS-linked familial ALS patient motor neurons, co-precipitated with FUS in ALS spinal cord lysates, and co-localized with FUS in the ER-Golgi compartments in neuronal cell lines. Fragmentation of the Golgi apparatus is linked to neurodegeneration in ALS and here we show that Golgi fragmentation is induced in cells expressing mutant FUS. Moreover, Golgi fragmentation was enhanced, and the early stages of apoptosis were triggered, when ataxin-2 Q31 was co-expressed with mutant FUS. **Conclusion:** These findings describe new cellular mechanisms linking ALS with ataxin-2 intermediate length poly Q expansions and provide further evidence linking disruption to ER-Golgi compartments and FUS pathology in ALS.

ORAL-11-01

SEX DIFFERENCES IN REELIN/BDNF INTERACTIONS IN THE VENTRAL HIPPOCAMPUS

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Purpose: Schizophrenia is a neurodevelopmental disorder most likely caused by a combination of genetic and environmental factors. Reduced expression of the growth factors, Reelin and Brain-Derived Neurotrophic factor (BDNF), have both been associated with schizophrenia in human post-mortem studies. However, it is unclear how Reelin and BDNF interact and whether this is sex-specific. **Methods:** We used Western blot to investigate BDNF-TrkB signalling in the hippocampus of male and female Reelin heterozygous ($Rln^{+/-}$) mice and the effects of gonadectomy ($n=6-10$ /group). **Results:** BDNF protein expression was significantly higher in the ventral hippocampus (VHP) of female $Rln^{+/-}$ compared to wildtype (WT) controls while expression of both truncated and full length TrkB was unchanged. However, phosphorylated TrkB (pTrkB) levels were significantly lower in the VHP in female $Rln^{+/-}$ compared to WT, and this translated to downstream effects with a significant decrease in phosphorylated ERK1 (pERK1). Ovariectomy had no effect on BDNF expression in the VHP of WT controls, but caused a significant decrease in $Rln^{+/-}$ mice. Ovariectomy reduced TrkB and ERK1 phosphorylation in the VHP of WT but not $Rln^{+/-}$ mice, where levels were already reduced. Interestingly, estradiol replacement had no effect on restoring BDNF-TrkB expression and downstream signalling, indicating a role for other ovarian hormones such as progesterone. No changes in BDNF-TrkB signalling were observed in the dorsal hippocampus or in male $Rln^{+/-}$ mice. **Conclusion:** These data demonstrate important gene x gene x sex interactions that are specific to the VHP. This study may provide a better understanding of the molecular biology which underlies stress and anxiety-related behaviours which involve ventral hippocampus function.

ORAL-11-02

A COGNITIVE NEUROME: NEUROPSYCHIATRIC DISORDERS REPRESENT AN INTERCONNECTED MOLECULAR SYSTEM

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Purpose: Many putative genetic factors that confer risk to cognitive disorders such as autism spectrum disorders (ASDs) and X-linked intellectual disability (XLID), and to neuropsychiatric disorders including attention deficit hyperactivity disorder (ADHD) and schizophrenia have been identified in individuals from diverse human populations. Although there is significant aetiological heterogeneity within and between these conditions recent data show that genetic factors contribute to their co-morbidity. Although many studies have identified candidate gene associations for these disorders, this is often done in a piecemeal fashion with little regard to the inherent molecular complexity. Here we sought to abstract relationships from our knowledge of systems level biology to help understand the unique and common genetic drivers of these conditions. **Methods:** We undertook a global and systematic molecular systems approach to build and integrate available data in gene networks associated with ASDs, XLID, ADHD and schizophrenia. Complex network concepts and computational methods were used to investigate if candidate genes associated with these conditions were related through mechanisms of gene regulation, functional protein-protein interactions, transcription factor and microRNA (miRNA) binding sites. **Results:** Our analyses show that genetic variations associated with the four disorders are more likely to occur in the same molecular pathways and functional domains, including synaptic transmission, cell-cell communication and transcriptional regulation. Of particular interest are DNA variations located in intergenic regions that comprise regulatory sites for transcription factors or microRNAs. **Conclusion:** Our approach provides a hypothetical framework, a proposed 'cognitive neurome', which will help discovery and analysis of candidate genes associated with cognitive and neuropsychiatric disorder.

ORAL-11-03

TIME-DEPENDENT GHRELINERGIC SIGNALING AND HYPERPHAGIA INDUCED BY OLANZAPINE

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Olanzapine is widely used to treat schizophrenia and other mental diseases. Interestingly, although increased body weight is evident throughout the course of olanzapine treatment, elevated food intake only appears in relatively shorter term. Olanzapine can modulate ghrelin levels, which may upregulate neuropeptide Y (NPY) secretion at hypothalamus. Brain-specific homeobox transcription factor (BSX) may also be involved in ghrelin-induced hyperphagia. **PURPOSE:** To investigate the effect of olanzapine on central NPY, BSX and plasma ghrelin levels, and their relationship to food intake. **METHODS:** Rats were treated with olanzapine (1mg/kg, orally, 3x/day, $n=12$ /group) or vehicle for 1 week, 2 weeks, or 5 weeks. Daily food intake was measured on the final day of treatment for further analysis. Postmortem blood and brain samples were collected for measurement of plasma ghrelin (EIA), hypothalamic NPY protein (EIA) and BSX mRNA levels (real-time PCR) ($n>5$ /group). **RESULTS:** Olanzapine increased daily food intake ($p<0.001$) and ghrelin ($p<0.01$) after 1-week, but not 2-week ($p=0.052$ for ghrelin) or 5-week treatment. Hypothalamic NPY protein increased after 1-week ($p<0.001$) and 2-week ($p<0.05$), but not 5-week olanzapine treatment. Hypothalamic BSX mRNA was increased by 3.6 folds ($p<0.001$) after 1 week, but not after 2 weeks or 5 weeks. NPY positively correlated to ghrelin levels after 1 week ($r=0.943$, $p<0.001$). Ghrelin levels positively correlated to final daily food intake at all times ($r=0.702$, $p<0.001$) and 1 week post treatment ($r=0.801$, $p<0.01$). **CONCLUSION:** Olanzapine increased ghrelin secretion in a time-dependent manner, which may explain its time-dependent hyperphagic effect. Olanzapine also increased hypothalamic NPY expression time-dependently, possibly through elevated plasma ghrelin and hypothalamic BSX levels.

ORAL-11-04

ADVANCES IN MODELING AN ENDOPHENOTYPE OF SCHIZOPHRENIA IN RODENTS: MISMATCH RESPONSES TO FREQUENCY DEVIANTS IN AWAKE, FREELY-MOVING RATS

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Purpose: Reductions in the amplitude of mismatch negativity (MMN) are one of the most robust neurophysiological endophenotypes observed in patients with schizophrenia. The purpose of this study was to develop a rat model of MMN to facilitate future investigations of the underlying neurobiology and pharmacology of MMN. **Methods:** EEG data were recorded from a surgically-implanted electrode above the right auditory cortex of wistar rats ($n=9$), and was referenced to another electrode over contralateral cerebellum. The rats were presented with auditory stimuli including an ascending oddball sequence, where a train of low frequency 'standard' stimuli was interrupted by a high frequency 'deviant', and a descending oddball sequence of high frequency standards interrupted by low frequency deviants. Auditory event-related potentials (ERP) were produced for each of the different stimulus types. **Results:** Three components of ERP were identified: a negative component from 16-31ms, a positive component from 33-48ms, and a long-lasting component from 60-100ms. Evidence of deviance-detection (a larger response to the deviant compared to the standard) was observed in all components for high frequency, but not low frequency deviants. **Conclusions:** These findings replicate previously published data from our lab (Nakamura et al, 2011), in which mismatch responses were observed in awake rats in response to high frequency, but not low frequency deviants. Together with the previously published data, these data demonstrate that robust mismatch responses can be observed in the awake rat, enabling future investigations of the MMN in a rat model of an environmental risk factor for schizophrenia, and will also focus on elucidating the major neurotransmitter and cellular mechanisms underlying deviance-detection in rats.

ORAL-11-05

FOCAL ADHESION DYNAMICS ARE ALTERED IN SCHIZOPHRENIA

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We showed previously that the expression of genes in the focal adhesion kinase signalling pathway was significantly dysregulated in neural stem/progenitor cell cultures ("neurosphere-derived cells") from olfactory mucosa of schizophrenia patients. There is also evidence from genome-wide association studies to suggest that variants in genes related to this pathway are associated with schizophrenia risk. The aim of the current study was to investigate focal adhesion signalling functions in cells derived from patients with schizophrenia comparing them with cells derived from healthy controls. Olfactory neurosphere-derived cells from nine male schizophrenia patients and nine male healthy control subjects were assayed for cell adhesion and cell motility and analysed for focal adhesion kinase (FAK). Focal adhesions were counted and measured in fixed cells and time-lapse imaging was used to assess cell motility and focal adhesion dynamics. Patient and Control cells expressed similar levels of total FAK but Patient cells expressed less pFAK, the activated form ($p < 0.001$). Patient-derived cells were less adhesive ($p = 0.008$) and more motile ($p = 0.0007$) than cells derived from healthy controls and their motility was reduced to Control cell levels by integrin-blocking antibodies and by inhibition of FAK. Vinculin-stained focal adhesion complexes were significantly smaller ($p = 0.001$) and fewer ($p = 0.024$) in Patient cells. Time-lapse imaging of cells expressing FAK tagged with green fluorescent protein revealed that the disassembly of focal adhesions was significantly faster ($p < 0.0001$) in Patient cells. These results demonstrate that FAK-mediated cell adhesion and cell motility are dysregulated in schizophrenia patient-derived neural stem cells. Alterations in cell adhesion dynamics and cell motility could bias the trajectory of brain development in schizophrenia or place the developing brain at risk to environmental factors also affecting brain development.

ORAL-11-07

AN ANALYSIS OF METABOTROPIC GLUTAMATE RECEPTORS 2/3 AND 5 IN SCHIZOPHRENIA, MAJOR DEPRESSION AND BIPOLAR DISORDER FROM THE STANLEY NEUROPATHOLOGY CONSORTIUM

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Purpose: Metabotropic glutamate receptors (mGluRs) are proposed novel therapeutic targets for a variety of brain disorders such as schizophrenia (SZ), bipolar disorder (BP) and major depression (MD). Despite their potential, the involvement of these receptors in these pathological processes is uncertain. This information is crucial to understand the efficiency of drugs that target these receptors. **Methods:** Using post-mortem human brains, mGluR2/3 and mGluR5 binding densities were measured in the anterior cingulate cortex of SZ, BP, MD and matched controls (CT) ($n = 15/\text{group}$) by receptor autoradiography. **Results:** Whilst preliminary analyses indicated no diagnostic effect in mGluR2/3 or mGluR5 binding densities, mGluR2/3 binding negatively correlated with age at death in the CT ($r = -0.695$, $p = 0.004$) and SZ ($r = -0.528$, $p = 0.043$) groups, but not MD or BP. Contrarily, mGluR5 displayed a borderline positive correlation with age at death in SZ ($r = 0.505$, $p = 0.055$) and MD ($r = -0.453$, $p = 0.090$) subjects. Furthermore, mGluR2/3 and mGluR5 binding correlated in subjects with SZ ($r = -0.516$, $p = 0.049$), but not BP, MD or CT groups. **Conclusion:** Targeting mGluRs may provide a therapeutic mechanism to modulate glutamatergic activity in psychiatric disorders. Our findings of unaltered levels of mGluRs in SZ, BD, and MD may be beneficial by potentially providing an unhindered therapeutic target. However the association between age and both mGluR2/3 and mGluR5 binding in SZ subjects may have age-dependant therapeutic implications for the use of these drugs in SZ patients. There are no published investigations examining mGluR function, which may be integral to interpreting absence of change in mGluR protein levels.

ORAL-11-06

PLASTICITY INDUCED IN THE PREFRONTAL CORTEX IS IMPAIRED IN PEOPLE WITH SCHIZOPHRENIA

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Purpose: Recent studies have attributed cognitive impairments in schizophrenia (SCZ) to deficits in neural plasticity. However, assessments of neural plasticity in brain regions relevant to cognition, such as the prefrontal cortex (PFC), are lacking in humans. The aim of this study was to compare plasticity in the PFC and working memory (WM) function in individuals with and without SCZ. **Methods:** Paired associative stimulation (PAS) was used to induce PFC plasticity in seven healthy participants (29.3 ± 10 years, 1 F) and seven people with SCZ (medicated, 37.0 ± 9 years, 2 F). PAS involved pairing peripheral nerve stimulation with transcranial magnetic stimulation (TMS, a non-invasive method of brain stimulation) over PFC (interstimulus interval (ISI) = 25 ms) every 10 s for 30 mins. TMS-evoked potentials (TEPs) following single- and paired-pulse (ISI = 100 ms) TMS were measured using electroencephalography (EEG) to assess PFC excitability and inhibition at baseline and 5 mins following PAS. Participants performed a WM task 30 mins before PAS to assess WM function. **Results:** SCZ showed slower performance during the WM task compared to controls ($p = 0.03$). Facilitation of PFC excitability following PAS (i.e. plasticity) was also significantly reduced in SCZ compared with controls ($p = 0.04$). There was no significant difference in baseline cortical inhibition between groups ($p = 0.6$), however lower cortical inhibition correlated with greater plasticity induction ($r = -0.6$, $p = 0.03$). **Conclusions:** These preliminary findings suggest that people with SCZ have impaired plasticity in the PFC. The impact of this impairment on WM function requires further investigation.

ORAL-11-08

EXPRESSION OF PROTEINS WITHIN THE NOGO RECEPTOR COMPLEX ARE ALTERED IN THE DORSOLATERAL PREFRONTAL CORTEX IN SCHIZOPHRENIA

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Purpose: Schizophrenia is a severe neuropsychiatric disorder with an elusive aetiology, thought to result from abnormal brain development. Nogo is an oligodendrocyte bound molecule that signals by binding to its receptor NgR, located on axonal membranes that interacts with its co-receptors p75 or TROY and Lingo-1. Nogo signalling is responsible for CNS myelin regulation and neurite outgrowth during neurodevelopment, and plasticity in the mature brain. This study examined NgR, p75, TROY and Lingo-1 protein levels within the human dorsolateral prefrontal cortex (DLPFC) in schizophrenia. **Methods:** Human DLPFC matched case control samples ($n = 37/\text{group}$) from the NSW Brain Bank Network were used to assess NgR, p75, TROY and Lingo-1 protein levels by immunoblotting. **Results:** NgR protein expression was significantly decreased by 16% ($p < 0.001$) and Lingo-1 protein expression was significantly increased by 12% ($p = 0.006$) in the DLPFC of schizophrenia subjects. Interestingly, neither the third receptor in this trimolecular receptor complex p75, nor its homolog TROY, showed any significant difference in levels of protein expression in schizophrenia subjects compared to controls ($p = 0.146$ and $p = 0.500$ respectively). There was a significant correlation between the protein levels of NgR and Lingo-1 ($r = -0.276$, $p = 0.017$); between Lingo-1 and p75 ($r = 0.263$; $p = 0.023$) and between NgR and TROY ($r = 0.329$; $p = 0.004$). **Conclusion:** This study shows strong evidence for the involvement of NgR/p75/Lingo-1 or NgR/TROY/Lingo-1 complex in schizophrenia, however further studies are required in order to investigate the implications of these proteomic alterations to the aetiology and symptomatology of schizophrenia.

ORAL-12-01

TDCS APPLIED TO THE FOREARM REGION OF M1 MODULATES CORTICAL EXCITABILITY AND INTRACORTICAL INHIBITION

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Purpose: Transcranial direct current stimulation (tDCS) is a promising tool for enhancing neurorehabilitation outcomes, especially after stroke. Here we aimed to develop a clinically relevant paradigm for testing the effectiveness of tDCS. Our sham-controlled crossover experiment was designed to test two hypotheses: 1) Anodal tDCS applied to the forearm region of M1 induces similar after-effects to that previously observed in the hand. 2) Anodal tDCS induced after-effects can be successfully measured in the target muscle in the presence of a controlled muscular contraction. **Methods:** In healthy subjects (n=16) we measured the after-effects of anodal tDCS in the right extensor carpi radialis (ECR) during resting and active motor states. Motor evoke potentials (MEPs) and short interval intracortical inhibition (SICI) measurements were collected before and after 20 min of 1 mA anodal/sham tDCS over M1. In the rest sessions, subjects maintained a relaxed posture in their forearm during TMS measurements. In the active sessions, subjects contracted their right ECR during TMS measurements. **Results:** We observed increases in MEP amplitude following the application of anodal tDCS in both resting (11.88% ± 33%) and active motor state (25.40% ± 23%). While these increases were correlated (p=0.026), they were statistically reliable in the active session only (p=0.001). SICI decreased significantly when at rest during the anodal session (p=0.043) but not when active. **Conclusion:** Our results show that application of anodal tDCS to the forearm region of M1 modulates cortical excitability in a similar manner to the hand region. Moreover, when TMS is used to probe the subject's response to tDCS, results might be more reliable when tested with a light pre-contraction of the target muscle. Our findings have important implications for the design of post stroke rehabilitation protocols that incorporate tDCS, suggesting that targeting more proximal muscles in the upper limb is achievable.

ORAL-12-03

GSK3 MEDIATED PHOSPHORYLATION OF PI4K2A REGULATES VESICLE TRAFFICKING: IMPLICATIONS FOR MOOD DISORDERS

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Mood disorders; including bipolar disorder, major depression and Dysthymia, are debilitating diseases that severely impair people's lives and severe cases can lead to exclusion from society and suicide. At present there are no clear genetic or environmental causes, therefore other strategies are required to develop novel therapeutics for improved treatments. Our approach is to determine mechanisms of action of current drug therapies to discover novel therapeutic targets. Glycogen synthase kinase-3 (GSK-3), a Ser/Thr protein kinase, has been shown to be deregulated in mood disorders, including bipolar disorder and schizophrenia. Lithium and other mood stabilizers target GSK-3, however the pathogenic targets downstream of GSK3 are not yet known. Our lab specialises in the discovery of novel GSK3 substrates that we hope could become novel therapeutics targets for improved treatment of mood disorders. We have recently discovered a novel substrate of GSK-3 in the brain, Phosphatidylinositol 4-kinase II alpha (PI4K2α) that is involved in synaptic vesicle trafficking. GSK3 phosphorylates PI4K2A at two distinct sites within its N-terminal domain that are regulated by therapeutic levels of lithium. GSK3 mediated phosphorylation of PI4K2A regulates its binding affinity to Adaptin δ of the AP3 protein complex, which mediates trafficking between endosomes, TGN and lysosomes. Phosphorylation promotes binding of PI4K2A to Adaptin δ and trafficking to the lysosome where it is degraded. When phosphorylation is blocked, trafficking of PI4K2A is directed away from the lysosome, increasing PI4K2A abundance and translocation from the cell body to neurites and synapses of neurons. We are currently investigating the effects this has on PI4K2A cargo proteins, especially neurotransmitter receptors and subsequent effects of neurotransmission. Thus, GSK3 mediated phosphorylation of PI4K2A regulates vesicle trafficking in cells with potential implications for neurotransmission in mood disorder patients.

ORAL-12-02

CHANGES IN SYNAPTIC INPUTS TO RETINAL GANGLION CELLS DURING RETINAL DEGENERATION

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Purpose: In animal models of retinal degeneration, significant morphological and physiological changes occur in inner retinal neurons, including ganglion cells (GCs). The aim of this project was to investigate whether the balance of excitatory and inhibitory synaptic inputs to alpha ON & OFF GCs are altered in the rd1 mouse model of retinal degeneration. **Methods:** Aldehyde fixed, retinal wholemounts from transgenic rd1-Thy1-YFP and Thy1-YFP were processed for immunocytochemical labelling of the excitatory synaptic marker, RIBEYE, or the inhibitory synaptic protein, gephyrin. Using Metamorph software, 50 cells and 122 partial dendritic arbours from rd1 and control mice aged 1 month and 3 months were analysed and differences in synaptic density across each cell were quantified. **Results:** On average, the ratio of excitatory to inhibitory synapses for control ON & OFF GCs was interestingly very similar to those previous reports on respective synaptic conductance ratio. In 3m old rd1 retina, ON GCs showed significant reductions in both excitatory and inhibitory synapse density (p<0.001). In contrast, most of the OFF GCs showed an increase in excitatory synapse density. As a result, while ON cells maintained their excitatory to inhibitory synapse density ratio, OFF cells exhibited an altered ratio by 3 months of degeneration. **Conclusion:** These changes imply that functional changes in ganglion cells following photoreceptor loss may develop because of anomalies in synaptic inputs.

ORAL-12-04

ACTO-MYOSIN II CONSTRICTING RINGS CONTRIBUTES TO ACTIVITY DEPENDENT BULK ENDOCYTOSIS IN CHROMAFFIN CELLS

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In chromaffin cells, the cortical actin network is an active player in neuroexocytosis. However, its role in compensatory endocytosis has not been investigated. In particular, the mechanism by which the necks of bulk membrane invagination narrows to allow dynamin to wrap around and proceed to pinch the endocytic vesicle off the plasma membrane, is not known. **Purpose:** To analyse the role of actin and myosin II in bulk membrane retrieval in response of secretagogue stimulation in neurosecretory cells. **Methods:** Using Lifeact-GFP transfected into bovine adrenal chromaffin cells, we imaged the dynamic changes occurring at the level of the cortical actin network in response to secretagogue stimulation by time-lapse confocal microscopy, in the presence of inhibitors of actin (cytochalasin D) and myosin II (blebbistatin). We perform FRAP analysis on single identified bulk endosomes as a mean to test the level whether they have undergone pinching off from the plasma membrane. **Results:** Following a phase of depolymerization, a reorganization of actin fibers into short-lived ring-like structures (0.8 μm ± 0.05 diameter, n=31; 2.2 mins ± 0.2 duration, n=31) takes place 7-9 minutes after the onset of stimulation. Their contractile nature, pointed to the involvement of myosin-II in the formation of these rings. Using the myosin II inhibitor blebbistatin, we found that the number of LifeAct-GFP rings decreased (from 12.3 ± 1.9 rings, (n=6) to 1.6 ± 1.4 rings (n=5), p<0.01) in response to secretagogue stimulation, and the small number of rings that formed persisted through out entire experiment and failed to contract. The role of acto-myosin-II in activity-dependent bulk endocytosis was confirmed using high molecular weight dextrans or GPI-GFP, found to be internalised in large compartments encircled by contractile Lifeact rings. FRAP analysis of dextran or GPI positive compartments at different stages of the acto-myosin II ring constriction process revealed differential fluorescence recovery suggesting a role for the acto-myosin rings in constricting the neck of bulk endocytic structures prior to their pinching off from the plasma membrane. **Conclusion:** Our results point to a selective role of acto-myosin II in promoting activity-dependent bulk endocytosis in chromaffin cells.

ORAL-12-05

PICK1 INTERACTS WITH PACSIN1 TO REGULATE AMPA RECEPTOR TRAFFICKING

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The trafficking of AMPA receptors (AMPA) into and out of synapses is crucial for synaptic transmission and synaptic plasticity in the brain. The PDZ and BAR domain containing protein PICK1 directly interacts with GluA2/3 subunits of the AMPAR. Despite the involvement of PICK1 in AMPAR membrane trafficking, the exact molecular mechanism underlying this process remains unclear. Here, we show that PICK1 interacts with PACSIN1 and forms a complex with AMPARs. PACSIN1 knockdown reduces the NMDA-induced GluA2 internalisation and accelerates GluA2 recycling back to the plasma membrane. Structure and function analyses of PACSIN1 mutants reveal a unique requirement for its variable region for GluA2 recycling, while the F-BAR and SH3 domains are both required for proper internalisation and recycling of GluA2 upon NMDA stimulation. Disruption of PICK1-PACSIN1 interaction also accelerates GluA2 recycling after NMDA application. Moreover, the recycling defect upon knocking down PACSIN1 is occluded in PICK1 KO neurons, suggesting that PICK1 and PACSIN1 work cooperatively to regulate AMPAR recycling. Overall, our data suggest that PACSIN1 acts as a novel regulator of AMPAR trafficking and provides a link to the endocytic and endosomal trafficking machinery.

ORAL-12-07

ELECTROPHYSIOLOGICAL AND MOLECULAR DEVELOPMENT OF SUBSTANTIA NIGRA PARS COMPACTA DOPAMINERGIC NEURONS

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Substantia nigra pars compacta (SNc) dopaminergic neurons are involved in movement control, sleep, reward and learning, while the dysfunction and degeneration of these neurons has been implicated in various disorders and diseases including Parkinson's Disease. However, a comprehensive analysis of SNc dopaminergic neuronal development, including electrical properties and the complement of ion channel subunits expressed, is lacking. **Methods:** Patch-clamp electrophysiology and immunohistochemistry were used to assess developmental changes in rat SNc dopaminergic neurons. **Results:** The regularity of spontaneous pacemaking increased across postnatal development (decreased CV ISI, $p < 0.05$, $n = 193$). When action potentials from P5-8 ($n = 64$) and P16-22 ($n = 161$) SNc dopaminergic neurons were compared, the peak amplitude, rise and decay slopes significantly increased ($p < 0.001$), while the spike half-width significantly decreased ($p < 0.001$). Using immunolabelling ($n = 3$ for P6, P21, P40) we identified ion channel subunits that contribute to the somatodendritic delayed rectifier (Kv1.3, Kv2.1, Kv3.2, Kv3.3), A-type (Kv4.3) and calcium-activated SK (SK1, SK2, SK3) potassium currents, I_h (mainly HCN2, HCN4) and the L- (Cav1.2, Cav1.3) and T-type (mainly Cav3.1, Cav3.3) calcium currents in SNc dopaminergic neurons. Across postnatal development the major changes were an increase in the immunolabelling intensity and the dendritic range of HCN, T-type calcium channels, Kv4.3, delayed rectifier and SK channels. These data were supported by a significant increase in the after-hyperpolarization amplitude ($p < 0.001$; P5-6 ($n = 30$) vs P17-22 ($n = 122$)), the amplitude of I_h at -40mV ($p < 0.001$; P6 ($n = 47$) vs P21 ($n = 82$)) and the conductance of I_h ($p < 0.001$, P6 ($n = 25$) vs P21 ($n = 93$)). **Conclusions:** This study comprehensively characterises the changes in firing activity and ion channel subunits expressed by SNc dopaminergic neurons across development.

ORAL-12-06

ISOLATING THE BULK ENDOSOME FROM NERVE TERMINALS

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Purpose: Activity dependent bulk endocytosis (ADBE) occurs at neuronal synapses during high activity to compensate for large amounts of internal membrane that fuse with the plasma membrane. ADBE is more rapid than constitutive clathrin mediated endocytosis and is triggered by high activity. New synaptic vesicles (SVs) derived from the bulk endosomes (BEs) primarily refill the recyclable pool of vesicles yet the intervening steps from BEs to SVs are unknown. Also unclear is whether internalized BEs are distinct from early endosomes where internalized receptors and cargo are sorted. Our aim is to identify the proteins localised to BEs as the first step towards understanding ADBE regulation. To achieve this requires the development of a purification method to isolate BEs from SVs for subsequent mass spectrometry (MS) analysis. **Methods:** P2 synaptosomes obtained from whole rat brains were treated with control or high K^+ stimulation, at a level known to predominantly induce BE formation. This was done in the presence of HRP as a fluid phase uptake marker to load BEs. The synaptosomes were lysed and organelles separated on a density gradient. The fractions were analysed by western blot, electron microscopy (EM) and MS. **Results:** We confirmed increased HRP uptake in high K^+ stimulated synaptosomes ($n = 7$). Preliminary EM analysis indicated the organelles are intact prior to loading onto the gradients. HRP detection by western blot indicated BEs were concentrated in lower density fractions from high K^+ stimulated, but not unstimulated synaptosomes. MS analysis of putative BE and SV fractions confirmed the presence of HRP in the putative BE fractions. **Conclusion:** We have successfully isolated BEs from synaptosomes.

ORAL-12-08

HUNTINGTIN-ASSOCIATED PROTEIN 1 (HAP1) REGULATES EXOCYTOSIS, VESICLE LOCALISATION AND INTERACTS WITH MULTIPLE VESICLE PROTEINS

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Purpose: Huntingtin-associated protein 1 (HAP1) has a greater binding affinity for mutant huntingtin, a key player in Huntington's disease, than normal huntingtin. Subcellular localisation and protein interaction data indicate that the HAP1 may be important in vesicle trafficking and cell signalling. However, no physiological evidence exists to verify this possibility. **Methods:** We used cells derived from HAP1^{-/-} (KO), and HAP1^{+/+} (WT) mice to measure exocytosis using carbon-fibre amperometry and whole cell capacitance. We also measured vesicle number and localisation using electron microscopy and created recombinant protein fragments of HAP1 to identify novel interaction partners using proteomic analysis. A glutamate ELISA assay measured neuronal glutamate release and vesicle localisation in neurons was analysed via immunocytochemistry. **Results:** Levels of exocytosis in KO cells ($n = 35$; $p < 0.01$) cells are 40% smaller than in WT ($n = 29$) cells. The duration of transient fusion pore opening is prolonged by 30% in KO cells ($p < 0.05$) cells and the size of the readily releasable pool (RRP) is 65% smaller in KO ($n = 7$) compared to WT cells ($n = 7$, $p < 0.01$). This is due to the reduced number of docked vesicles in KO cells ($p < 0.01$). Using a proteomics approach, novel interactions between HAP1 and known trafficking-related proteins have also been discovered. Glutamate release from KO cortical brain slices is 50% smaller than WT ($p < 0.01$) and synaptic vesicle mislocalisation is observed in neurites of cultured KO neurons. **Discussion:** We demonstrate that HAP1 is a regulator of the rate of exocytosis, fusion pore stability and vesicle localisation. These roles may be explained by HAP1 binding to proteins involved in vesicle trafficking.

ORAL-13-01

THE ROLE OF CX3CR1 SIGNALLING IN REGULATING PHOTORECEPTOR INTEGRITY

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Purpose: Recently microglia have been shown to modulate neuronal development and synaptic integrity in the CNS. The fractalkine/Cx3cr1 signalling pathway is thought to be an important mediator of microglial-neuronal communication. This study characterised the role retinal microglia, specifically the Cx3cr1 signalling pathway, in photoreceptor development and maintenance. **Methods:** Cx3cr1null (Cx3cr1GFP/GFP), Cx3cr1 heterozygous (Cx3cr1GFP/+) mice and control C57Bl6J mice were studied at times ranging from P14 (eye opening) to 12 months of age. Retinal function was assessed (N=10) using the electroretinogram and microglial photoreceptor associations were detailed using immunohistochemistry (N=5-10 per age, per strain). The photoreceptor opsins were quantified using real-time PCR (N=9-10 per strain per age). **Results:** The Cx3cr1 receptor is expressed exclusively on microglia in the retina. The Cx3cr1null animals showed an increased number of microglia in the outer nuclear layer (ONL) from eye opening (P14), and abnormal microglial processes frequently extended into the ONL from eye opening at P14 ($p<0.001$). Cone pathway function was reduced in the Cx3cr1null animals at 3 months (-53%; $P<0.014$) and this correlated with a loss of cone numbers (30% reduction $p<0.001$). These cone-related deficits were maintained out to 12 months, by which time the rod pathway also exhibited a functional decrease (-22%). Between P14 and 1 month of age, wild type (C57Bl6J) control retinæ showed an increase in opsin expression, reflecting outer segment maturation, while expression remained at pre-eye opening levels in the Cx3cr1null retina. **Conclusion:** These data suggest that very early changes in microglial-photoreceptor interaction via Cx3cr1 signalling may result in abnormal photoreceptor maturation and cell death. These early developmental alterations may play a role in the later retinal degeneration observed in this animal model and may help explain the association of Cx3cr1 polymorphisms with age related macular degeneration.

ORAL-13-03

PLASTICITY OF THE PRIMATE RETINOTHALAMO-MT PATHWAYS FOLLOWING LESIONS OF THE STRIATE CORTEX (V1) DURING DEVELOPMENT AND IN ADULTHOOD

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Purpose: To investigate how perturbations of the primary visual cortex (V1) early in life can lead to altered connectivity between the cortex and subcortical areas which may afford the preservation of vision. **Methods:** Connectivity between the medial portion of the inferior pulvinar (Plm), lateral geniculate nucleus (LGN) and the middle temporal (MT) area of cortex following longstanding partial unilateral removal of the primary visual (V1) cortex was investigated through a combination of probabilistic tractography (dMRI) and neuroanatomical tracing. dMRI data was obtained from perfused fixed brains 12 months after unilateral V1 ablation at PD14 (n=2), adult (n=2) and adult control (n=2) animals and probabilistic tractography was run from each voxel in the Plm and LGN to the ipsi- and contralateral hemisphere (area MT). Intracocular anterograde tracer and cortical retrograde tracer injections into area MT were performed 12 months after unilateral V1 ablation in postnatal day 14 (PD14, n=4) and adult (n=3) marmoset monkeys (*Callithrix jacchus*) and compared to adult control animals (n=4). cFos immunoreactivity was used to determine activated visual pathways following a stimulation paradigm. **Results:** Compared to the adult control and adult V1 ablated animals, neonatal removal of V1 resulted in an increase in Plm to area MT connectivity, a sparing of retinal input to Plm and an increase in the volume of Plm. It was confirmed that relay cells in Plm ipsilateral to area MT proximal with labelled retinal ganglion cell terminals were activated by light stimulation. **Conclusion:** These data provide evidence of a putative pathway involving the pulvinar and area MT that may underpin the improved visual capacity observed in humans following a lesion of V1 early in life (prior to the closing of the critical period) compared with adults.

ORAL-13-02

CONE BIPOLAR TYPES IN PRIMATE RETINA: QUANTITATIVE ANALYSIS

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Purpose: Cone bipolar cells are interneurons that transfer signals from photoreceptors to the inner retina. At least ten bipolar types have been described in primate retina, most of which can be identified using immunohistochemical markers. Densities of bipolar cell types were estimated and compared between central (1mm) and mid-peripheral (2-6mm) retina. **Methods:** Bipolar cells were labeled immunohistochemically in vertical cryostat sections of marmoset (*Callithrix jacchus*) and macaque (*Macaca fascicularis*) retina. Bipolar cell densities were estimated using the method described by Rose and Rohlich (1988). 19 sections were analysed in marmoset and 20 sections in macaque. **Results:** The total density of bipolar cells in marmoset was 46,000 cells/mm² at 1mm and 21,000 cells/mm² at 2-6mm. In marmoset retina, the proportion of calbindin positive cells (DB3 type) was comparable between the two eccentricities (9.0% at 1mm, 7.7% at 2-6mm). The proportion of CD15 positive cells (flat midget bipolar (FMB) and DB6 types) decreased from 44.24% at 1mm to 23.36% at 2-6mm. Protein kinase Cα positive bipolar cells (DB4 and rod bipolar (RB) cells) exhibited an increase in their proportion with increasing eccentricity (6.92% at 1mm, 26.03% at 2-6mm). The proportions of bipolar cells in macaque at 1mm were 7.7% for DB3 cells, 32.3% for FMB cells, 3.4% for DB6 cells, 12.4% for DB1 cells and 15.2% for DB4 and RB cells combined. **Conclusion:** The results suggest that the proportion of FMB cells falls with distance from the fovea, while the proportion of RB cells rises. The proportion of DB3 cells remains constant. Rose, R.D. & Rohlich, D. (1988). J. Comp. Neurol., 272 (4), 365-386.

ORAL-13-04

TIMESCALES OF LONG-RANGE RESPONSE CORRELATIONS IN VISUAL AREAS V2 AND DM

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Purpose: Electrophysiological recordings from single neurons have long been the standard method used to understand the neuronal underpinnings of sensory perception. However, further progression in sensory neuroscience requires characterising the interactions between large populations of neurons within multiple cortical regions. Here, we characterise "noise" or "spike-count" correlations (r_{sc}) between the responses of pairs of multi-unit recordings obtained simultaneously in the second (V2) and dorsomedial (DM) visual areas. **Methods:** Marmosets were anaesthetized with sufentanil (8 µg/kg/h) and N2O (70% in oxygen), and viewed visual stimuli subtending 40° of the visual field. Extracellular recordings were obtained using a 96-channel microelectrode array (Blackrock Microsystems), which spanned the border of V2 and DM. **Results:** Recordings yielded 13 orientation-tuned units in V2 and 68 orientation-tuned units in DM. Across all 3240 pairs of units, the mean spike-count correlation associated with responses to 400 ms of a moving sine-wave gratings was $r_{sc} = 0.30 \pm 0.22$ (SD). We observed that spike-count correlation between pairs of neurons depends on neuronal separation in cortex, the degree of receptive field overlap, and the similarity in preferred orientation of the neuronal pair. Critically, we also show that spike-count correlations are higher when neurons are actively driven by visual stimulation compared to periods of no stimulation, and that significant increases in correlation occur after just 200 ms of visual stimulation. **Conclusion:** Our results indicate that the neurons separated by up to 5 mm share common inputs, and that the strength of these connections is weakly, but significantly, dependent on tuning similarity, spatial separation and stimulus duration. These results help constrain models of cortical read-out, which use neuronal activity to predict both stimuli and perception.

ORAL-13-05

SACCADE DIRECTION MODULATES POST-SACCADIC ENHANCEMENT OF OCULAR FOLLOWING EYE SPEED

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Purpose: In primates, neural responses to visual stimuli in many visual areas are reduced during saccadic eye movements and enhanced afterwards. Similarly, reflexive ocular following eye speed is also enhanced after saccades. What are the mechanisms mediating post-saccadic enhancement? **Methods:** Monkeys (macaca mulatta; n=2) viewed vertical cosine gratings covering 40° of their visual field. Eye position was recorded via an infrared video eye tracker. The monkeys fixated a small target positioned 10° to the left, the right or below the center of the screen. The peripheral target was then removed and replaced with a central target to which the monkeys made saccades. The monkeys then fixated the central target for 50ms (short-delay condition) or 300ms (long-delay condition) after which the grating began moving to the left or the right, triggering robust ocular following eye movements. **Results:** Ocular following eye speed was always enhanced in the short-delay condition compared to the long-delay condition (p<0.001). This enhancement was modulated by the relative direction of the preceding saccade: enhancement was greater when the saccade and subsequent post-saccadic test stimulus were in the same direction (i.e., retinal image motion during the saccade and the test stimulus were in opposite directions) compared to when the saccade and test stimulus were in opposite directions (p<0.001). **Conclusion:** Post-saccadic enhancement of ocular following eye speed is modulated by peri-saccadic visual input. Moreover, this modulation is sensitive to the direction of the retinal image motion during the saccade relative to that of the subsequent motion test stimulus.

ORAL-13-07

CORRELATIONS AND SYNCHRONY IN THE MIDDLE TEMPORAL AREA DEPEND ON FUNCTIONAL DISTANCE AND TUNING

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Purpose: Shared connectivity causes the activity of sensory cortical neurons to fluctuate together. These correlations depend on the functional similarity of the measured neurons and their relative position in the cortex. We investigated how magnitude and timing of correlations in the middle temporal (MT) area depend on distance and direction tuning. **Methods:** Extracellular recordings were made from area MT in 8 adult male marmosets, *Callithrix jacchus*, anaesthetised with sufentanyl forte (9ug/kg/hr). In 6 animals (8 recordings), a 10x10 array of single electrodes (spacing 0.4mm) was inserted. In 3 animals (12 recordings), laminar probes (spacing 0.2mm) were inserted perpendicular to the cortical surface. The stimulus was a large dot field drifting for 2s in each of 4 directions. Spike-count correlations and cross-correlograms between pairs of direction-selective neurons were estimated. **Results:** The arrays yielded 2497 pairs of single-units, and the laminar probes 458. Spike-count correlations between similarly tuned pairs were high, and decreased with distance. Nearby pairs (<0.4mm) within the same column were more correlated than pairs in different columns. This high correlation was largely due to synchronous spiking at short timescales (<100ms). The difference in correlations for neurons within compared to between columns persisted even when we only considered similarly tuned pairs. **Conclusion:** At short timescales, the functional connectivity of neurons in area MT of marmoset is dominated by the position of those neurons in the cortical matrix. These correlations could arise from strong 'vertical' connections between neurons in the same column, or because more of the inputs to neurons in the same column arise from the same source.

ORAL-13-06

THE ROLE OF PROTEIN ARGININE METHYLTRANSFERASE 8 IN VISUAL CORTICAL CRITICAL PERIOD

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Much of our adult behavior is shaped by experience during critical period (CP) in early postnatal development. The visual system has been the premier model for studying activity-dependent and CP plasticity. We hypothesize that the dynamic epigenetic state of the brain is influenced by the external environment impacting CP plasticity. One such epigenetic modification is protein methylation. Protein methylation can be catalyzed by protein arginine methyltransferases (Prmt), that methylate arginine residues in histones. Prmt8 is particularly interesting because it is selectively expressed in the central nervous system (CNS). We found that Prmt8 is up-regulated in the visual cortex during developmental CP and in a dark-rearing paradigm, indicating that it is experience-dependently regulated. We next investigated Prmt8 null mice and observed that they have a defect in synapse formation as compared to their wildtype counterparts. These Prmt8 mutants also have an increased latency of visual discrimination. We compared Prmt8 mutants and their wildtype counterparts using a proteome-wide approach, Tenascin-R (TNR), an extracellular matrix glycoprotein essential for the formation of perineuronal nets (PNN) around parvalbumin interneurons, was found to be significantly upregulated in Prmt8 null mice. It has been shown that artificial removal of PNNs in adult mice can reactivate CP and we are further investigating this in Prmt8 null mice. This will allow us to better understand the role of PRMT8 in synaptic plasticity and in establishing CP in the visual system.

ORAL-13-08

COMPARISON OF FLUORESCIN ANGIOGRAPHY DYNAMICS BETWEEN THE EYE AND BRAIN

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Purpose: To compare the time course of sodium fluorescein transit through retinal and cortical vasculature. To determine if repeated injections of sodium fluorescein return repeatable outcomes. **Methods:** Sodium fluorescein (1%, 0.07ml, 1.05ml/min) was delivered into the femoral vein of anaesthetised Long-Evans rats (eye: n=5, brain: n=6, ketamine:xylazine, 60:5mg/kg). The skull was thinned to allow cortical vessels to be visualised. The Micron-III camera (Phoenix Labs) was used to acquire images at 23 frames/sec. Post-hoc analysis was performed with FIJI software to calculate transit times (fluorescence >10% of background) and fluorescence profiles for different blood vessel types. Repeated fluorescein injections were performed at 15-minute intervals for 1-hour to investigate the stability of fluorescence profiles from baseline. Profiles were modelled and bootstrapped to determine 95% confidence limits (CL). **Results:** The eye and brain returned similar transit times in the artery (P=0.5), vein (P=0.6) and capillaries (P=0.6). Fluorescence profiles normalised to the peak showed a sharp, ascending limb (half-height (95% CL): eye=1.6sec (1.5-1.8), brain=1.9sec (1.7-2.0)) followed by an exponential decay (half-decay: eye=7.1sec (6.4-8.5), brain=7.2sec (6.3-8.5)). Retinal fluorescence profiles following repeated injections showed similar kinetics to the first dose. In contrast, brain fluorescence profiles showed poor repeatability due to a gradual reduction in signal-to-noise ratio from increasing background fluorescence with each dose. **Conclusion:** The transit time for fluorescein in retinal and cortical vasculature are comparable. Fluorescence profiles could be quantified to reveal similar dynamics between both areas. Following repeated injections, fluorescein profiles were stable in the eye but not the brain. This approach allows for quantitative assessment of fluorescein angiography dynamics in rat retinal and cortical blood vessels.

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 interneurons. **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-14-03

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-14-02

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-14-04

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, 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 underlying 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 sutureless 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 ($\lambda=532\text{nm}$, 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 $0.37\pm0.15\text{ N}$, that was unchanged 1-week after transection (n=30), with minimal heating ($<6^\circ\text{C}$, 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 $0.015\pm0.015\text{ (n=15)}$. **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-14-07

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-14-06

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-14-08

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 a 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 = $5.4 \pm 3.1\%$ vs. control = $36.7 \pm 2.5\%$ & MK-801 = $10.0 \pm 2.0\%$), but enhanced the reversal produced by clozapine (clozapine + MK-801: $24.3 \pm 4.0\%$; + BQCA: $34.4 \pm 5.1\%$), olanzapine (olanzapine + MK-801: $21.1 \pm 2.7\%$; + BQCA: $36.6 \pm 3.3\%$), haloperidol (haloperidol + MK-801: $21.2 \pm 2.6\%$; + BQCA: $35.0 \pm 3.2\%$) or aripiprazole (aripiprazole + MK-801: $18.9 \pm 3.3\%$; + BQCA: $25.7 \pm 3.3\%$). In the Y-maze, BQCA alone had no effect, whereas BQCA and clozapine was the only combination that restored memory loss (novel: $38.8 \pm 2.0\%$, familiar: $29.0 \pm 2.0\%$) in MK-801 treated mice (novel: $32.0 \pm 1.7\%$, familiar: $34.1 \pm 2.1\%$) to a similar level as in the control group (novel: $38.7 \pm 1.1\%$, familiar: $28.1 \pm 1.8\%$). **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 DISCRIMINATIONPaolini A.G.^{1,2} and Morgan S.³

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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 than 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 CORTEXChaplin T.A.¹, Yu H.H.¹, Pinskiy V.², Tolpygo A.², Mukherjee A.², Mitra P.P.² and Rosa M.G.P.¹¹Department of Physiology, Monash University, VIC3800, Australia.²Cold Spring Harbor Laboratory, Cold Spring Harbor, NY11724, USA.

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 neuron, 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-15-07

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-15-06

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-15-08

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. ¹Etemad 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 (A β) 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 A β 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 β ₁₋₄₀ and A β ₁₋₄₂ 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 1.5×10^5 density. Experiments were performed at 21 days in culture (neurons) and 15 days in culture (astrocytes). A range of concentrations of Zn, Cu, A β ₁₋₄₀ and A β ₁₋₄₂ 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 biomaterials. **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 μ M), or Rho kinase inhibitors Y27632 (30 μ M) or Fasudil (100 μ M) 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 biomaterials 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-16-07

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-16-06

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-16-08

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 photothrombotic 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-17-03

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-17-02

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:xylozine (k:x) or isoflurane were measured in the same cohort at days 7 and 14. All data are expressed as mean (\pm SEM) and parameters between groups are compared via mixed linear analysis. **RESULTS:** Conscious ERG returned maximal a-wave ($-15 \pm 1 \mu V$), rod b-wave ($39 \pm 5 \mu V$) and cone b-wave ($17 \pm 2 \mu V$) amplitudes, which were significantly smaller than that under k:x (a-wave $-22 \pm 4 \mu V$; rod b-wave $56 \pm 9 \mu V$; cone b-wave $24 \pm 3 \mu V$) but larger than responses under isoflurane (a-wave $-10 \pm 2 \mu V$; rod b-wave $24 \pm 5 \mu V$; cone b-wave $8 \pm 2 \mu V$). Isoflurane produced less sensitive a-waves compared to conscious (1917 ± 334 vs 398 ± 126 $m^2 \cdot cd^{-2} \cdot s^{-3}$). VEP amplitudes were similar in all conditions, with only P2-N1 amplitude larger in k:x ($15 \pm 2 \mu V$) compared with conscious ($12 \pm 2 \mu V$) and isoflurane ($14 \pm 2 \mu V$). Isoflurane yielded significantly slower VEP (implicit times: P1 25 ± 3 ms, N1 53 ± 4 ms, P2 81 ± 6 ms) than conscious (P1 18 ± 1 ms, N1 34 ± 1 ms, P2 62 ± 1 ms). P2 implicit times were slowed under k:x (72 ± 1 ms) 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-17-04

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 (DPMs); 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 DPMs & 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-17-07

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 intrastratial injection of 2×10^9 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 intrastratial 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-17-06

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-17-08

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 candelas/m²) 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-18-03

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-18-02

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-18-04

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-18-07

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-18-06

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-18-08

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. Ganglioneogenesis 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 ganglioneogenesis. **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 \geq ENCC), movement, proliferation (neurons \leq 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 ganglioneogenesis, 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-19-03

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-19-02

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 neuroligins1 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-19-04

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 $5 \pm 2.0\%$ 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 $18 \pm 2\%$ 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 (1x10⁸ 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-19-07

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 hmg1 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-19-06

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-19-08

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 PERCEPTIONSuri M.¹, Jain S.² and Mathur R.²¹Department of Physiology, Institute of Home Economics, University of Delhi, New Delhi, India. ²Department of Physiology, All India Institute of Medical Sciences, New Delhi, India.

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 $11.6041 \pm 6.16\%$ at a flow rate of 1.5 μ l/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 PADSCondon M.^{1,2}, Hudson K.^{1,2}, Chelvanayagam D.¹, Mahns D.¹, Birznies I.^{2,3}, Olausson H.⁴, Lamotte R.H.⁵ and Macefield V.G.^{1,3}¹School of Medicine, University of Western Sydney. ²School of Health & Science, University of Western Sydney. ³Neuroscience Research Australia. ⁴Dept of Clinical Neurophysiology, University of Gothenburg, Sweden. ⁵Dept of Anesthesiology, Yale University, USA.

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 SAIL 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. FAI afferents also showed a linear decrease in firing during the loading but not unloading phase, though the slope was lower. Conversely, SAIL 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 SAIL 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 underlying cellular and synaptic mechanisms are not well known. In this study, we using intracellular recording in vivo 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 conductance state than UP state by calculating total conductance (n=5, p=0.001), and an excitatory synaptic conductance dominated state by testing synaptic reversal potential (n=6, p=0.012), comparing with UP state. This synaptic conductance state fundamentally influenced cellular input resistance and membrane potential, thus modulated neuronal 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

BARRETTES, BARRELOIDS AND BARRELS: STRUCTURES AND CONNECTIVITY OF THE SOMATOSENSORY PATHWAY REVEALED USING SUPER RESOLUTION TRACTOGRAPHYRichards K.L.¹, Calamante F.², Tournier J.-D.², Kurniawan N.D.³, Reutens D.C.³, Reid C.A.¹, Connelly A.² and Petrou S.^{1,4}¹Florey Institute of Neuroscience and Mental Health. ²Brain Research Institute. ³Centre for Advanced Imaging, University of Queensland. ⁴Centre for Neural Engineering, The University of Melbourne.

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 100 μ m 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 20 μ m. **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-20-07

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-20-06

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 25 μ m 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 250 msec. 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-20-08

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.