ORAL SESSIONS

ORAL-01-01

SPINAL INHIBITION DURING A SUSTAINED SUBMAXIMAL EFFORT

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We recently demonstrated rapid complete inhibition of the response to the second of paired motor cortical stimuli during a fatiguing maximal contraction (McNeil et al., J Physiol, in press). The inhibition occurred at the spinal cord but precluded measurement of a potential increase in intracortical inhibition. **Purpose:** To assess cortical and spinal contributions to long-interval inhibition during a submaximal contraction. **Methods:** Eight healthy subjects performed a 10min contraction of the elbow flexors matched to the level of EMG at 25% of maximal effort. The motor cortex was activated by transcranial magnetic stimulation (TMS). Single test and paired conditioning-test stimuli (100ms interval) were delivered 5s apart at the end of each minute. On a separate day, subjects repeated the protocol but the TMS test pulse was replaced with cervicomedullary stimulation between the mastoids. TMS motor evoked potentials (MEPs) and cervicomedullary motor evoked potentials (CMEPs) were recorded from the biceps brachii. Inhibition was calculated as the size of the conditioned test response divided by the preceding unconditioned test response. **Results:** Unconditioned (single test) MEPs were unaffected by fatigue whereas the CMEPs decreased toward the end of the contraction by ~15%. Conditioned (paired) MEPs and CMEPs decreased gradually with fatigue at a similar rate. At the end of the contraction, they had decreased by 74±25% and 65±40%, respectively. Inhibition increased with fatigue and mirrored the magnitude and time course of change of the conditioned responses. **Conclusion:** Long-interval inhibition increased gradually with progressive muscle fatigue. As in a maximal effort, the fatigue-related increase in inhibition occurred primarily at the motoneurones rather than the motor cortex.

ORAL-01-02

PAIN CHANGES THE GAIN OF POSTURAL RESPONSES

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Movement changes during pain, but the mechanisms remain unclear. One hypothesis is that the nervous system adopts a protective strategy during pain to reduce the risk for further pain/injury. This could be achieved by adopting a new strategy of muscle activation, or by increasing the gain of postural responses (i.e. the strategy reserved for higher load tasks is adopted at a lower load). This study investigated these possibilities in ten male volunteers. Electromyographic (EMG) activity of gluteus maximus and tensor fasciae latae were recorded with fine-wire and surface electrodes. Subjects stepped down from steps of 0, 5, 15 and 30 cm onto a force plate, and down from a 5 cm step during trials in which pain was induced by electrical stimulation over the sacrum (7/10) trigger by foot contact on the force plate. An extra condition was completed that involved painful stimulus during 75% of trials and resulted in trials in which the pain was anticipated. EMG onset and amplitude were compared between conditions. Hip muscles were active in advance of foot contact. Stepping down from higher steps (15 and 30 cm) involved earlier and larger muscle activation. Hip muscle activity from a 5 cm step during real or anticipated pain was identical to activity from a 15 cm step during the no pain condition. The findings suggest that changes in muscle response with pain are consistent with an increased gain of the postural response. Changes in activation with anticipation of pain exclude a direct effect of nociceptor activation.

ORAL-01-03

FORCE CONTROL IN THE LOWER LIMB AFTER STROKE

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Stable low-level voluntary contractions with a constant force output are essential for many activities of daily living. Force steadiness has been measured previously after stroke, but only over short intervals such as 5-20 s. This study investigated force stability over a more functionally relevant duration in hemiplegic stroke subjects. 10 subjects between 1-13 years post stroke, sat with the knee of the test leg extended to 120° and the ankle at the mid-point of passive range of motion. Subjects held either 5 or 10% of their maximum voluntary dorsiflexion force during an isometric contraction for up to 6 minutes under various conditions including with and without visual feedback, and during a distraction task. The less affected side was tested first. Force stability was measured as the root mean square error from the target force. Surface EMG was recorded from tibialis anterior, peroneus longus and triceps surae and normalised to the maximum muscle activity. Significant differences in force stability were seen between sides ($p<0.001$) and between conditions ($p<0.001$). The amount of EMG varied between conditions ($p<0.01$) but not between sides. The perceived effort required to generate force was assessed using a modified Borg scale. The physical effort was significantly greater on the more affected side ($p<0.001$) as was the reported concentration effort necessary to produce this force ($p=0.005$). These data suggest that force control during sustained low-level voluntary contraction is less steady on the more affected side after stroke. Force unsteadiness increased when distraction tasks were introduced and when visual feedback was reduced. These results emphasise the importance of visual feedback in motor control after stroke and the need for minimal distractions during complex tasks.

ORAL-01-04

SUBSTANTIA NIGRA ECHOMORPHOLOGY AND MOTOR CORTEX EXCITABILITY

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The aim of our study was to investigate motor cortex excitability in individuals with a substantia nigra (SN) abnormality identified with transcranial sonography. Nigral hyperechogenicity in healthy adults is believed to represent abnormal iron accumulation. Our study investigated 20 healthy older subjects (72-84 yrs) with normal (n=10) or abnormal (n=10) SN echomorphology. All subjects were neurologically normal. Motor cortical excitability and intracortical inhibition were assessed with transcranial magnetic stimulation (TMS) of the motor cortex. Single and paired stimuli were delivered over the first dorsal interosseus motor area during relaxation. Single stimuli were also delivered during voluntary contraction. Each cortical hemisphere was analysed separately. The response to single pulse TMS (in motor cortex ipsilateral to the target SN) did not differ between groups. However, a significant difference between groups was observed with paired pulses (conditioning stimulus intensity: 70% resting motor threshold; interstimulus interval: 2 ms). The conditioned motor evoked potential amplitude was significantly larger ipsilateral to the hyperechogenic SN compared with controls ($P=0.014$). These data suggest that healthy subjects with SN hyperechogenicity exhibit reduced $GABA_A$-mediated intracortical inhibition within the motor cortex compared with subjects with normal SN echomorphology. Decreased intracortical inhibition is also observed in Parkinson's disease patients. This study provides further evidence that SN hyperechogenicity in healthy individuals is associated with changes characteristic of Parkinson's disease and supports its role as a marker of vulnerability for this disorder.

ORAL-01-05

GABA-DEFICIENT MICE REGULATE SURVIVAL OF HYPOGLOSSAL AND BRACHIAL, BUT NOT LUMBAR, MOTONEURONS DURING EMBRYONIC DEVELOPMENT

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Purpose: GABAergic/glycinergic synaptic transmission undergoes a developmental switch from excitation to inhibition due to altered transmembrane chloride gradient. Previous work on mice lacking glycinergic transmission suggested that altered motoneuron activity levels correspondingly regulated motoneuron survival during embryonic development (Banks et al., 2005). To determine if GABAergic transmission plays a similar role, we quantified motoneuron number and activity in GAD67-deficient mice which show significantly lower GABA production. **Methods:** Hypoglossal, brachial and lumbar motoneurons were counted in GAD67-deficient and wild-type littermate embryos (E18; *n*=6/age and genotype for each motor pool). We recorded periodic motor burst activity in hypoglossal (XIIn), cervical (C6-8), and lumbar (L3-6) via suction electrodes (*n*=15-18) from ventral nerve rootlets in brainstem-spinal cord preparations. **Results:** At E18, GABA-deficient mice showed a significant decrease in hypoglossal motoneuron number (1753 ± 40) compared to wild-type (2054 ± 100; *n*=6, *P*=0.013, *t*-test). This decrease correlated with increased mean nerve burst frequency ($^{+/-}$ 20%; $^{-/-}$ 38%) and half-width ($^{+/-}$ 48%; $^{-/-}$ 25%) in both heterozygous and mutant mice, as well as increased activity (11%) (total charge/minute) for heterozygous mice. Brachial motoneuron number in GABA-deficient mice were increased (3316 ± 97) compared to wild-type (2506 ± 102, *n*=6, *P*=0.0002, *t*-test). This increase correlated with a decreased mean nerve burst frequency (4%), instantaneous frequency (28%) and activity (2%) in heterozygous mice. Lumbar motoneuron number showed no significant difference between the wild-type and mutant mice (2359 ± 133; 2226 ± 167; *n*=6 *P*=0.5500, *t*-test). This was associated with increases in instantaneous frequency (22%), mean nerve burst frequency (25%), and decreases in mean nerve activity (27%), nerve burst half-width (39%) in heterozygous mice. **Conclusion:** During development, GABAergic transmission can regulate motoneuron numbers, but further investigation is required to fully understand the role that motoneuron pool activity plays in this regulation.

ORAL-01-06

MYOSTATIN INHIBITION ATTENUATES ATROPHY AND LOSS OF MUSCLE FUNCTION IN MICE WITH CANCER CACHEXIA

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Cancer cachexia describes the progressive skeletal muscle wasting and weakness in many cancer patients. Cancer cachexia impairs mobility, causes severe fatigue, and accounts for >20% of cancer-related deaths. We tested the hypothesis that antibody-directed myostatin inhibition would attenuate the atrophy and loss of function in skeletal muscles of tumour-bearing mice. Twelve week old C57BL/6 mice received a subcutaneous injection of saline (Control) or $7.5x10^5$ Lewis Lung Carcinoma (LLC) tumour cells. One week later, mice began once-weekly injections of saline (Control, n=12; LLC, n=9) or a mouse chimera of anti-human myostatin antibody (PF-354, 10 mg/kg/week, Pfizer Global Research and Development, Groton; USA; LLC+PF-354, n=11), which continued for 5 weeks. Compared with controls, LLC mice had an 8-10% lower muscle mass ($P<0.05$) which was prevented with PF-354 ($P>0.20$). Peak tetanic in situ force production of tibialis anterior (TA) muscles of LLC mice was reduced by 8% ($P<0.05$), but this deficit was attenuated with PF-354 treatment ($P>0.05$). PF-354 increased the cross-sectional area (CSA) of Type IIx/b fibres in TA muscle from LLC mice by 12% ($P<0.05$), but there was no difference between groups in CSA of Type IIa fibres ($P=0.56$). Apoptosis in cross-sections of TA muscle from LLC mice was increased by 140% ($P<0.05$), but this increase was prevented with PF-354 treatment ($P>0.05$). Antibody-directed myostatin inhibition attenuated the skeletal muscle atrophy and loss of muscle force-producing capacity in a murine model of cancer cachexia, in part by reducing apoptosis. These findings highlight the therapeutic potential of myostatin inhibition for cancer cachexia.

ORAL-01-07

STORE-OPERATED Ca^{2+} ENTRY IN INTACT SKELETAL MUSCLE FIBRES FROM HEALTHY AND DYSTROPHIC MICE

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Store-operated Ca^{2+} entry (SOCE) is a mechanism that involves an influx of extracellular Ca^{2+} in response to store-depletion during work in skeletal muscle. It has been suggested that SOCE may be deregulated in dystrophic skeletal muscle activating proteolytic enzymes and triggering necrosis. To test this we stained the cytoplasm of wild-type (wt) and mdx fibres with fluo-4AM to image Ca^{2+}. By adding Ca^{2+} to the external solution of the Ca^{2+}-depleted fibres, we observed a low amplitude fluorescence transient in wt fibres (n= 5). In contrast, mdx showed no such transient (n=8) under the same conditions. Upon the application of caffeine, the sarcoplasmic reticulum (SR) of both wt and mdx released SR Ca^{2+} as indicated by an increase in fluorescence showing that the SR has refilled with Ca^{2+}. The results also show that SOCE must deactivate when the SR is refilled with Ca^{2+} in both wt and mdx fibres and that mdx fibres must have a more efficient SR terminal cisternae for sequestering Ca^{2+} from the junctional space as it enters through the transverse tubules during SOCE, preventing Ca^{2+} from escaping to the bulk cytoplasm. SOCE influx was also found to be increased three times in mdx (n=3) compared to wt fibres. This finding is consistent with previous work that found SOCE proteins Stim 1 and Orai 1 to be increased three-fold in mdx fibres (Friedrich et al., 2008, *Proc Aust Physiol Soc*). We conclude that while SOCE is upregulated in mdx muscle, it is not functionally compromised, consistent with SOCE playing a compensatory role in mdx mouse muscle.

ORAL-01-08

IN VITRO INTERACTIONS BETWEEN THE β1A SUBUNIT OF THE SKELETAL MUSCLE DHPR AND RYR1

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The β_{1a} subunit of the skeletal muscle DHPR plays two important roles in excitation-contraction (EC) coupling in skeletal muscle. β_{1a} was originally found to target the α_{1S} subunit of the DHPR to sarcolemmal tetrads which oppose skeletal ryanodine receptor (RyR1) channels in the sarcoplasmic reticulum. It was later found to also aid in transmission of EC coupling between the α_{1S} subunit and RyR1, through its C-terminal tail residues. This presumably depends on an interaction between the C-terminal tail of β_{1a} and RyR1 since the β_{1a} subunit binds to RyR1 in affinity chromatography experiments, but direct binding of the C-terminal tail to RyR1 has not been reported. Nor have direct functional interactions between the proteins been demonstrated. We show here, using affinity chromatography, that a peptide corresponding to the native sequence of the C-terminus of β_{1a}, but not a peptide with a scrambled sequence, binds to RyR1 (N=3). In addition the peptide and the full β_{1a} subunit at 0.1 to 1 nM significantly increase both [^{3}H]ryanodine binding (N=7-18) and single RyR1 channel activity, with maximum 3- to 5-fold activation at ~10nM (N=7-10 at each of eight concentrations). The increase in RyR1 channel activity with both full length β_{1a} and the C-terminal peptide was irreversible within the lifetime of the bilayer, indicating high affinity binding. Therefore the C-terminal tail of the β_{1a} subunit is capable of directly binding to and activating RyR1, suggesting that β_{1a} may enhance EC coupling by virtue of its ability to activate RyR1.

ORAL-02-01

SPATIOTEMPORAL ALIASING AND THE NEURAL DELAY OF MOTION DETECTORS IN NOCTURNAL HAWKMOTHS

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The physiological mechanisms underlying motion detection require non-linear comparison of the signal at one location with the delayed signal from an adjacent location, yet mechanisms underlying the delay remain poorly understood. In most insects, motion detectors use a temporal delay mechanism that exploits the phase shift associated with a low-order, low-pass filter. This has the advantage over a simpler delay mechanism (e.g. a pure delay such as would be introduced by an additional neuron in the delayed pathway) that as pattern speed increases, responses to periodic gratings roll-off without aliasing. In nocturnal hawkmoths (Sphingidae), however, we find that motion detectors are tuned to unusually low temporal frequencies compared with most other insects, consistent with their need to detect very low speed motion in dark conditions [1]. Interestingly, in these species direction selectivity to high-speed periodic patterns reverses compared with that at low speed, suggestive of a fundamentally different neural delay filter. In two species, *Deilephila elpenor* (n=4) and *Manduca sexta* (n=6) we find temporal optima of 0.7-1.5 Hz but reversal of directionality at temporal frequencies of 6-10 Hz in individual neurons. Using computational models employing different types of delay filter, we find that such temporal aliasing is not easily accounted for by a simple pure delay model, but can be well fitted by a lognormal delay model similar to those used previously to account for the 2nd messenger system that underlies photoreceptor kinetics.
1. Theobald, Warrant & OCarroll (2009) Wide-field motion tuning in nocturnal hawkmoths. Proc. Roy. Soc. B. (in press).

ORAL-02-02

DISTINCT SYNAPTIC MECHANISMS MEDIATE ORTHOGONAL ORIENTATION SELECTIVITY IN RABBIT RETINAL GANGLION CELLS

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Cells sensitive to the orientation of contrast edges are common in the visual cortex and retina. Orientation-selective retinal ganglion cells were first described several decades ago, but the synaptic mechanisms generating orientation selectivity remain largely unknown. In this study we measured physiological light-evoked responses in an isolated, in vitro rabbit retinal preparation. We show that two populations of off-centre ganglion cell, which are morphologically and physiologically distinct, have preferred orientations that are orthogonal to each other. Apart from the difference in preferred orientation, the spiking responses of the two groups, measured from extracellular recordings, are essentially indistinguishable. However, the light-evoked synaptic current, revealed using whole-cell patch-clamp recording from a group of 35 cells, displayed distinct differences between the two groups. Physiological and pharmacological data demonstrate, in agreement with the earlier extracellular studies, that GABAergic transmission is critical for orientation selectivity. Surprisingly, we find that excitation of the off-centre orientation-selective ganglion cells is driven in part by glycinergic dis-inhibition that crosses over from the on-pathway. The results demonstrate that orientation-selectivity is not generated by synaptic integration at the level of the ganglion cell, but arises presynaptically, most likely within specific orientation-selective amacrine cells. Overall, the results show that very similar spiking phenotypes can be generated by diverse synaptic mechanisms.

ORAL-02-03

ACTIVITY INDEPENDENT DEVELOPMENT OF DIRECTION-SELECTIVE CIRCUITRY IN THE MAMMALIAN RETINA

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In the mammalian retina, several subtypes of retinal ganglion cells (RGCs) code image motion directions by responding strongly to movement in one direction and weakly to the movement in the opposite direction. For the ON-OFF direction selective ganglion cells (DSGCs), the mechanism underlying coding motion directions has been shown to result from selectively connection between dendrites of the RGCs and a particular sector of processes of the overlapping starburst amacrine cells, a type of interneuron. Recent results demonstrated that the formation of the retinal DS circuitry does not rely on visual experience or light driven activities, or nicotinic acetylcholine receptor β2 subunits. Here, we report that with pharmacological blockade of synchronous cholinergic activity and/or action potentials for over two weeks from birth, DS circuitry can develop normally. Our results strongly indicate the formation of retinal DS circuitry is genetically programmed.

ORAL-02-04

TEMPORAL LIMITS FOR PREDICTING STIMULUS CHANGES AND PERCEPTION FROM SINGLE MT/MST NEURONS

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To investigate the timescales of neural processing during behavior, we trained 2 macaque monkeys to perform a combined detection, discrimination and reaction-time task. Animals made a saccadic eye movement when they detected a change in stimulus speed, with their choice of saccade target indicating if they perceived an increase or decrease in speed. Simultaneously, extracellular recordings were made from 180 single neurons in cortical areas MT and MST. The slope of a neuron's speed tuning curve correlated with neurometric performance, the amount of information that the neuron could convey about the sign of the stimulus change. Importantly, the most informative response period was ~60-180 ms after the speed change; neurometric performance did not necessarily increase with the duration of the spike counting window. Similarly, we found that the ability to use a neuron's spiking rate to predict the nature of the animal's response (choice probability - CP) and reaction times (reaction time spike rate correlations - $r_{RT,R}$) was best when spikes were counted in short time windows (80-160 ms) starting at the end of the neuron's latent period (50-80 ms after the speed change). CP and $r_{RT,R}$ were higher for neurons with better neurometric performance. Surprisingly, both behavioural measures were significant even when spikes were counted in windows as short as 10 ms. Our results suggest that on short time scales, CP is likely to reflect variability in sensory inputs to an area, and that behavior can be influenced by very transient periods of sensory activity in the brain.

ORAL-02-05

NEURAL EVIDENCE FOR AN EXTRA-RETINAL MECHANISM UNDERLYING POST-SACCADIC ENHANCEMENT OF VISUAL SENSITIVITY

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Visual sensitivity is reduced before saccades and enhanced afterwards. Here we investigate the mechanism underlying post-saccadic enhancement. We recorded neural activity from 145 neurons in the dorsal medial superior temporal area (MSTd) of two monkeys (Macaca mulatta). Monkeys made rewarded saccades from a peripheral to a central fixation target separated by 10°. Fixation targets were presented on a full-field random texture pattern of either 0%, 10% or 100% contrast. After the saccade the monkeys were required to maintain fixation for a nominal interval of either 50ms (short delay condition) or 300ms (long delay condition), after which the target was removed and the contrast of the background texture was set to 100%. The background texture simultaneously began moving at the preferred speed in the preferred direction of the recorded cell, evoking robust spiking responses in the recorded cell and triggering reflexive ocular following. Neural responses to the motion stimuli were significantly enhanced in the short delay condition compared to the long delay condition (t-tests, 0%, $p = 0.009$; 10%, $p = 0.003$; 100%, $p = 0.001$). Initial speeds of the ocular following responses were also enhanced for the short delay condition (t-tests, 0%, $p = 0.002$; 10%, $p < 0.001$; 100%, $p < 0.001$). There was no significant difference in the enhancement observed for the different stimulus contrasts. Our results suggest that post-saccadic enhancement is independent of afferent visual input during saccades and provide compelling evidence that post-saccadic enhancement is mediated by an extra-retinal mechanism.

ORAL-02-06

ANISOTROPIES IN THE RESPONSE OF HUMAN VISUAL CORTEX TO GRATING ORIENTATION

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Neuroimaging of human visual cortex has shown that responses to variations in pattern orientation are anisotropic; different orientations do not evoke equivalent responses. However, the form of orientation anisotropy is inconsistent across studies and remains uncertain. A complicating factor may be that anisotropic responses are also obtained when the conjunction of pattern orientation and angular position in the visual field is considered, with greater responses obtained when the pattern orientation and preferred visual field meridian are parallel compared to tangential; this meridian-relative anisotropy is known as the radial bias. Here, we performed an integrated investigation of absolute and meridian-relative orientation anisotropy using fMRI (n=4). Maps of BOLD responses to an observed sinusoidal grating of varied orientation were coregistered with maps of the preferred visual field meridian obtained from responses to a rotating wedge. We report significant anisotropies in the responses of V1, V2, V3, and V3A/B to both absolute and meridian-relative orientation. The anisotropy to absolute orientation is characterised by a reduced response to horizontal orientations, which is inconsistent with reports of a direct association between the behavioural oblique effect (increased perceptual salience of horizontal and vertical relative to oblique orientations) and the V1 BOLD response, while the form of meridian-relative anisotropy is consistent with previous findings of a radial bias. We conclude that the BOLD response in early human visual cortex displays anisotropies to both absolute and meridian-relative pattern orientation, and that further investigation is required to link the disparate forms of BOLD and behavioural anisotropies in absolute pattern orientation.

ORAL-02-07

VISUAL MOTION GRADIENT SENSITIVITY REVEALS INTEGRATION PROPERTIES OF GLOBAL MOTION PERCEPTION

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We investigated the properties of the perceptual integration of visual motion psychophysically using band-pass filtered noise stimuli containing spatially segregated bands of motion. The bands were created by adding together two noise samples moving in opposite directions which were each multiplied by out of phase contrast modulators which determined the dominant motion direction in each spatial band (Watson, A.B., & Ahumada, A.J. JOSA, A(2) 322-342. 1985). A 2-AFC detection task containing a noise interval with motion but no modulators and a signal interval with the modulators present was used to characterise the integration properties by obtaining the luminance and motion contrast detection thresholds for the stimulus. We found that psychophysical observers have optimal sensitivity to stimuli in which the ratio of carrier to modulator spatial frequency is a factor of 10. This ratio is consistent with a mechanism involving two neural processing stages, one in Primary Visual cortex and the other in the Middle temporal area. Both luminance and motion contrast sensitivity showed similarities in tuning properties probably because of their similar effect on the signal strength of the local motion detection stage.

ORAL-02-08

LOW INTENSITY TRANSCRANIAL MAGNETIC STIMULATION CAN IMPROVE DETECTION OF VISUAL STIMULI

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Transcranial Magnetic Stimulation (TMS) of visual cortex can induce an illusory visual percept known as a phosphene. The phosphene threshold is the level of TMS which induces a phosphene on an arbitrary proportion of trials (typically 50%). Stimulation above the phosphene threshold usually impairs the detection of visual stimuli. We examined the effect of magnetic stimulation of the visual cortex at or below the phosphene threshold on the detection of visual stimuli. Four human participants completed a two-interval two-alternative forced-choice task detecting plaid patterns which were presented at the position that coincided with the perceived location of the phosphenes. The contrast of the plaid varied from trial to trial according to an adaptive staircase procedure that optimised the information gain on each trial. TMS was delivered to the occipital lobe 120 ms after the stimulus onset. We found that the stimulus detection was better, compared with control stimulation (Cz), when the occipital lobe was stimulated at 95-100% of phosphene threshold. The results suggest that TMS at or below the phosphene threshold can act as a pedestal and improve the detection of visual stimuli.

ORAL-03-01

REGIONALLY-SPECIFIC CHANGES IN LEVELS OF TUMOUR NECROSIS FACTOR IN THE DORSOLATERAL PREFRONTAL CORTEX OBTAINED POSTMORTEM FROM SUBJECTS WITH MAJOR DEPRESSIVE DISORDER

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Background: Peripheral studies have reported changed levels of tumour necrosis factor (TNF) in individuals with major depressive disorders (MDD). These data were interpreted as suggesting a role for proinflammatory pathways in the pathophysiology of depressive symptoms[1]. We therefore measured levels of TNF in the frontal cortex of subjects with MDD. **Methods:** Tissue homogenates were prepared from Brodmann's areas (BA) 24 and 46 from the left hemisphere of 10 subjects with MDD and 10 control subjects. Levels of trans-membrane (tmTNF) and soluble TNF (sTNF) were measured by Western blot analysis. **Results:** tmTNF was significantly increased in BA 46 (mean±SEM: 7.70 ± 0.92 vs. 3.18 ± 0.87; ratio internal control (RIC), $p < 0.001$), but not BA 24 (2.80 ± 0.53 vs. 1.50 ± 0.23 RIC, $p = 0.15$), from subjects with MDD compared to controls. There was no change in levels of sTNF in either region (BA 46: 1.60 ± 0.10 vs. 1.50 ± 0.23; $p = 0.55$; BA 24: 1.40 ± 0.13 vs. 1.12 ± 0.10; $p = 0.11$). **Conclusions:** Our data supports the hypothesis that changes in pro-inflammatory pathways may be involved in the pathophysiology of MDD and that targeting these pathways may be a new approach to treating the disorder[1]. Further experiments to determine the extent of changes in tmTNF in MDD and whether levels of that protein are changed in other psychiatric disorders are ongoing.

[1]Leonard, B.E. (2009) Hum.Psychopharmacol. 3:165-175.

ORAL-03-02

THE BRAIN-SPECIFIC MICRORNA, MIR-128-2, BELONGS TO AN ACTIVITY-DEPENDENT GENE REGULATORY NETWORK ASSOCIATED WITH FEAR EXTINCTION

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Extinction, the gradual reduction in conditioned fear responses generated by repeated presentation of a non-reinforced conditioned stimulus, is a process of inhibitory learning with enormous clinical importance, since it represents the explicit model of behavioural therapy for phobia, panic, and post-traumatic stress disorder. Understanding the neural mechanisms by which fear can be extinguished is crucial if we are to develop better therapeutic protocols for the treatment of affective disorders. MicroRNAs (miRs) are a newly discovered family of endogenous small non-coding RNAs (~23 nucleotides long) that regulate gene function either by degrading target mRNAs or by directly binding to the 3'UTR of protein-coding genes, resulting in post-transcriptional silencing. In the past decade, several lines of evidence have implicated various miRs in the pathogenesis of human brain disorders, including schizophrenia; however, their functional role in fear-related learning and memory remains unknown. We've discovered that the brain-specific microRNA, miR-128-2, is highly expressed within excitatory cortical neurons that express its host gene, regulator of calcium signaling (RCS), and that are innervated by dopamine within the infralimbic prefrontal cortex (ILPFC); a brain region heavily involved in encoding fear extinction memories. We also show that, unlike the non-specific expression pattern of brain-specific miR-134, miR-128-2 is preferentially increased in the ILPFC after extinction but not after the acquisition of conditioned fear. Using an in vivo lentiviral-mediated gene transfer approach and in vitro luciferase assay, we are currently testing the functional relationship between miR-128-2 and several predicted target genes known to be involved in learning-related synaptic plasticity. Our data suggest an important role for miR-128-2 in regulating long-term memories associated with fear extinction.

ORAL-03-03

MAPPING MICROGLIAL ACTIVATION FOLLOWING CHRONIC STRESS AND EXPLORING ITS IMPLICATIONS FOR THE REGULATION OF MOOD STATE

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A number of recent reports have identified that exposure to stress can both structurally and functionally alter microglia. Microglial cells are pivotal to the production and maintenance of a neuroinflammatory state in the brain. These cells are the first line of defense against pathogens and other threats to the integrity of CNS and have pronounced cytotoxic capabilities and have consistently been identified as major contributors in several neurodegenerative disorders. As stress is recognized to be a major antecedent of mood disorders and in particular depression our research group has begun to examine the relationship between stress induced microglial changes and alterations in mood state in the rat. Across several experiments we have identified that chronic stress, relative to brief handling, (i) decreases an animal's preference for sucrose (ii) reduces their motivation to explore a novel environment while (iii) significantly increasing microglial activation (as indicated by ionized calcium binding – 1 labelling) in several mood regulatory forebrain nuclei including the medial prefrontal cortex and amygdala ($p<0.05$ in all cases). Additionally, we have now observed that increased levels of microglial activation in the medial prefrontal cortex are significantly correlated with enhanced levels of neuronal activity, as indicated by delta-fosB labeling ($p<0.05$). This result indicates that repeated or excessive neuronal activation may contribute to microglial activation. Currently, our group is now functionally characterizing, using a variety of ex-vivo techniques, the inflammatory status of microglia within the mood regulatory nuclei where we have observed differences following exposure chronic stress.

ORAL-03-04

OLANZAPINE-INDUCED METABOLIC DYSFUNCTION: A ROLE FOR CENTRAL MUSCARINIC M3 RECEPTORS

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Olanzapine has proven efficacy in controlling the positive and negative symptoms of schizophrenia, but can cause body weight gain and diabetes by unknown mechanisms. The arcuate (Arc) and ventromedial hypothalamic nucleus (VMH), and dorsal vagal complex (DVC) of the brainstem are key areas regulating energy balance and glucose homeostasis. Antipsychotic affinity to muscarinic M3 receptor (M3R) can be used to predict its weight gain and diabetogenic liability. However, the effects of olanzapine on central M3R are unknown. METHODS: Rats were treated with olanzapine (0.75, 1.5, 3.0, 6.0mg/kg/day, orally 3x/day, n=12/group) or vehicle (control) for 14-days. M3R binding density (n=6/group) was measured in the Arc, VMH and DVC. Correlations between M3R binding density and plasma concentrations of ghrelin and insulin were examined. RESULTS: Olanzapine significantly increased M3R binding density in the Arc (1.5-6.0mg/kg/day), VMH (0.75-6.0mg/kg/day) and DVC (1.5-6.0mg/kg/day). Body weight gain, food intake and ghrelin were increased, whereas insulin decreased. M3R binding density in the Arc and DVC (but not the VMH) positively correlated to body weight and food intake. M3R binding correlated to ghrelin, but not insulin. CONCLUSION: Increased M3R binding density may be a compensatory up-regulation in response to olanzapine antagonism. Olanzapine increased M3R binding density in regions of the brain that regulate metabolism. Increased M3R in these regions may enhance ghrelin secretion, which stimulates food intake and subsequent weight gain. Decreased insulin despite increased central M3R suggests peripheral M3R located on pancreatic beta cells may have a greater impact on insulin secretion than central M3R following short-term olanzapine treatment.

ORAL-03-05

ANTIPSYCHOTICS REDUCE THE POWER OF ONGOING GAMMA FREQUENCY OSCILLATIONS, BUT ONLY LY379268 ATTENUATES KETAMINE–INDUCED INCREASES IN GAMMA POWER

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A single non-anaesthetic injection of ketamine, a non-competitive NMDA receptor (NMDAr) antagonist with hallucinogenic properties, induces cognitive impairment and psychosis in healthy volunteers, and aggravates schizophrenia symptoms in patients. In conscious rats, an equivalent dose of ketamine increases the power of ongoing gamma oscillations in the neocortex and concomitantly induces abnormal behaviour, including ataxia and hyperlocomotion, key features of animal models of acute psychosis. This study investigated whether NMDAr antagonist-induced aberrant gamma oscillations were reversible with antipsychotic treatment. Extradural brass electrodes were surgically implanted into the skull of adult male Wistar rats. After recovery, rats were connected to ECoG recording electrodes and placed in an open arena for 30 minutes (baseline recording). They were then administered either clozapine (1-5 mg/kg sc, n=7), haloperidol (0.05 – 0.25 mg/kg sc; n=8), LY379268 (0.3 – 3 mg/kg sc; n=5) or the appropriate vehicles, and 30 minutes later received an injection of ketamine (5mg/kg sc) or vehicle. Quantitative measures of gamma power and locomotor activity were assessed throughout the experiment for all rats. All doses of antipsychotics rapidly and significantly reduced the power of ongoing gamma oscillations by 30-50%, an effect most prominent after LY379268. Ketamine produced an increase in gamma power, but only pretreatment with LY379268 was able to attenuate this effect of ketamine. All antipsychotics were able to inhibit ketamine-induced locomotor activity. The present study suggests that antipsychotics reduce cortical gamma power, a property which may be related to their clinical efficacy. The exclusive ability of the newer generation antipsychotic, LY379268, to inhibit the rise in gamma power elicited by ketamine may be related to its stabilising effects on cognitive function.

ORAL-03-06

DIFFERENTIAL EFFECTS OF THE SYNTHETIC CANNABINOID HU210 IN ADOLESCENT AND ADULT BRAIN: FOCUS ON SEROTONIN

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The endocannabinoid and serotonin systems share a high level of overlap in terms of the physiological processes they regulate (including mood, anxiety and cognition) however little is known about their functional interactions particularly during adolescence, a vulnerable period for both the development of psychosis and for initiation to substance use. In the present study, the effects of cannabinoid treatment on serotonin $5HT_{1A}$ receptor density were investigated in two age groups: Adolescent (postnatal day 35) and adult (postnatal day 70) rats were injected with the synthetic cannabinoid HU210 (25, 50 or 100 µg/kg) or vehicle for 1, 4 or14 days and sacrificed 24 hours after the last injection. $5HT_{1A}$ receptor density was measured in different brain regions using [^{3}H]8-OH-DPAT and quantitative autoradiography. A single high-dose (100 µg/kg) HU210 treatment did not produce any effect on $5HT_{1A}$ receptor binding in any of the brain regions examined. In contrast, subchronic (4 days) high dose HU210 treatment increased $5HT_{1A}$ receptor density in the CA1 region of the hippocampus by 23% ($p<0.01$) whereases chronic (14 day) treatment with 50 µg and 100 µg resulted in increase $5HT_{1A}$ receptor binding in the CA1 (22%, $p<0.05$) and dentate gyrus (26%, $p<0.01$) of the hippocampus respectively. Interestingly, the same treatment regimen did not affect $5HT_{1A}$ receptor binding in the brain of adolescent animals in any of the brain regions examined. Similar effects were observed on $5HT_{1A}$ mRNA expression measured in adjacent brain sections to those used in the present study. These results suggest that the adolescent brain seems to be less sensitive to the effects of cannabinoids compared to the adult brain, in agreement with previous data from our group showing that adolescent animals fail to reduce their cannabinoid CB1 receptors after exposure to a CB1 agonist, to the same degree as adults do. The effects in the adult brain may compromise hippocampal functioning and may account for the cognitive deficits seen in habitual heavy cannabis users.

ORAL-03-07

COGNITIVE ENHANCEMENT BY $GABA_C$ RECEPTOR ANTAGONISTS

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Phosphinic acid analogues of the nootropic $GABA_{B/C}$ receptor antagonist SGS742 are potent and selective $GABA_C$ receptor antagonists. Examination of these analogues may help elucidate a role for hippocampal $GABA_C$ receptors in memory processing. (R)- and (S)- 4-(aminocyclopent-1-enyl)-butylphosphinic acid (ACPBPA) were examined for cognitive enhancement in 8-9 week old male Swiss mice using the novel object recognition test. During training (10min) the amount of time spent with two identical objects was recorded. 24-hours later during the retention trial (10min), the amount of time spent exploring one familiar and one novel object was recorded and a recognition index (RI) calculated. Compounds were injected ip 20min prior to training to examine effects on learning. Compounds were injected ip immediately post training to examine effects on memory. Results showed that (R)- and (S)-4-ACPBPA can improve learning with a significant enhancement of the retention RI for both compounds at doses of 10 and 100mg/kg ($n=10$, $P<0.05$ and $P<0.001$ respectively), compared to saline. 100mg/kg of both compounds when administered post training show a significant enhancement of the retention RI ($n=10$, $P<0.01$), compared to saline. 100 mg/kg of the compounds were also examined to determine if they could reverse amnesia induced by 0.3mg/kg scopolamine, or by 4mg/kg baclofen. (R)-4-ACPBPA significantly reversed the chemically induced amnesia of scopolamine and baclofen ($n=10$, $P<0.001$ and $P<0.01$ respectively). (S)-4-ACPBPA was not as effective reversing the amnesia by scopolamine ($n=10$, $P<0.01$), but was as effective as (R)-4-ACPBPA when administered with baclofen ($n=10$, $P<0.01$). The nootropic properties of (R)- and (S)-4-ACPBPA indicate an involvement of the $GABA_C$ receptors in memory processing.

ORAL-03-08

CHEMOTHERAPY CAUSES MEMORY AND EXECUTIVE FUNCTION DEFICITS IN RATS

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Improvements in cancer care mean people are living longer post treatment. However, a significant subset of patients who received adjuvant chemotherapy as a part of their treatment experience problems in working memory, executive function and processing speed that persist for 5-10 years after treatment has finished. This so-called chemo-brain is a cause of considerable distress and disability for some cancer survivors. However, it has been tricky pulling out a causative role for chemotherapy in this cognitive decline, as there are many other psychosocial or disease-related reasons why cancer patients and survivors may show impairments on standardised neuropsychiatric tests. Here we show that laboratory rats (Wistars) show similar deficits in rodent models of memory and executive function after treatment with a range of chemotherapy drugs. In particular, rats injected with methotrexate (n=10; 200mg/kg i.p.) show deficits in the novel object test (NOR; recognition memory), the water maze (WM; long-term memory), and in a delayed go-nogo task (executive function and working memory). These deficits were apparent for 9 months after treatment. In addition, rats injected with oxaliplatin and 5-flurouracil (FOLFOX; n=10) show deficits in the NOR, in the WM, and fear conditioning (long-term memory). In separate studies, rats injected with oxaliplatin alone (n=10) showed deficits in learning contextual discriminations within 2 months of treatment, and reduced performance in a Pavlovian-to-Instrumental Transfer test at 9 months after treatment. Taken together, these results demonstrate that chemotherapy has a direct and persistent impact on a range of cognitive functions in isolation from psychosocial distress or other disease-related complications.

ORAL-04-01

IDENTIFICATION OF COMPONENTS OF A GLUTAMATE HOMEOSTASIS COMPLEX IN ASTROCYTES

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Efficient glutamate transport into astrocytes is essential for termination of glutamatergic neurotransmission. Uptake by proteins such as GLAST (EAAT1) requires a sodium gradient, formed by the actions of a sodium-potassium ATPase (NKA), and the rapid catabolism of the accumulated glutamate by glutamine synythetase (GS). We demonstrate, by co-immunoprecipitation experiments (n=12) that GLAST associates with NKA α 1 in the rat brain. GST-pulldown assays demonstrate that the cytoplasmic loop region (residues 350 – 775) of NKA α 1 pulls down GLAST from brain lysate. Similarly, the extreme C-terminal tail (residues 513 -543) of GLAST pulls down NKA α 1 from brain lysate. Confocal microscopy studies revealed that GLAST co-localised with NKA α 1. We have examined D-aspartate uptake in transfected Cos-7 cells to study the effects of co-expression of GLAST and a construct encoding the cytoplasmic loop region (residues 350 – 775) of NKA α 1. The construct was predicted to block normal interactions between GLAST and NKA α 1. Co-expression led to a significant decrease (~20%) in D-aspartate uptake when compared to the control. The interaction between the cytoplasmic loop region of NKA α 1 and the C-terminal tail of GLAST is likely to involve other accessory proteins. Similarly we demonstrate by immunoprecipitation studies that the enzyme GS is part of this multi-molecular complex. We have previously demonstrated that NHERF1 anchors GLAST to the cytoskeleton, and confirm here that NHERF1 is part of the complex containing GS, GLAST and NKA α 1. These findings indicate that multiple protein interactions may be required for efficient glutamate transport and that these interactions may represent novel pharmacological targets in conditions such as stroke.

ORAL-04-02

PHENYLALANINE 124 AT $GABA_C$ RHO1 RECEPTORS INFLUENCES THE ON-OFF TIME CONSTANTS FOR GABA

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$GABA_C$ rho1 receptors are members of the Cys-loop receptor superfamily that includes nicotinic acetylcholine, 5-hydroxytryptamine type 3, $GABA_A$ and glycine receptors. The agonist and/or competitive antagonist binding site of Cys-loop receptors is formed by residues within the N-terminal region and is located at the interface of two subunits. Five discontinuous regions of the N-terminal, referred to as loops A-E, have been shown to be critical for agonist binding to rho1 receptors. Loops A-C are located on one subunit, while loops D and E form the complementary region on the adjacent subunit (Sedelnikova et al., J. Biol. Chem. 2005, 280: 1535). Phenylalanine 124 (F124) is located within loops A and D on rho1 receptors. Modelling studies predict that this residue may form part of a cavity that binds agents such as gabazine (Abdel-Halim et al., Chem. Biol. Drug. Des. 2008, 71: 306). In this study, we investigated the influence of this residue on receptor activity for a series of agonists and antagonists. F124 was mutated to tyrosine and valine. GABA affinity was significantly reduced at the mutant receptors compared to wild-type (rho1F124Y:EC_{50}=2.5±0.1μM; rho1F124V: EC_{50}=2.5±0.1μM; and WT: EC_{50}=1.0±0.1μM; $p<0.05$; n=3-4 oocytes). In contrast, gabazine activity was unchanged at rho1F124Y, but was 3-times weaker at rho1F124V. Interestingly, the GABA on-off time constants were altered. In particular, the deactivation time constant was reduced by 5-fold at rho1F124Y receptors compared to wild-type (n=3-8). Thus, F124 may impact on the strength of the interactions of GABA within the binding site.

ORAL-04-03

DIFFERENTIAL EXPRESSION OF GLYCINE RECEPTOR SUBUNITS IN THE RAT BASOLATERAL AND CENTRAL AMYGDALA

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The amygdalar complex is a limbic structure that plays a key role in emotional processing and fear conditioning. Although inhibitory transmission in the amygdala is predominately GABA-ergic, neurons of the amygdala are also known to express glycine receptors. The subtype and function of these glycine receptors within the synaptic circuits of the amygdala is unknown. In this study, we have investigated the relative expression of the four major glycine receptor subunits (α1-3 and β) in the rat basolateral (BLA) and central amygdala (CeA), using real-time PCR and protein biochemistry, and characterised the subunit composition of functional glycine receptors expressed by the major cell types in the BLA and CeA using subunit specific pharmacology. We demonstrate that α1, α2, α3, and β subunits are all expressed in the BLA and CeA with α2 being the predominant α-subunit in both nuclei. Electrophysiological recordings from BLA and CeA neurons in acute brain slices indicated that differences in relative expression of these subunits were correlated with the pharmacological properties of native glycine receptors expressed on these neurons. We conclude that glycine receptors assembled in BLA neurons are largely α1β-containing heteromultimers whereas receptors assembled in neurons of the central amygdala are primarily α2β-, α3β- or α1β-containing heteromultimers, with a minor component of α2 or α3 homomeric receptors also expressed.

ORAL-04-04

LOOP 9 RESIDUES ADJACENT TO LOOP 2 ARE INVOLVED IN GLYCINE RECEPTOR ACTIVATION

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Inter-subunit interactions within the extracellular domain of Cys-loop receptors, such as the glycine receptor (GlyR), are important for receptor activation properties. Our GlyR homology model predicts inter-subunit contacts between loop 9 and loop 2 of adjacent subunits. Our aim was to investigate the role of this interaction. In particular, residues L184 and Q186 (loop 9) are predicted to be within 4-5Å of T55 and M56 (loop 2). Cysteine residues were introduced at these positions alone and in pairs. The observed glycine EC_{50} was only modestly increased in M56C, L184C and Q186C mutants compared to wild-type (*n*=4-6), but was increased by 29-fold in T55C. The availability of cysteines for covalent modification was investigated using MTSES or MTSET. Changes in current evoked by EC_{30} and I_{max} glycine concentrations were monitored. MTSET application had no effect on M56C and Q186C, but increased the current response to an EC_{30} concentration in T55C (*n*=4). In contrast, the current response of L184C to EC_{30} and I_{max} concentrations was decreased (*n*=4). MTSES application had no effect on T55C and Q186C, but increased the EC_{30} current and decreased the I_{max} response of M56C and L184C (*n*=4). No glycine-evoked currents were detected for the double mutants T55C/Q186C and M56C/L184C (*n*=4). These results suggest either Q186C is not accessible to MTS reagents or the covalent modification has no functional effects. L184C is covalently modified and demonstrates differential effects of charge. Our results confirm the role of loop 2 in gating and suggest a role for loop 9 in GlyR gating.

ORAL-04-05

MODULATION OF KCC2 FUNCTION BY TYROSINE PHOSPHORYLATION

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Neuronal chloride homeostasis by the transporters NKCC1 and KCC2 plays a critical role in determing the response to the transmitters GABA and glycine. The expression levels of KCC2 decrease in response to neuronal injury, but less is known about the mechanisms mediating more dynamic modulation of KCC2 function. Using gramicidin-perforated patch-clamp recordings and the GABA reversal potential (E_{GABA}) as a measure of intracellular Cl^-, we investigated how tyrosine phosphorylation affects KCC2 function (Watanabe et al., 2009, J Biol Chem 284, 27980-8). Application of the tyrosine kinase inhibitor genistein (100 µM) to cultured hippocampal neurons results in a positive shift of E_{GABA} by 10.5 ± 1.2 mV (n=5). Transfecting KCC2-EGFP into GT1-7 cells resulted in Cl^- efflux and a more negative E_{GABA} (-66.6 ± 2.4 mV, n=6) compared to untransfected cells (-36.6 ± 0.9 mV, n= 5). In GT1-7 cells, genistein also shifted E_{GABA} to more positive values (n=2, by 10 mV and 15 mV). Mutating the putative KCC2 tyrosine phosphorylation site (Y1087D) resulted in non-functional KCC2 (E_{GABA} was -36.6 ± 0.9 mV, n=5). This mutation also resulted in a translocation of surface KCC2 from a punctate pattern associated with lipid rafts, to a more diffuse distribution. Neuronal stress is known to induce a rapid loss of KCC2 tyrosine phosphorylation and transport function (Wake et al., 2007, J Neurosci, 27, 1642-1650) and we conclude that 1) GT1-7 cells represent a good cellular model for further studies of the regulation of KCC2 in models of neuronal injury, and 2) loss of KCC2 tyrosine phosphorylation is associated with loss of function and altered membrane surface localisation.

ORAL-04-06

THE EPILEPSY ASSOCIATED $GABA_A$ RECEPTOR γ_2R43Q MUTATION INCREASES SENSITIVITY TO ZN^{2+} INHIBITION

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$GABA_A$ receptors mediate rapid inhibitory signalling in the central nervous system, and mutations in various subunits of these pentameric receptors are associated with epilepsy. Receptors composed of α and β subunits are highly sensitive to inhibition by extracellular Zn^{2+} ions; however incorporation of a γ subunit disrupts two of three known Zn^{2+} binding sites and greatly reduces Zn^{2+} sensitivity. Here we demonstrate that the epilepsy associated γ_2(R43Q) mutation greatly increases the susceptibility of heterologously expressed receptors to Zn^{2+} inhibition while preserving functional characteristics underpinned by presence of the γ_2 subunit. $\alpha_1\beta_2\gamma_2$ receptors are believed to contain a single N-terminal Zn^{2+} binding site. Mutation of residues contributed to this site by the α subunit ameliorated the effect of γ_2(R43Q) on Zn^{2+} sensitivity, indicating that γ_2(R43Q) allosterically affects Zn^{2+} binding or affects signal transduction, rather than directly interacting with Zn^{2+}. This assertion was bolstered by the increased Zn^{2+} sensitivity of mutations predicted, by molecular modelling, to interact with γ_2(R43Q). We also examined other epilepsy-associated γ_2 mutations, γ_2(K289M) and γ_2(R139G), and found that they did not substantially increase sensitivity to Zn^{2+} inhibition. Increased Zn^{2+} sensitivity may be physiologically important in hippocampal neurones, where synaptic Zn^{2+} reaches high enough levels to modulate GABAergic signalling, and may represent a novel mechanism underlying the increased occurrence of febrile seizures reported in patients harbouring the γ_2(R43Q) mutation.

ORAL-04-07

ALTERED CRAC CHANNEL GATING IN THE ORAI1 E106D MUTANT

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Ca^{2+}-release activated Ca^{2+} (CRAC) channels represent the main avenue for Ca^{2+} entry in non-excitable cells, and play an important role in Ca^{2+} dependent processes of both excitable and non-excitable cells, such as immune cell differentiation, regulating vascular smooth muscle tone, and exocytosis in neurons. The minimal molecular components of functional CRAC channels are Stromal interaction molecule 1 (STIM1), a Ca^{2+} binding protein that plays the role of Ca^{2+} sensor, and Orai1, the pore forming subunit. Investigation of the structure of Orai1 has identified key residues in the transmembrane domains that control the selectivity and gating of CRAC channels (1). In this work we have generated and expressed V102I, E190Q and E106D mutants along with STIM1 in H4IIE rat liver cells to investigate their selectivity and gating. In contrast to previous reports we have found that that V102I and E190Q mutant channels retain normal fast Ca^{2+}-dependent inactivation when co-expressed with saturating amounts of STIM1, similarly to WT (2). Compared to WT Orai1, E106D mutation, however, shifted voltage dependence of gating to more positive potentials by 80 mV ($p<0.01$, n=10) and significantly reduced the time constant of current inactivation at negative potentials ($p<0.01$, n=6). Furthermore, while WT Orai1 is highly selective for Ca^{2+} over Na^+ and is completely blocked at pH 6.0, we have found that E106D conducts Na^+ in the presence of Ca^{2+} and is not blocked by low pH. These results show that glutamate at position 106 in WT Orai1 determines selectivity of the channel and its dependence on external pH. 1. Yamashita *et al.* (2008) *J Gen Physiol.* **13**:525-40 2. Scrimgeour *et al.* (2009) *J Physiol.* **587**:2903-18.

ORAL-04-08

FURTHER ANALYSIS OF COUNTERION PERMEATION THROUGH ANION CHANNELS: LIQUID JUNCTION POTENTIALS AND OFFSET CORRECTIONS

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To determine how positive counterions permeate through anion-selective channels it is necessary to accurately determine their anion-cation permeability ratios. These are readily obtained from whole-cell patch-clamp situations by determining the shifts in zero-current reversal potentials in salt solution dilutions, with the Goldman-Hodgkin-Katz (GHK) equation used to analyse the data. We have already shown that the anion-cation permeability ratios (P_{Cl}/P_{cation}) of wild-type (WT) and mutant (with larger pore diameter) glycine receptor (GlyR) channels in the presence of Li^+, Na^+ and Cs^+ counterions, were inversely related to counterion hydration diameter, with P_{Cl}/P_{cation} increasing as hydration diameter approached channel minimum pore diameter (Sugiharto et al., 2008; *Biophys. J.* 95, 4698-4715). Corrected for liquid junction potentials (LJPs; using ion activities), the P_{Cl}/P_{cation} values were 23.4±2.8 (n=6; LiCl), 10.9±0.3 (n=32; NaCl) and 5.0±0.5 (n=6; CsCl) for the smaller WT channel (note that ignoring LJPs reduced each permeability ratio to about 4). Further analysis to incorporate an initial potential offset correction, to fully allow for slight differences between internal cell composition and external control salt solution, changed the above P_{Cl}/P_{cation} values to 29.0±4.4 (LiCl), 11.8±0.4 (NaCl) and 5.0±0.5 (CsCl), adding enhanced support for the relationship between counterion permeation and the difference between pore diameter and ion hydration size. Also, new direct measurements of LJPs (e.g., for NaCl salt dilutions) using a 3M KCl-agar reference salt bridge (with freshly-cut end for each solution change) have shown precise agreement (within 0.1 mV experimental error) with calculated LJPs (using ion activities), validating such calculated values. We suggest that counterion cations permeate with chloride ions as neutral pairs.

ORAL-05-01

THE TAU AMINO-TERMINUS IN Aβ TOXICITY IN MOUSE MODELS OF ALZHEIMER'S DISEASE

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The microtubule-associated protein tau is predominantly found in axons. Both tau and β-amyloid (Aβ) form aggregates in Alzheimer's disease (AD) brains. Aβ mediates the tau pathology, but at the same time Aβ toxicity is tau-dependent. To further address this interplay, we have generated novel transgenic mice expressing truncated tau that lacks the microtubule binding domains (Δtau74). Although Δtau cannot bind to microtubules, Δtau localized to the cell membrane of cell bodies, but was virtually excluded from diffusing into dendrites, suggesting interaction with proteins that are restricted to the cell body. Crossing Δtau74 mice with mutant human APP expressing mice (APP23) rescued the premature mortality of APP23/Δtau74 mice to the same extend as hetero-deficiency of tau in APP23/tau+/- mice. This was accompanied by a markedly reduced susceptibility to excitotoxic seizures of Δtau74 mice. Interestingly, we found that similar molecular mechanisms lead to the improved resistance to excitotoxicity in tau-deficient and Δtau expressing mice. In conclusion, our findings shed new light on how tau is involved in mediating Aβ toxicity.

ORAL-05-02

UPPER AIRWAY DYSFUNCTION AND BRAINSTEM TAUOPATHY IN AGEING TAU-P301L MICE: POSSIBLE IMPLICATION FOR ALZHEIMER DISEASE

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Tauopathy comprises hyper-phosphorylation of the microtubule associated protein tau causing intracellular aggregation and accumulation as neurofibrillary tangles and neuropil treads. Primary tauopathies are linked to mutations in the *MAPT* gene coding for protein tau. In Alzheimer's disease, the most frequent secondary tauopathy, neither the cause nor the pathological mechanisms are understood. Transgenic mice expressing mutant Tau-P301L suffer cognitive and motor defects and die prematurely from unknown causes. Here, using a multidisciplinary approach, we report that ageing Tau-P301L mice also suffer upper airway dysfunction. First, *in vivo* plethysmography showed normal breathing pattern in twelve young Tau-P301L mice (age 3 months) but significant ($p<0.05$) upper airway dysfunction in eighteen older Tau-P301L mice (age 8 months) with dramatically reduced airflow and enlarged chest movements. Second, *in situ* electrophysiology in six old Tau-P301L mice compared to age-matched non-transgenic mice, revealed significant abnormal laryngeal motor discharges. Third, immunohistology in seven old Tau-P301L mice showed accumulations of hyper-phosphorylated and aggregated protein tau in brainstem nuclei involved in upper airway motor control, i.e. Kölliker-Fuse, periaqueductal grey, and intermediate reticular nuclei. Thus, our results in ageing Tau-P301L mice demonstrate a pathological link of upper airway dysfunction and aggregated tau in identified neural circuits. This is of interest for understanding breathing and upper airway alterations in patients suffering from tauopathy, including Alzheimer's disease.

ORAL-05-03

RESCUE OF SPATIAL LEARNING PERFORMANCE IN ALZHEIMER'S DISEASE MICE BY KNOCKDOWN OF THE P75 NEUROTROPHIN RECEPTOR

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We investigated spatial learning in 14 month old Tg2576 Alzheimer's disease (AD) model mice that were made heterozygous for a null mutation in the p75 neurotrophin receptor (p75),and thus carried just one working copy of the p75 gene. Their performance was compared to Tg2576 mice that carried the normal two copies of the p75 gene. Tg2576 mice have been documented to show impaired performance in various behavioral tests, including tests of spatial learning, from 10 months of age. This is accompanied by considerable amyloid deposition in the brain. To ensure that all mice in our study had identical genetic backgrounds, Tg2576 and p75 knockout mice were each backcrossed onto the inbred 129Sv strain until they achieved congenicity with the strain. They were than mated together, and Tg2576 mice that were heterozygous for p75 were selected and retained until the age of 14 months. Mice were then tested on the Barnes Maze, a test of spatial learning and memory that reflects hippocampal function. As expected, 14 month old Tg2576 mice performed poorly. After 8 days, only 7% of Tg2576 mice had reached the learning criterion. By the end of the 10-day trial, only 27% had reached the criterion. In contrast, 73% of 14 month old Tg2576/p75$^{+/-}$ mice reached criterion by day 10. This difference in performance was highly significant ($p = 0.007$; Wilcoxon matched pairs test). Thus, genetic knockdown of p75 improved spatial learning and memory in this AD model. We are undertaking further studies to identify neurobiological correlates of the enhanced cognitive performance associated with p75 knockdown in Tg2576 mice.

ORAL-05-04

INTRAMUSCULAR DELIVERY OF A SINGLE CHAIN ANTIBODY GENE PREVENTS BRAIN Aβ DEPOSITION AND COGNITIVE IMPAIRMENT IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Anti-Aβ-amyloid (Aβ immunotherapy) is effective in removing brain Aβ but was associated with detrimental effects. We have demonstrated that AAV-mediated intramuscular delivery of an anti-Aβ single chain antibody (scFv) gene was as effective as intracranial delivery in clearing brain Aβ of old APPSwe/PS1dE9 transgenic mice. In the present study, we tested the efficacy and safety of intramuscular delivery of scFv gene in preventing brain Aβ deposition. ScFv gene was intramuscularly delivered at three months of age when the brain Aβ deposition was not formed. Six months later, we found that the transgenes were expressed in a stable form in the delivered sites, with small amount of ectopic expression in the liver and olfactory bulb. Brain Aβ plaque formation, Aβ accumulation, AD-type pathologies and cognitive impairment were significantly attenuated in scFv-treated APPSwe/PS1dE9 transgenic mice relative to EGFP-treated mice. Intramuscular delivery of scFv gene was well tolerated by the animals, did not cause inflammation and microhemorrhage in the gene expression site and brain, and did not induce neutralizing antibodies in the animals. These findings suggest that peripheral application of scFv is effective and safe in preventing the development of Alzheimers disease (AD), and would be a promising non-inflammatory immunological modality for prevention and treatment of AD.

ORAL-05-05

Aβ DECREASES AMPARS ON THE CELL SURFACE BY INCREASING INTRACELLULAR CALCIUM AND PHOSPHORYLATION OF GLUR2

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α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are key regulators of synaptic functions and cognition. In Alzheimer disease (AD), the amount of AMPARs and activity are downregulated, but the mechanism of this downregulation is not clear. In the present study, we found that Aβ significantly decreased cell-surface glutamate receptor subunits 2 (GluR2), and increased the concentration of intercellular calcium. Blockers of L-type voltage-gated calcium channels (L-VGCC) attenuated the effect of Aβ on cell-surface GluR2 expression and cytosolic calcium, whereas activators of VGCC resulted in a decrease in cell-surface GluR2 similar to Aβ. Aβ and Bay K8644, an activator of L-VGCC significantly increased phosphorylation of serine-880 (S880) on GluR2, whereas blockers of L-VGCC decreased the effects of Aβ on S880 phosphorylation. Finally, we found that bisindolylmeimide I (GF 109203X, GFX), an inhibitor of protein kinase C (PKC) blocked the decrease in cell-surface GluR2 and the increase in phospho-S880 induced by Aβ or Bay K-8644. Taken together, these results demonstrate that Aβ decreases cell-surface AMPARs by increasing PKC-mediated phosphorylation of S880 on GluR2. Our data suggests that the rise in cytosolic calcium induced by Aβ could impair synaptic function by decreasing the availability of AMPARs at the synapse. This decrease in AMPARs could account for some of the decline in cognitive function seen in Alzheimer's Disease.

ORAL-05-06

COFILIN AND PHOSPHORYLATED TAU CO-LOCALIZE IN ALZHEIMER-LIKE CYTOSKELETAL INCLUSIONS TRIGGERED BY ENERGY DEPLETION OR AMYLOID PEPTIDES

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Purpose: In Alzheimer disease (AD), thread-like inclusions (neuropil threads) of phosphorylated microtubule-associated protein (pMAP) tau are generated in affected brain regions and correlate with cognitive decline and disease progression. Tau lesions are accompanied by cytoskeletal abnormalities that include rod-like cofilin accumulations (cofilin-actin rods). The relationship between these pathological structures is poorly understood, yet important for understanding the causes of sporadic AD. **Methods:** Primary neuronal cell culture (derived from human, chick or rat brain), confocal and electron microscopy were used to model the assembly of pathological cytoskeletal inclusions induced by neurodegenerative stimuli (mitochondrial inhibition, oxidative stress, exposure to oligomerized amyloid peptides). **Results:** We demonstrate that during mitochondrial inhibition or treatment with amyloid peptides, activated actin-depolymerizing factor (ADF)/cofilin assemble into rods along processes of cultured primary neurons that recruit pMAP/tau and mimic neuropil threads. Fluorescence Resonance Energy Transfer (FRET) analysis revealed co-localization of cofilin-GFP (green fluorescent protein) and pMAP in rods, suggesting their close proximity within a cytoskeletal inclusion complex. **Conclusions:** Our results suggest that cofilin-actin rods form a scaffold for the recruitment of pMAP thus revealing a common pathway for pMAP and cofilin accumulation in neuronal processes. We propose that neuropil thread structures in the AD brain may be initiated by elevated cofilin activation and F-actin bundling that can be caused by oxidative stress, mitochondrial dysfunction or Aβ peptides, all suspected initiators of synaptic loss and neurodegeneration in AD.

ORAL-05-07

THE EXCITOTOXIN QUINOLINIC ACID INCREASES TAU PHOSPHORYLATION. A NEW NEUROTOXIC MECHANISM FOR ALZHEIMER'S DISEASE

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Background: Some of the tryptophan catabolites produced through the kynurenine pathway (KP), and more particularly the excitotoxin quinolinic acid (QA), are likely to play a role in the pathogenesis of Alzheimer's disease (AD). We have previously shown that the KP is over activated in AD brain and that QA accumulates in amyloid plaques and within dystrophic neurons. We hypothesized that QA in pathophysiological concentrations affect tau phosphorylation. Methods & Results: Using immunohistochemistry, we found that QA is co-localized with hyper phosphorylated tau (HPT) within cortical neurons in AD brain. We then investigated in vitro the effects of QA at various pathophysiological concentrations on tau phosphorylation in primary cultures of human neurons. Using western blot, we found that QA treatment increased the phosphorylation of tau at serine 199/202, threonine 231 and serine 396/404 in a dose dependent manner. Increased accumulation of phosphorylated tau was also confirmed by immunocytochemistry. This increase in tau phosphorylation was paralleled by a substantial decrease in the total protein phosphatase activity. A substantial decrease in PP2A expression and modest decrease in PP1 expression were observed in neuronal cultures treated with QA. These data clearly demonstrate that QA can induce tau phosphorylation at residues present in the PHF in the AD brain. To induce tau phosphorylation, QA appears to act through NMDA receptor activation similar to other agonists, glutamate and NMDA. The QA effect was abrogated by the NMDA receptor antagonist memantine. Using PCR arrays, we found that QA significantly induces 10 genes in human neurons all known to be associated with AD pathology. Of these 10 genes, 6 belong to pathways involved in tau phosphorylation and 4 of them in neuroprotection. Conclusion: Altogether these results indicate a likely role of QA in the AD pathology through promotion of tau phosphorylation. Understanding the mechanism of the neurotoxic effects of QA is essential in developing novel therapeutic strategies for AD. (see PloS One 2009, e6344).

ORAL-05-08

DIFFERENT DOMINANT EFFECTS FROM DIFFERENT TRUNCATIONS OF PRESENILIN1

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The human *PRESENILIN1* (*PSEN1*) gene is the major locus for mutations causing familial Alzheimer's disease. Distinct mutations in *PSEN1* also cause frontotemporal dementia. Presenilin proteins facilitate the λ-secretase cleavage of APP to produce neurotoxic amyloidβ peptide. Presenilins also cleave Notch and many other transmembrane proteins and interact with GSK3β and tau to control tau phosphorylation. We use gene manipulation in zebrafish embryos and HEK293 cells to investigate the biochemical and phenotypic effects of changes in Presenilin splicing and protein structure. Previously, we showed that truncation of zebrafish Psen1 in the region of exon 7 sequence causes dominant suppression of Notch signalling. We now show that, in human cells, expression of Psen1 truncated after exon 4 sequence (similar to the homologous hypoxia-induced "PS2V" isoform of *PSEN2*) dramatically reduces APP-C-terminal fragments [n=3] suggesting marked alterations in APP cleavage. Further, truncation of Psen1 after exon 8 sequence apparently inhibits APP cleavage [n=3] but not Notch cleavage [n=4] indicating differential activity of such truncations on different signalling pathways. Psen1 protein truncations after exon 5, 6, 7 or 8 sequences all incorporate into SDS-resistant high molecular weight complexes in zebrafish [n=3]. Truncations after exon 5 sequence - as found in one characterised case of frontotemporal dementia (Pick disease) - are especially prone to incorporation into higher MW complexes in both zebrafish and human cells but, counter-intuitively, have least apparent effect on Notch [n=4] or APP [n=3] cleavage. This suggests that frontotemporal dementia mutations in *PSEN1* may interfere, primarily, with PSEN1's non γ-secretase activities.

ORAL-06-01

DEVELOPMENT OF AN IMPROVED IVERMECTIN-ACTIVATED RECEPTOR FOR NEURONAL SILENCING

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Purpose: Reversible silencing of particular neuron groups should elucidate functions of neurons within a circuit and could possibly tune excess neurotransmission in disease. One method is to express an inhibitory signalling receptor and activate that receptor with a selective pharmacological agent. The α1 glycine receptor (GlyR) is a ligand-gated ion channel mediating inhibitory neurotransmission, and is also activated by the antihelminth, ivermectin. We developed a mutant GlyR that is insensitive to glycine but sensitive to ivermectin. Methods: We used a high throughput fluorescence based assay to screen numerous mutant GlyRs expressed in HEK293 cells and found that one mutant with a single amino acid substitution was less sensitive to ivermectin than wildtype. Using site-directed mutagenesis we generated a mutant, A288G, that increased sensitivity to ivermectin. We combined this mutation with F207A, which is known to abolish glycine binding, to produce a receptor (FAAG) that is both insensitive to glycine and highly sensitive to ivermectin. We tested this new receptor by electrophysiology in HEK293 cells and cultured hippocampal neurons. Results: In HEK293 cells, FAAG showed 50-fold lower EC50 values than WT (FAAG, 19±6 nM; WT, 1.1±0.3 μM; n=5). FAAG was only activated by high millimolar concentrations of glycine (EC50 values, FAAG, > 10 mM; WT, 39±6 μM; n=5). Cultured neurons transfected with FAAG showed chloride currents upon application of 10 and 100 nM ivermectin, while current in WT-transfected neurons were only activated by application of 1 μM ivermectin and higher. Conclusions: FAAG appears to be a suitable pharmacological silencing receptor, as it conducts chloride current upon applications of low concentration ivermectin and is responsive to only high millimolar concentrations of glycine.

ORAL-06-02

FUNCTIONAL ENHANCER TRAPPING IN THE ZEBRAFISH OPTIC TECTUM

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One of the fundamental goals of neuroscience is to describe how the brain produces behaviour. This has proved difficult at the level of individual cell types because anatomical descriptions of single cells have traditionally not addressed behavioural function, and functional studies have generally studied regions rather than single cell types. Here, we present a flexible transgenic approach that offers one solution to this problem. We have carried out a Gal4 enhancer trap screen in zebrafish, and have generated 184 stable transgenic lines with interesting expression patterns throughout the nervous system. We continued with a further analysis of three lines with expression in the optic tectum. Detailed morphological analysis of single cells, using a genetic “Golgi-like” labelling method, revealed four common cell types (superficial, periventricular, shallow periventricular, and radial glial), along with a range of other less common neurons. We find that it is specifically periventricular neurons with dendrites in the deep tectal neuropil that generate tectal efferent projections to the reticular formation. Our results show that the larval tectum, both broadly and at the single cell level, represents a miniature version of its adult counterpart, and that it has all of the necessary anatomical characteristics to inform motor responses based on sensory input. This anatomical study in the tectum sets the stage for functional analyses of these cells’ roles in behaviour. Since they express Gal4, we can drive UAS-linked transgenes of our choice, including calcium and voltage sensors as probes of activity and channelrhodopsin and halorhodopsin for activating and silencing the neurons during behavioural assays. Our anatomical, functional, and behavioural results, along with this general approach for analyzing circuit structure and function, will be presented.

ORAL-06-03

EXTRACELLULAR RECORDING OF VISCEROFUGAL NEURONS IN COLON

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Viscerofugal neurons form the afferent arm of reflex circuitry between the gut and prevertebral ganglia that modulate gastrointestinal motility. The majority of studies to date have investigated viscerofugal function indirectly by recording of fast synaptic potentials in prevertebral ganglion nerve cell bodies. These studies have suggested viscerofugal neurons are first or higher order neurons that encode mechanosensory data. Extracellular recordings and biotinamide tracings were made of mesenteric nerves in isolated segments of distal colon. Preparations were used fresh or were maintained in organ culture for 3-6 days prior to recording to allow degeneration of extrinsic afferent axons. Biotinamide labelling indicated that the proportion of filled viscerofugal axons within colonic nerves increased from 8.6% (n=6) to 51.4% (n=5) after 3-5 days of organ culture, reflecting degeneration of extrinsic axons. Most preparations showed viscerofugal neuron firing in coordinated bursts (8 of 10 preparations) with interburst intervals of 2.0 – 2.6s (mean: 2.42 ± 0.24). Application of hexamethonium (100 micromolar), but not nicardipine with hyoscine (both at 1 micromolar) blocked burst firing. Viscerofugal neurons could be directly activated by DMPP (100 micromolar applied locally). In fresh preparations, capsaicin (0.3 micromolar) caused vigorous firing: in cultured preparations it evoked no response, confirming degeneration of extrinsic sensory neurons. Two of 6 preparations showed modest increases in firing during circumferential stretch, manifested as an increase in spikes per burst. Identified viscerofugal neurons have been recorded extracellularly; they are clearly activated by cholinergic enteric neuronal pathways but it is not clear whether they are directly mechanosensitive.

ORAL-06-04

HIGH RESOLUTION FIBRE OPTIC COLONIC MANOMETRY IN PATIENTS WITH SLOW TRANSIT CONSTIPATION INDICATES THE POTENTIAL OF SIGNAL ALIASING AND MISINTERPRETATION OF PROPAGATING EVENTS

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Colonic manometry has helped define dysmotility in patients with constipation. However, measurement of human colonic motility poses substantial methodological challenges and therefore understanding of both physiology and pathophysiology of colonic motor patterns remains relatively primitive. This is partly due to poor spatial resolution of recording sites in current colonic manometry catheters. Typically colonic manometry is recorded with sensor spacing ≥7cm, potentially twice that of many of the propagated events, which may result in short propulsive activity being missed. To overcome this we have developed a unique fibre-optic manometry catheter with up to 144 sensors at 1 cm interval. Preliminary prototypes of this highly flexible catheter have been used in 3 female patients with slow transit constipation. The catheters were positioned colonoscopically and clipped to the caecum or mid-transverse colon. Manometric recordings were made over a 24hr period and antegrade and retrograde propagating sequences (PS) were identified. Data from 7 and 10cm spaced sensors were analysed and compared to 1cm spaced data. At 7cm spacing there was a 26% reduction in antegrade and 49% reduction in retrograde PSs identified compared to the high resolution 1cm spaced data. At 10cm spacing this difference was more pronounced with a 78% and 96% reduction in antegrade and retrograde PSs. The data demonstrate the potential of aliasing (incorrect interpretation of pseudo-periodic signals due to inadequate sample rates) of PSs when inadequate sensor spacing is used. High resolution colonic manometry may help to correctly identify specific biomarkers of colonic disorders and ultimately guide treatment.

ORAL-06-05

SYNCHROTRON X-RAY FLUORESCENCE MICROSCOPY: HIGH-DEFINITION MAPPING OF TRACE METAL ELEMENTS IN THE HIPPOCAMPUS IN A MODEL OF CLOSED-HEAD TRAUMATIC BRAIN INJURY

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Closed-head traumatic brain injury (TBI) is common and morbidity is caused by neurological dysfunction. Bioactive metals may be altered in TBI so X-ray fluorescence microscopy (XFM), performed on the Microspectroscopy Beamline at the Australian Synchrotron, was used to map metal elements in brain slices in a model of TBI. Fe, Cu and Zn were mapped in the hippocampus of sham (n=4) and TBI (n=3) animals to determine neuronal changes and whether zinc mapping is superior to Timm staining of neuronal tracts. Six months post-TBI, animals were killed, transcardially perfused with paraformaldehyde and brains sectioned (40 microns). Brain slices were raster scanned over the 4 x 4 mm2 samples (pixel size 1.25µ x1.25µ) using the Maia detector developed by CSIRO. Fe, Cu and Zn were distributed in distinct layers of the hippocampus; Zn was expressed in the hilus extending from the dentate gyrus to the CA3, tracking mossy fibre neurons. Levels of Zn in the hilar region of CA3 (28.89±1.30 ppm) were high compared to the molecular layer of the dentate gyrus (9.77±1.55 ppm) and CA3 region (22.4±0.75 ppm). Preliminary data indicates increased Zn in the hilus in the TBI group. Cu was in discrete foci associated with hilar neurons. This study illustrates synchrotron-XFM can be used to investigate metal elements in brain revealing aspects of the neurobiology and that XFM may be valuable in the study of TBI and other neurodegenerative diseases.

ORAL-06-07

IN VIVO 2-PHOTON IMAGING OF LASER-MEDIATED MICRO-LESIONS IN THE ADULT BRAIN

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We are interested in the mechanisms of regeneration and repair in the adult central nervous system (CNS), with particular emphasis on the dynamics of connectivity in the brain when challenged by injury. To gain a comprehensive view of axonal responses to injury in the adult CNS, we have developed a paradigm to induce discrete axonal lesions in the intact mouse brain using high-energy femtosecond lasers. By combining neuron-specific green fluorescent protein transgenic mice and *in vivo* 2-Photon (2P) microscopy through a cranial window, we are monitoring the temporal dynamics of cortical rearrangements post-injury in the living brain. Our interests are twofold - to study the temporal dynamics of degeneration of the severed axon whilst concurrently monitoring the integrity and synaptic turnover in the surviving axon. The detached axon rapidly undergoes beading post-lesion (n≥40axons, 25mice), commences fragmentation within 186±32 min (n=19axons, 8mice) and typically disappears within 24 hours (n≥30 axons, 20mice). Interestingly, different axon subtypes respond differently to lesion, with some cortical axons undergoing fragmentation within minutes of insult, and others surviving for longer than 24 hours post-lesion. Immunohistochemical characterisation of the lesioned cortex indicates a rapid localised microglial response at the lesion site. We have not observed any regrowth at the proximal side of the lesion. However, in the intact axon we have observed increased synaptic turnover in the days and weeks post lesion (n=9axons, 7mice). Insight into the cellular and molecular details of axon responses to injury through this approach has potential clinical value to better manage not only traumatic brain injuries but also a wide variety of neurological diseases characterised by axonal injuries.

ORAL-06-06

FAST METHOD FOR PRECISE PHOSPHENE THRESHOLD IDENTIFICATION IN RESEARCH USING TRANSCRANIAL MAGNETIC STIMULATION

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While transcranial magnetic stimulation (TMS) is being used in diverse research settings, the stimulators themselves can be limiting in providing options for setting up and running experiments. Here we present a method of controlling Magstim Rapid[2] stimulator via a serial connection using a library of freely available Matlab functions. We also present an application of that library which uses a graphical user interface and an adaptive staircase method to automatically adjust the pulse intensity and estimate the phosphene threshold in less than one minute. A comparison of the current method for estimating the phosphene threshold with previously used techniques is provided, advantages and disadvantages are discussed, and suggestions for standardising the phosphene threshold measurements are outlined.

ORAL-07-01

TOPIRAMATE MODULATES AXONAL ION CHANNEL FUNCTION IN VIVO

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BACKGROUND: Topiramate, an anticonvulsant medication extensively used in the treatment of migraine and epilepsy, is efficacious in the management of neuropathic pain. The mechanisms underlying the therapeutic effects of topiramate on peripheral nerve function remain unclear, although a possible effect mediated by axonal Na+ channels has been raised. In the present study, nerve excitability techniques, which provide information related to axonal ion channel function and membrane potential, were undertaken in patients to provide insight into the mechanisms of action of topiramate on human peripheral axons. METHOD: Nerve excitability studies were undertaken in five patients treated with topiramate for the management of migraine. The excitability properties of median nerve motor and sensory axons were assessed at baseline and repeated at two months and 4 months following the commencement of therapy. The following excitability parameters were recorded: stimulus-response curve; strength-duration time constant (SDTC), a marker of the expression of persistent sodium channels; threshold electrotonus (TE) reflecting internodal properties; a current-threshold relationship; and the recovery cycle of excitability, including refractoriness, a marker of inactivation of transient Na+ channels. RESULTS: There were significant changes in axonal excitability evident at two months following the commencement of topiramate. Specifically, there was significant prolongation of SDTC in sensory studies (P <0.05), accompanied by a subtle increase in hyperpolarizing TE at 90-100ms. These changes were accompanied by the development of paraesthesiae. Recordings undertaken at 4 months demonstrated normalization of SDTC and resolution of sensory symptoms. In certain subjects studied beyond 4 months, further reductions in SDTC were evident despite continued increases in topiramate dose. No changes were evident in recovery cycle measures, including refractoriness, either at two or four months. CONCLUSIONS: The present study has demonstrated changes in nerve excitability in patients treated with topiramate. The prominent changes in SDTC, in the absence of changes in refractoriness, suggest a preferential action on persistent Na+ conductances. While the initial prolongation of SDTC suggests increased expression of this conductance, the subsequent reduction suggests that topiramate may exert its long-term therapeutic effects by downregulation of persistent Na+ conductances.

ORAL-07-02

REGION- AND TRANSMITTER-SPECIFIC INVOLVEMENT OF PRESYNAPTIC SODIUM CHANNELS IN SELECTIVE INHIBITION OF GLUTAMATE VS. GABA RELEASE BY VOLATILE ANAESTHETICS

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Neurotransmitter release is a multistep process, where presynaptic voltage-gated Na^+ channels (VGSC) play a pivotal role. Volatile anaesthetics that selectively inhibit glutamate over GABA release also block VGSC, which suggests fundamental differences between mechanisms of excitatory and inhibitory transmitter release that are coupled to presynaptic VGSCs. To test whether variable sensitivities of volatile anaesthetics to transmitter release result from different sensitivities to presynaptic Na^+ channel inhibition, we compared the effects of isoflurane and the VGSC blocker tetrodotoxin (TTX) on depolarisation-evoked [^{3}H]glutamate and [^{14}C]GABA release from 4 regions of rat CNS. Isolated nerve terminals from striatum, hippocampus, cerebrocortex, and spinal cord were superfused at 37°C and depolarised with 4-aminopyridine, which indirectly activates VGSCs. Relative expression of VGSC subtypes (Na_v1.1-1.9) that vary in sensitivity to TTX, significantly differed ($P<0.05$) between CNS region. The potency of TTX to inhibit evoked glutamate release differed significantly ($P<0.05$) between CNS regions (striatum>hippocampus>cortex>spinal cord), but only glutamate release evoked from spinal cord was more sensitive ($P< 0.001$) to inhibition by isoflurane (striatum=hippocampus=cortex> spinal cord). Isoflurane was more potent ($P<0.05$) in inhibiting evoked glutamate vs. GABA release in all brain regions, but not spinal cord. TTX potency did not always differ between evoked glutamate and GABA release inhibition, but greater efficacy of TTX to inhibit evoked glutamate vs. GABA release was observed in all brain regions ($P<0.01$), except spinal cord. These findings suggest a greater role for VGSCs in depolarisation-induced glutamate vs. GABA release, and that volatile anaesthetics selectively reduces excitatory transmitter release based on greater VGSC contribution rather than relative potency.

ORAL-07-03

DOPAMINERGIC AND GABAERGIC NEURONS IN THE MOUSE VENTRAL TEGMENTAL AREA

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The ventral tegmental area (VTA) is involved in substance abuse. Based on cellular physiology, VTA neurons are reportedly either dopaminergic or GABAergic, but overlapping phenotypes between them have only been claimed by inference. We used GAD67-GFP knock-in mice with post-hoc tyrosine hydroxylase and GFP immuno-staining to examine cellular physiology and pharmacology of identified GABAergic and dopaminergic neurons during patch-clamp recording. Adult male mice were deeply anaesthetised with isoflurane, decapitated and VTA slices prepared. All protocols were approved by Ethics Committee of the University of Sydney. GFP neurons exhibited more spontaneous activity than non-GFP cells (GFP 3.9 ± 0.7 Hz, n = 41; non-GFP 1.5 ± 0.1 Hz, n = 31, $p < 0.05$). GFP cells fired briefer action potentials in both on-cell mode (GFP 0.42 ± 0.02 ms, n = 14; non-GFP 1.51 ± 0.05 ms, n = 21, $p < 0.05$), and whole-cell mode (GFP 0.45 ± 0.02 ms, n = 21; non-GFP 1.28 ± 0.07 ms, n = 17, $p < 0.05$). GFP neurons also had larger action potential amplitude (GFP 73 ± 2 mV, n = 21; non-GFP 66 ± 2 mV, n = 18, $p < 0.05$), but a smaller proportion of GFP neurons displayed Ih (GFP 24%, 9/37 cells; non-GFP 74%, 20/27 cells, $p < 0.05$). GFP neurons were predominantly inhibited by DAMGO (3 µM, 12/13 cells) but not dopamine (100 µM, 8/9 cells), whilst non-GFP cells were not inhibited by DAMGO (21/21 cells) but inhibited by dopamine (22/22 cells). These distinct cellular properties in DAergic and GABAergic neurons increase confidence in physiological classification of GABAergic neurons in VTA.

ORAL-07-04

CORTICOSTRIATAL PLASTICITY AFTER VISUAL ACTIVATION OF SUBCORTICAL PATHWAYS

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A recently proposed mechanism of reinforcement learning involves the tagging of active synapses by a spike-timing-dependent plasticity (STDP)-based mechanism and the induction of synaptic changes by a subsequent reinforcement signal, mediated in part by phasic dopamine (DA) signals. We used an established paradigm in which disinhibition of the midbrain superior colliculus (SC) and visual stimulation can evoke phasic DA release in urethane-anaesthetised animals to test this hypothesis. Postsynaptic potentials (PSP) were evoked in intracellularly recorded striatal spiny neurons (n=28) by electrical stimulation of the contralateral motor cortex. PSPs often consisted of up to three distinct components, probably representing distinct corticostriatal pathways. After 15 min baseline recording, the SC was locally injected with bicuculline. Each cortical stimulation pulse (>60; 0.2 Hz) was then paired with a delayed (250ms) reinforcing light flash to the contralateral eye. Consistent postsynaptic spike discharge was induced by a 5 ms current pulse (+0.8 to +2 nA) 8-15 ms after cortical stimulation (n=13). Changes in PSPs were measured as the maximal slope normalised to baseline (5 min pre). Reinforcement-related changes ranged from significant short-lasting potentiation of PSPs (<15 minutes, ~120%, n=2) and depression (~80%, n=2) to selective modulation of individual PSP components. The direction of change depended on the relative timing between PSP components and spike such that PSP components coinciding or closely following the spike were potentiated. These effects were seen only in experiments with successful bicuculline-mediated disinhibition (n=10). The present results provide first support for STDP-based reinforcement learning mechanisms *in vivo*.

ORAL-07-05

ADENOSINE RECEPTOR MEDIATED CELLULAR AND SYNAPTIC CHANGES UNDERLYING LOCAL NETWORK MODULATION BY ADENOSINE RECEPTOR ACTING DRUGS

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It has been shown that adenosine receptor active drugs alter gamma frequency (30-80 Hz) oscillation strength in vitro. We investigated the underlying mechanisms responsible for modulating hippocampal network activity through adenosine receptors by using intracellular and patch-clamp recordings from pyramidal neurons in the rat hippocampus. Resting membrane potential was hyperpolarized by adenosine (50 µM, by -2.68±0.54 mV, p=0.028) and the adenosine A1-receptor agonist N6-cyclopentyladenosine (CPA, 50 nM, by -3.76±1.16 mV, p=0.047). Adenosine decreased EPSP slope measured at -90 mV (by -2.64±0.53 V/s, p=0.002) while the A1-receptor antagonist 8-cyclopentyltheophylline (8-CPT, 5 µM) has the opposite effect (by 1.08±0.47 V/s, p=0.048). Maximum IPSP amplitude (measured at -65 mV) was increased by adenosine (by 2.73±0.5 mV, p=0.006) and adenosine shifted the stimulus-effect relationship to higher values (by 0.69±0.16 V, p=0.016). Patch clamp recordings revealed that monosynaptic IPSC conductance was not affected by adenosine receptor modulation. A1-receptor modulation caused a shift in IPSC reversal potential, which correlated with the change in membrane potential (r=0.476, p=0.019). A1-receptor mediated resting membrane hyperpolarization is most likely caused by activation of the G-protein coupled Inward Rectifying potassium (GIRK) channels. The measured increase in maximum IPSP is likely caused by an increase in driving force, coupled to a hyperpolarized resting membrane potential, possibly though increased activity of the K-CL cotransporter 2 (KCC2). The shift in the IPSP stimulus-effect relationship is likely caused by a reduced activation of interneuron's due to a decrease in EPSP strength. These results indicate that endogenous adenosine modulates cell excitability and synaptic strength. The resulting changes in network activity are likely to alter cognitive function.

ORAL-07-06

PRESYNAPTIC SCALING: A NOVEL HOMEOSTATIC SCALING PLASTICITY

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Purpose: Throughout their lifetime, neurons are continuously modified and rebuilt. Despite these changes, the outputs of their targets are reproducable, driving the same repertoire of behaviours. How is this achieved? Homeostatic adaptations at synapses significantly contribute to the process. Here we describe an unusual adaptation that we discovered which operates in such a capacity at the *Drosophila* larval neuromuscular junction, termed presynaptic scaling. **Methods**: Larval muscles receive glutamatergic synaptic inputs from the highly and less active 1b and 1s motor neurons, respectively. In transgenic animals, the activity pattern of just the 1s neuron innervating muscle 2 is chronically reversed in vivo by expressing modified K+ channel transgenes. In our analysis of control (C) and transgenic (T) animals, we combined electrical recordings from single 1b and 1s synaptic boutons, whole cell recordings, and light and serial electron microscopy. **Results**: Genetically enhancing just 1s neuronal activity reduced 1s bouton neurotransmitter output (quantal content) by 28.3% (P<0.02; n: C=9; T=8). Unexpectedly, a similar reduction (25.1%; P<0.04; n: C=9; T=8) is also observed at synaptic boutons formed by the unmanipulated 1b neuron. The proportional downscaling of neurotransmitter output at both bouton types is referred to as presynaptic scaling. Presynaptic scaling maintains the relative 1b-1s neurotransmitter output differences at a 'set point' to potentially maintain differential muscle activation for driving different movements. Presynaptic scaling also reduces total synaptic drive (21.9%; P< 0.001; n: C=8; T=6) in a direction to renormalize the muscle's activity. **Conclusion**: Presynaptic scaling stabilizes the circuit by operating to maintain both target activity and presynaptic diversity at set points, despite manifestations of abnormal activity even in just single input neurons.

ORAL-07-07

ALTERED SYNAPTIC PLASTICITY IN INTERSECTIN-1 NULL MICE

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Intersectin-1 (Itsn1) is upregulated in individuals with Down syndrome (DS) and this has been suggested to contribute to the pathogenesis of both Down syndrome and Alzheimer's disease. Itsn1 interacts with proteins involved in endocytosis, regulating synaptic vesicle recycling presynaptically, and receptor and ion channel turnover postsynaptically, and in modulating second messenger coupling. Our aim was to determine in more detail the role of Itsn1 by examining the effects of Itsn1 knock-out (KO) on synaptic function in mouse hippocampus. **Methods:** Wild type (WT) and Itsn1 KO mice were anaesthetized and their brains rapidly removed to an ice slurry. Hippocampal slices, 300 µm thick, were prepared, mounted in a recording chamber, and continuously superfused with artificial cerebral spinal fluid (aCSF) at 35°C. Field excitatory post synaptic potentials (fEPSPs) were recorded with extracellular electrodes from the stratum radiatum of the CA1 region in response to stimulating the Schaffer collaterals. **Results:** Paired pulses were applied with interstimulus intervals of 25–500 ms and resulted in facilitation. The degree of facilitation was not changed in slices from 5 KO mice compared with 10 WT animals (P = 0.09). Bursts of tetanic stimulation resulted in potentiation of the fEPSPs that persisted for at least 3 hr. The potentiation was 2.1 ± 0.5 fold greater (P = 0.02) in slices from KO (n = 5) compared with WT (n = 10). **Conclusions:** These results demonstrate altered postsynaptic plasticity in slices from Itsn1 KO mice, with a marked increase in fEPSP amplitude. This could be explained in terms of enhanced postsynaptic receptor density involving reduced endocytosis, or altered second messenger coupling.

ORAL-07-08

SUPPRESSING TONIC INHIBITION IN VIVO MEDIATES POST-STROKE FUNCTIONAL IMPROVEMENTS

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Post-stroke neural repair and rehabilitation remains ongoing for weeks and includes changes in, synaptic plasticity, axonal sprouting, and cortical reorganization. Extrasynaptic $GABA_A$ receptors, consisting of alpha5 or delta containing subunits, mediate a tonic form of neuronal inhibition and dampen neuronal responsiveness to afferent stimulation. We tested the role of tonic $GABA_A$-mediated inhibition in functional recovery after stroke using pharmacological and genetic manipulations of alpha5 and delta-containing $GABA_A$ receptors. Photothrombotic stroke was induced in the mouse forelimb motor cortex. Significant forelimb deficits (P<0.001; n=8) were observed for at least 6-weeks post-insult using two behavioral measures: rearing in the cylinder, and accurate foot placement on the grid-walking task. Treatment with L-655,708, a selective $GABA_A$ alpha5 inverse agonist (2.5-5mM), starting 3-days after stroke resulted in a significant dose-dependent decrease in forelimb deficits from week-1 post-insult. Assessment of Gabra5-/- mice (n=10) showed a similar pattern of recovery, whilst Gabrd-/- mice (n=10) only showed a mild gain of function. The neuroanatomical tracer biotinylated dextran amine (BDA) was injected into pre-motor cortex 6-weeks after the insult in order to assess the reorganization (axonal sprouting) within the injured brain. The distribution of BDA-labeled cell bodies were plotted in tangential sections through the ipsilateral cortical hemisphere. Assessment of BDA-labeled cell bodies in L655,708-treated and Gabrd-/- mice showed no difference in the pattern of labeling, indicating tonic inhibitory currents are not involved in post-stroke axonal sprouting. These results demonstrate that delayed suppression of tonic inhibitory currents affords an early and sustained reversal of forelimb motor deficits after experimental stroke.

ORAL-08-01

LYSOSOMAL FUNCTION IN PARKINSON'S DISEASE

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Neurodegenerative cascades in Parkinson's disease (PD) are thought to involve dysfunction of cellular waste removal processes. Changes within the ubiquitin-proteasome system in PD have been intensively investigated, yet to date the second major waste removal organelle in the cell, the lysosome, has received relatively little attention. Recently it was reported that immunoreactivity for the lysosomal proteins lysosome associated membrane protein (LAMP1), cathepsin D and Heat shock protein 73 is reduced in remaining substantia nigra neurons in PD, particularly in neurons containing α-synuclein inclusions (1). These data suggest that lysosomal function is decreased prior to neuronal loss in PD. To investigate if the quantity and/or activity of lysosomes is altered primarily due to α-synuclein deposition, a similar assessment of the anterior cingulate cortex, a region with limited neuronal loss in PD, was undertaken and compared with a brain region which exhibits neither α-synuclein deposition nor neuronal loss in PD. Lysosome-enriched tissue fractions from each region were prepared from frozen brain from eight PD patients and eight age-matched controls. Western blotting for LAMP1 protein (H228, sc-5570, Santa Cruz, U.S.A.) demonstrated no disease or regional differences. Cathepsin D activity measured using an assay kit from Sigma-Aldrich, revealed a trend towards increased (45%) enzymatic activity in the anterior cingulate cortex in PD (p=0.08). These data show no reduction in lysosomes in a region with significant α-synuclein deposition in PD, but suggest an increase in lysosomal activity as a consequence of abnormal α-synuclein accumulation. It may be that an inability to sustain such increased lysosomal activity under conditions of α-synuclein accumulation has detrimental consequences on neurons which leads to neuronal degeneration as observed in the PD substantia nigra. 1. Chu, Y., et al. 2009 Neurobiol Dis. 35, 385-98.

ORAL-08-02

EXCITABILITY REGULATES TYROSINE HYDROXYLASE EXPRESSION IN SUBSTANTIA NIGRA NEURONS

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in dopamine (DA) synthesis and its level of expression is a major determinant of neuronal DA synthesis. TH expression is regulated by modulation of ion-channels, electrical stimulation, membrane polarization, and intracellular Ca^{2+} in a variety of catecholaminergic (including DAergic) neurons, but whether the same occurs in substantia nigra pars compacta (SNc) DAergic neurons is not known. In this study ion-channel agonists and antagonists (or vehicle) were infused unilaterally into the SNc of mice (n=3-6 in each treatment) for 2 weeks using osmotic pumps then TH expression in SNc cells was measured using immunohistochemistry. Cellular TH expression increased significantly in response to the SK channel antagonist apamin (300nM) and the $GABA_A$ receptor agonist muscimol (20μM). In contrast, infusion of the D2 DA receptor agonist quinpirole (100nM) into dorsal striatum (the efferent target of SNc DAergic neurons) decreased SNc cellular TH expression. Interestingly, the number of TH immunoreactive (TH+) SNc cells was altered in the opposite direction by these treatments; i.e. apamin and muscimol decreased TH+ SNc cells whereas quinpirole increased TH+ SNc cells. We also observed changes in the number of TH+ SNc cells in response to SK channel agonists (100μM 1-EBIO and 30μM riluzole), L-type Ca^{2+} channel agonist (100nM FPL64176) and antagonist (10μM nimodipine), 30mM extracellular K+, and $GABA_A$ receptor antagonist (100μM picrotoxin). We conclude that SNc neuronal excitability regulates their TH expression in a relatively direct way, presumably via changes in intracellular Ca^{2+} leading to changes in the amount of TH protein, whereas changes in TH+ cell number are a result of a homeostatic mechanism maintaining a constant amount of nigrostriatal DA.

ORAL-08-03

ANALYSIS OF TYROSINE HYDROXYLASE ISOFORMS AND PHOSPHORYLATION IN PARKINSON DISEASE

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Parkinson disease (PD) is a neurodegenerative disease which predominantly targets dopaminergic neurons of the substantia nigra (SN). The rate limiting enzyme for dopamine synthesis is tyrosine hydroxylase (TH) which is activated primarily through phosphorylation of Ser40. Unlike other species, TH occurs in four isoforms in humans (hTH1-4) which are differentially regulated. The relative distribution and role of these TH isoforms in PD remains unknown. Therefore, brain tissue samples from the SN and nearby ventral tegmental area (VT) and their striatal target regions (caudate and putamen) of four PD and three age-matched disease-free controls were obtained from the Prince of Wales Medical Research Institute Brain Bank following study approval. Quantitative Western blotting in controls revealed that hTH1 and hTH2 comprise ~95% of the total amount of TH in these regions, while hTH3 and hTH4 represent ~5%. Regional comparison revealed that hTH1 was significantly lower than hTH2 in control SN ($p<0.01$) whereas in other regions analysed, the levels were the same. As expected, the levels of total TH were significantly decreased in PD compared to controls ($p< 0.01$). hTH1 levels were disproportionately lower in the nigrostriatal system in PD compared with controls ($p<0.05$), with no change in TH Ser40 phosphorylation in the SN. In contrast the levels of phosphorylation of Ser40 in the mesostriatal system was significantly increased in PD vs. control brains ($p<0.05$). This data suggests that hTH1 containing neurons preferentially degenerate in the PD SN and that surviving TH-containing neurons in this region and nearby unaffected VT neurons compensate by increasing TH activation through Ser40 phosphorylation.

ORAL-08-04

ALPHA-SYNUCLEIN AND PARKINSON'S DISEASE: INTER-RELATIONSHIPS OF MUTANTS THAT SENSITISE CELLS TO ALPHA-SYNUCLEIN TOXICITY

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Parkinson's disease (PD) is a neurodegenerative disease affecting more than 50,000 Australians who have lost approximately 30-40% of dopaminergic neurons at the time of diagnosis. Earlier diagnosis and new treatments are needed as current therapies are only partially effective. Alpha-synuclein (aSyn) is a natively non-toxic protein of unknown function that associates with synaptic vesicles. aSyn is a central component in PD and is the main constituent of Lewy bodies, the primary pathological hallmark of PD. The propensity of aSyn to become cytotoxic likely involves a complex interaction of unknown predisposing genes (risk factors). The identification of these risk factors would potentially permit early diagnosis as well as implicate the molecular pathological mechanisms by which PD develops. Using an aSyn based PD model system expressing non-toxic levels of aSyn, we screened amongst 5000 genes for those whose deletion render the cell toxic to this level of aSyn. Preliminary results indicate that the majority of candidate gene deletions that result in significant increases in aSyn toxicity involve ROS-independent mitochondrial dysfunctions, endosomal membrane trafficking and endocytosis/actin cytoskeleton dysfunctions. These pathways may represent either potential targets of aSyn in its cytotoxic state and / or cellular pathways whose dysfunction contributes to aSyn toxicity.

ORAL-08-05

PROTEASOME INHIBITION INDUCED RE-LOCALIZATION AND AGGREGATION OF TDP-43 IN NEURONAL CELLS

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The protein TDP-43 is the primary component of pathological inclusions found in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin inclusions (FTLD-U). In both of these disorders TDP-43 is cleaved, hyperphosphorylated and ubiquitinated and forms cytoplasmic and intranuclear inclusions in affected neurons and glial cells. These inclusions are found throughout the hippocampus, frontal and temporal lobes and/or the spinal cord of ALS and FTLD-U patients. Furthermore, in these disorders TDP-43 relocalizes from its normal location in the nucleus to the cytoplasm. In this study, we aimed to develop a robust cell culture model of TDP-43 proteinopathy as a basis for subsequent studies. Primary murine hippocampal and cortical cultures as well as an immortalized murine motor neuron cell line (NSC-34) were treated with a proteasome inhibitor and then evaluated through immunohistochemistry and western blots (n=4 per cell type). We found that treatment of all cell types caused a progressive decrease in the levels of soluble TDP-43 and caused a progressive increase in the formation of high-molecular weight TDP-43 aggregates and lower-molecular weight TDP-43 fragments. Furthermore, TDP-43 was found to relocalize from the nucleus to the cytoplasm. These results demonstrate that the TDP-43 proteinopathy observed in FTLD-U and ALS can be induced in cell culture, and this model can therefore be utilized to study the pathomechanisms involved in these disorders. Furthermore, these results suggest that proteasome dysfunction may contribute to the pathogenesis observed in FTLD-U and ALS.

ORAL-08-06

TRPM8 CHANNELS ARE NECESSARY FOR TRANSTHYRETIN-INDUCED CALCIUM INFLUX IN SENSORY NEURONS

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Familial amyloidotic polyneuropathy (FAP) is a peripheral polyneuropathy caused by the extracellular accumulation and deposition of insoluble, misfolded transthyretin (TTR) protein. Although relatively rare, FAP is invariably fatal with profound neural disturbances to autonomic and sensory nervous system circuits. TTR variants implicated in the pathogenesis of FAP show aberrant aggregation and we have previously shown that significant cytosolic calcium dysregulation occurs in cell lines treated with a particularly aggressive variant, L55P TTR. It has been hypothesised that oligomers, rather than amyloid fibrils, interact with plasma membrane receptors causing calcium influx. The exact molecular mechanism responsible for these effects is unclear and the aim of the present study was to investigate the actions of TTR in a peripheral cell model. Using dynamic light scattering, we show that L55P contains oligomeric (50-200nm) species not present in wild-type forms. While voltage gated calcium channels (VGCC) have been implicated in TTR-induced calcium entry, their activation by TTR has not been explained. Here we show that L55P induces significant extracellular calcium entry in rat spinal neuron growth cones in a mechanism mediated by Transient Receptor Potential (TRPM8) cation channels. Using single-wavelength calcium imaging we show that L55P induces a calcium influx sensitive to pharmacological inhibition of L-type VGCC, Voltage Gated Sodium Channels (Nav 1.8) and TRPM8 cation channels. Furthermore, knockdown of TRPM8 channels on DRG growth cones to 40% of control levels utilising a specific TRPM8 siRNA aproach demonstrated the necessity of these channels for TTR-induced calcium entry in DRG growth cones. These results suggest that TRPM8 channels and voltage-gated channels interact at the cell membrane to induce calcium influx in response to TTR.

ORAL-08-07

HYPOTHALAMIC CHANGES IN HUNTINGTON DISEASE

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Recent observations of early metabolic disturbances have fundamentally changed our view of the fatal and hereditary Huntington disease (HD); a condition previously considered a movement disorder caused by selective basal ganglia pathology. Dysfunction in areas outside this region, in particular the hypothalamus, has started to emerge as an important aspect of HD. As non-motor symptoms and signs involving depression, anxiety, sleep disturbances and increased appetite precede motor onset in HD, we hypothesized that these may be due to hypothalamic changes. We have previously described loss of the hypothalamic neuropeptide orexin, involved in the regulation of sleep and metabolism, in both HD patients and mouse models of the disease. In order to test whether hypothalamic alterations indeed occur before the onset of motor symptoms, we have performed voxel based morphometric analyses of cross-sectional MR images from 220 HD gene carriers and 75 age and gender matched control individuals in the unique multi-center PREDICT-HD study. A predicted time to motor onset in HD can be calculated using a formula with age and CAG repeat length. We have found that changes in the hypothalamic region are detectable at least a decade before predicted time of diagnosis, which indicates that pathology occur both in the basal ganglia and in the hypothalamus early in the disease process. Using the novel BACHD mouse model expressing the disease causing mutant huntingtin, we have found that depressive- and anxiety like behaviour as well as increased appetite develop before the decline in motor function (n= 6-10/genotype). These non-motor features are due to neuroendocrine dysfunction. Taken together, our results show that hypothalamic changes occur early in the disease, and are important in the development of metabolic disturbances in HD.

ORAL-08-08

IL-10-819 POLYMORPHISM AND PATHOLOGY IN PARKINSON'S DISEASE

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Purpose: The loss of dopaminergic neurons in the substantial nigra (SN) and the presence of Lewy bodies are the cardinal pathological features of movement disorder Parkinson's disease (PD). The rate of decline in the motor symptoms of PD is associated with different IL-10 polymorphisms (Huang, et al. APSN, 2006) supporting a role for neuroinflammation in disease progression. The objective of this study was to confirm this association in pathologically-confirmed cases of PD. **Methods:** Genomic DNA was extracted from 112 (33 female and 79 male) PD brain samples, obtained following study approval by the UNSW Human Ethics Advisory Panel from the Australian Brain Bank Network. Clinical information including age of disease onset and duration of PD was requested. Macroscopic and microscopic reports were obtained for each case, which contained information on the degree of neuronal loss in the SN and Braak staging using alpha-synuclein immunoreactivity. IL-10-819 polymorphism was genotyped for each case and SPSS-Chi-square and SPSS-multivariant analysis were used for statistics analyses. **Results:** Increasing neuronal loss in SN and increasing severity of Braak stages of PD were correlated. Low IL-10 producers had more severe neuronal loss in the SN compared with high IL-10 producers (-819 CC genotype). IL-10 genotypes were not correlated ith the severity of Braak staging of PD. **Conclusion:** Our data indicate that IL-10 associated inflammation appears to be associated with the severity of neuronal loss in the SN and not Lewy body infiltration in PD.

ORAL-09-01

CONCURRENT RECORDING OF SPONTANEOUS MUSCLE SYMPATHETIC NERVE ACTIVITY AND WHOLE-BRAIN FMRI SIGNAL INTENSITY: REAL-TIME IMAGING OF CARDIOVASCULAR CONTROL IN AWAKE HUMAN SUBJECTS

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Introduction: We have recently demonstrated that it is possible to record spontaneous muscle sympathetic nerve activity (MSNA) at the same time as performing functional magnetic resonance imaging (fMRI) to functionally identify the operation of the medullary circuitry involved in spontaneous fluctuations in MSNA *(Macefield & Henderson, Human Brain Mapping 2009, in press).* In the present study we attempted to identify suprabulbar areas within the brain that may contribute to the generation of spontaneous MSNA at rest. **Methods:** MSNA was recorded via a tungsten microelectrode inserted into the common peroneal nerve in 8 subjects. Gradient echo, echo-planar fMRI was performed using a 3T scanner (Philips Achieva). 200 volumes (46 axial slices, TR=8 s, TE=40 ms, flip angle=90 deg, raw voxel size =1.5x1.5x2.75 mm) were collected in a 4s-ON, 4s-OFF protocol. Total sympathetic burst amplitudes were measured from the RMS-processed mean voltage amplitude during the 4 s period between scans. Blood Oxygen Level Dependent (BOLD) changes in brain signal intensity (SPM5: random effects, uncorrected $p<0.01$) were measured during the subsequent 4 s period to take into account the +5 s neurovascular coupling delay and the -1 s required for conduction of the sympathetic bursts from the brain to the peripheral recording site. **Results:** MSNA was positively correlated to BOLD signal intensity in the bilateral dorsolateral prefrontal cortex and the left insula, and inversely correlated to medial prefrontal cortex, hypothalamus and right insula. **Conclusions:** Using concurrent microneurography and fMRI we have shown that spontaneous fluctuations in muscle sympathetic nerve activity in awake human subjects are associated with spontaneous fluctuations in BOLD signal intensity in areas above the brainstem, with reciprocal connections being evident in the activity of the left and right insular cortices. The left insula and bilateral dorsolateral prefrontal cortex are positively coupled to MSNA, i.e. each could contribute to muscle vasoconstrictor drive, whereas the right insula, medial prefrontal cortex and hypothalamus are inversely coupled to MSNA, i.e. they are driven by baroreceptor inputs.

ORAL-09-02

CHRONIC FOOTSHOCK STRESS CAUSES ENDURING CHANGES IN CIRCADIAN RHYTHMS, WITHOUT PROVOKING HYPERTENSION IN RATS

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We aimed to determine whether chronic stress causes enduring hypertension and, if so, whether this is reflected in biochemical markers of sympathetic activity. Accordingly, adult male Wistar rats were subjected to six weeks of inescapable footshocks (FS+, n=5), with constant monitoring of AP and HR by telemetry; another group served as controls (FS-, n=5). Repetitive stress caused a 6% decrease in body weight in FS+ rats; they also started to emit ultrasound vocalisation (a sign of distress) in response to non-noxious stimuli. Chronic stress did not alter daily average values of AP or HR, mean AP being 122±1 and 116±2 mmHg for FS+ and FS-, respectively, on the final day of the experiment, compared to 120±3 and 119±3 mmHg, respectively, at the beginning of the experiment. Chronic stress also failed to alter cardiac susceptibility to arrhythmias. Consistent with this lack of cardiovascular consequences, chronic stress did not alter AT1 receptor expression or tyrosine hydroxylase activity in the stellate ganglia or the adrenal gland. However, chronic stress dramatically altered circadian rhythms, significantly disrupting the normal dark phase rises in AP and HR. These changes were already detectable after the first week of stress, and lasted for at least one week after termination of footshocks. We conclude that chronic footshock stress does not provoke sustained changes in daily average values of AP or HR, or in biochemical markers of sympathetic activity, but causes enduring disruption of circadian rhythms. The latter appears to be the earliest and most sensitive physiological index of chronic stress.

ORAL-09-03

DIET-INDUCED OBESITY ALTERS SYMPATHETIC NEUROTRANSMISSION IN RAT SMALL MESENTERIC ARTERIES

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In pressurized rat small mesenteric arteries, ATP released from sympathetic nerves, activating smooth muscle purinoceptors, constitutes the major mechanism underlying neurally-evoked vasoconstriction. However, during obesity, increased contractility has been linked to adrenergic hyperactivity, while the role of purinergic signalling in diet-induced obesity remains unexplored. In the present study, adult male Sprague-Dawley rats were fed either normal or high fat diet and small mesenteric arteries (341±10μm diameter, n=33) were isolated and pressurised (80 mmHg). Sympathetic nerves were activated with electrical field stimulation (1-10Hz), while intracellular recordings were made with sharp microelectrodes (120-185 Mohms), filled with fluoroscein to identify impaled cells, and simultaneous changes in vessel diameter monitored (DIAMTRAK). While resting membrane potential did not vary between groups (control: -41.7±0.6mV; obese: -40.2±1.3mV, n=33), ATP-mediated excitatory junction potentials (EJPs) were increased in amplitude in obese arteries (13.0±3.5mV; control: 8.2±0.5mV, n=4, $P<0.05$), with decrease in rise time (28.5±1.4ms; control: 66.5±2.5ms, n=4, $P<0.05$) and rate of decay (28.5±1.4ms; control: 66.5±2.5ms, n=4, $P<0.05$). Repetitive nerve stimulation (1 Hz, 30s) caused significantly greater vasoconstriction (obese: 4.3±1.3%D/Dmax; control: 1.7±1.9%D/Dmax) and larger EJPs in obese arteries (9.5±1.0mV; control: 7.2±0.8mV; n=6, $P<0.05$). Desensitisation with α,β-methylene ATP (0.1μM) abolished EJPs and vasoconstriction in both arteries ($P<0.05$). Increasing stimulation frequency (3, 5, 10Hz) increased constriction amplitude; the effects being greater in obese arteries (n=6, $P<0.05$). Intracellular recordings revealed EJPs superimposed on a slow depolarization. We conclude that sympathetic nerve-mediated vasoconstriction is augmented during diet induced obesity and that ATP plays a significant role. Data suggest that this occurs due to increased neurotransmitter release, perhaps accompanied by postsynaptic receptor alterations. Investigation of the underlying mechanisms may help to reduce and control obesity related cardiovascular disease.

ORAL-09-04

THE HEATER IS COLD, THE HOUSE IS WARM: NONSHIVERING THERMOGENESIS WITHOUT BROWN ADIPOSE TISSUE

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Conditioned fear is known to increase body temperature, but the source of this thermogenesis is not known. Because fear leads to freezing immobility, it is unlikely that heat derived from muscle contraction contributes to it. In the present study, we tested if brown adipose tissue (BAT), the main source of nonshivering thermogenesis during cold exposure, would be implicated. As BAT thermogenerates in response to β-adrenoceptor activation, Propranolol (a β-adrenergic antagonist) should prevent BAT activation. The largest BAT deposit in the rat lies in the interscapular area, directly under the skin. Hence, we hypothesized that BAT activation would i) increase the skin temperature in that area faster than in other skin areas not overlying BAT and ii) that increase would be blocked by Propranolol. Rats were shaved in the interscapular and lumbar back areas, and skin temperature was measured noninvasively by infrared thermography. Exposure to cold for 30 min increased the temperature difference between interscapular and lumbar skin (TiScap-TLumbar) by 2.7°C, which was reduced to 1°C after Propranolol treatment. Lumbar back skin temperature remained stable, but after Propranolol it fell by 2.58°C, suggesting that the organism lost its ability to withstand cold. In comparison, 30 min exposure to conditioned fear led to a TiScap-TLumbar increase of 1°C, which was not affected by Propranolol treatment. Surprisingly, Propranolol did abolish the 1.5°C lumbar skin temperature increase due to conditioned fear. These results confirm a role of BAT thermogenesis during cold defense, but suggest that fear-related hyperthermia, although β-mediated, is not related to BAT activation. Further research is needed to identify the source of this new thermogenic mechanism.

ORAL-09-05

WHY GUT SMOOTH MUSCLE NEEDS TO "EQUILIBRATE" BEFORE ENTERIC MOTOR INNERVATION BECOMES FULLY FUNCTIONAL

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Enteric inhibitory motor neurons evoke prominent inhibitory junction potentials (IJPs) in gut smooth muscle, however, preparations typically need to "equilibrate" for an hour or longer before IJPs reach their full amplitude. Intracellular recordings and dye fills (1% carboxyfluorescein in 2M KCl) were made from smooth muscle cells in isolated specimens of ileum, from humanely killed guinea pigs. IJPs were evoked by a stimulating electrode placed 1mm circumferentially. Cells were filled with 0.5nA hyperpolarising pulses (50% duty cycle, 2 minutes+ 1 minute diffusion) then dye labelled cells were counted. Preparations were dissected in cool Krebs solution: time 0 was when superfusion with Krebs solution at 35 degrees C started. Recordings in the first 20 minutes always showed IJPs <1mV in amplitude (n=6). After equilibration (up to 120 minutes) IJPs had a mean maximum amplitude of 18.0 ± 3.0mV. We compared cells with IJPs <1mV with those >10mV to identify correlates of the process of "equilibration". The mean resting membrane potential was significantly reduced in cells with IJPs>10mV (49.7±1.0 vs -55.5±2.0mV, p<0.5, 33 cells, n=7). The mean number of circular smooth muscle cells filled with carboxyfluorescein was significantly greater in cells with IJPs>10mV (6.7 ± 0.5 vs 3.2± 0.6 cells, p<0.001, 31 cells, n=7). Interestingly, longitudinal muscle cells showed little coupling even after "equilibration" (1.6 ± 0.4 cells filled). In conclusion, immediately after set up, smooth muscle cells are slightly hyperpolarised and have reduced gap junction coupling during a period when their IJPs are suppressed. During the "equilibration period" normal gap junction coupling is resumed, allowing full conduction of IJPs through the muscle layer.

ORAL-09-06

STEM CELL THERAPY TO TREAT INTESTINAL MOTILITY DISORDERS: IN VIVO STUDIES USING A MOUSE MODEL

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Gut motility is controlled by the enteric nervous system (ENS), and damage to the ENS (enteric neuropathies) results in digestive disorders. Hirschsprung's disease is a developmental disorder in which the ENS is absent from the distal-most regions of the bowel. Infants with Hirschsprung's disease require surgery to remove the affected region, but motility problems commonly persist. There is currently enormous interest in the potential of cell therapy to treat enteric neuropathies, including Hirschsprung's disease. Studies have shown that stem/progenitor cells can colonize segments of embryonic gut *in vitro* and give rise to enteric neurons and glia. However, it is unknown whether stem/progenitor cells can colonize the post-natal colon *in vivo*. In this study, neurospheres were generated by dissociating gut from E14.5 mice in which neural crest cells express the fluorescent protein, Kikume (Kik). Ednrb$^{-/-}$ mice lack neurons in the distal colon and are a model of Hirschsprung's disease. Kik+ neurospheres were transplanted into the external muscle layers of the distal colon of P14-P21 Ednrb$^{-/-}$ or wild-type mice. After 1-8 days, the recipient mice were killed and the colon examined. Kik+ cells migrated extensively within the wall of the colon and showed markers of differentiated neurons (Hu) and glia (S100β). Some neurons expressed nitric oxide synthase (NOS), which is also expressed by a sub-population of enteric neurons in mature mice. These data show that stem/progenitor cells can migrate and undergo neuronal differentiation in the post-natal bowel. Future studies will determine whether the transplanted cells restore normal motility patterns.

ORAL-09-07

EXPLORING SHORT TERM PLASTICITY IN GUINEA-PIG MYENTERIC NEURONS

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Enteric neurons undergo long-term increases in excitability during inflammation or infection, and can change the proportion of neurotransmitters responsible for fast synaptic transmission. We hypothesised that short-term, low frequency stimulation would be associated with an upregulation of non-nicotinic fast synaptic transmission (fast EPSPs). Two methods were used to monitor membrane potential changes in myenteric neurons from the guinea pig ileum: intracellular recordings, and fast CCD-based imaging with the potentiometric dye di-8-ANEPPS. Low frequency electrical stimulation of an interganglionic strand was used to stimulate activity in the myenteric network. Hexamethonium (200μM) was used to block nicotinic fast EPSPs. Imaging experiments revealed a control fast EPSP amplitude of 1.00±0.10ΔF/F (n=50 neurons). Following addition of hexamethonium for 10 minutes the fast EPSP was reduced to 0.55±0.07ΔF/F (55% of control). A train of electrical stimuli was then applied for 5 minutes (1Hz, 0.4ms) followed by a rest period of 2.5 minutes and fast EPSPs evoked again. After the first train fast EPSPs were still depressed (49% of control) and further trains of electrical stimulation did not improve this. Washout of hexamethonium resulted in fast EPSP amplitude returning to 67% of control (n=20). Electrophysiological experiments also showed that in the presence of hexamethonium fast EPSP amplitude remained depressed after stimulation (hexamethonium : 38% of control; after stimulation: 30% of control, n=5). In the presence of nicotinic blockade low frequency electrical stimulation of mixed excitatory/inhibitory fibres was not associated with an increase in fast EPSP amplitude. We predict that selective stimulation of excitatory fibres alone may be associated with an increase in non-nicotinic fast synaptic transmission.

ORAL-09-08

CHARACTERISATION OF VOLTAGE-GATED SODIUM AND CALCIUM CHANNEL EXPRESSION IN THE GUINEA-PIG ENTERIC NERVOUS SYSTEM AND CHANGES INDUCED BY INTESTINAL INFLAMMATION

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Inflammatory conditions drastically affect intestinal performance and alter enteric nervous system (ENS) neuronal phenotypes. Voltage-gated sodium and calcium channels are responsible for the upstroke of the neuronal action potentials and control calcium influx, triggering cellular responses, including neurotransmitter release. We have characterised the expression profiles of these channels in enteric neurons in the guinea-pig ileum by RT-PCR and combined in situ hybridisation and immunohistochemistry. RT-PCR indicated the presence of the TTX-sensitive sodium channels NaV1.2, 1.3, 1.6, and 1.7, the TTX resistant sodium channels NaV1.8 and 1.9, the N-type calcium channel CaV2.2 (α1B), the P/Q channel CaV2.1 (α1A), the R channel CaV2.3 (α1E) and the auxiliary calcium channel subunits α2δ1-4. In situ hybridisation indicated robust neuronal expression of NaV1.3, 1.7, 1.9, CaV2.2 and 2.3 and the α2δ1 subunit. Co-localisation experiments demonstrated strong expression of NaV1.7, NaV1.9 and CaV2.2 in Dogiel Type II neurons (IPANs) and modest-to-low expression of NaV1.3, CaV2.3, and α2δ1. nNOS-positive Dogiel type I neurons expressed NaV1.3, NaV1.7, CaV2.3 and α2δ1. 7 days after TNBS-induced ileitis expression of many channels (measured by qPCR, n=4 for all datapoints) appeared reduced, e.g. CaV2.2 and CaV2.1 were reduced to 42.9 and 40.2 % of control levels. By contrast, ileitis increased (2.6-fold) the expression of the IK_{Ca} potassium channel KCNN4. Our results indicate that specific channel types are expressed in enteric neurons and that changes in expression are associated with the phenotypic changes after inflammation.

ORAL-10-01

DENTAL PULP STEM CELL TRANSPLANTATION IMPROVES FUNCTIONAL OUTCOME IN A RODENT MODEL OF ISCHAEMIC STROKE

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Stroke is the leading cause of disability in the Australian community. We can prevent stroke and even treat an occlusion within the cerebral circulation with thrombolysis. The future challenge is how to improve functional outcome post-stroke when the brain has been irretrievably damaged. There is controversy as to the role of stem cell therapy in the answer to this challenge. Although many stem cell populations exist the optimum for neuro-regeneration is unknown. We have published extensively on the discovery and neural potential of a stem cell population derived from human teeth, i.e. the Dental Pulp Stem Cell (DPSC). In this study we used a reversible middle cerebral artery occlusion method to generate an ischaemic stroke in Sprague-Dawley rats. 24 hours post-stroke 500,000 DPSCs were transplanted into the peri-infarct region of the rodent brain. Cyclosporin was administered daily to DPSC treated and media-only treated, control animals. Multiple neuro-behavioural assessments were undertaken weekly on all animals before and following treatment and the assessor was blinded to treatment. At four weeks following treatment we found enhanced improvement in function in the DPSC treated animals (n=9) in comparison to the control animals (n=5). By this time a majority of DPSC had not survived in the stroke brain, when immunohistochemically labelled for human mitochondrial antigen, suggesting functional improvement may not depend upon direct neural replacement. All experiments were authorised through the University of Adelaide, Animal Ethics Committee.

ORAL-10-02

OXIDATIVE STRESS IN ASTROCYTES: A TRIGGER FOR SECONDARY DEGENERATION?

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Nervous tissue can be further damaged following brain and spinal cord injury, when tissue outside the trauma site succumbs to delayed damage known as secondary degeneration. We have comprehensively characterised a model of secondary degeneration in the central nervous system, partially transecting the optic nerve, resulting in clear spatial separation of tissue undergoing secondary degeneration from the initial injury. The first changes that occur in tissue vulnerable to secondary degeneration immediately after injury are likely to trigger further degeneration. We present results of an immunohistochemical survey of secondary cellular changes in and around axons and the somata of their related retinal ganglion cells (RGCs) in the first minutes and days after dorsal ON injury, before the secondary loss of other axons in the uninjured portion of ON. Within five minutes, MnSOD (a marker of oxidative stress) co-localized within the astrocytic network across the entire profile of the ON. Secondary astrocyte hypertrophy of immunofluorescent labelling was evident from 3 hours ($p \leq 0.05$). Increases in ED-1 positive activated microglia / macrophage and Iba1 positive reactive resident microglia/macrophage numbers were only seen in ON vulnerable to secondary degeneration by 3 days ($p \leq 0.05$). Changes within RGC somata exclusively vulnerable to secondary degeneration were detected at 24 hours, evidenced by increases in MnSOD immunoreactivity. Oxidative stress spreading via the astrocytic network and from injured axons to parent RGC somata is an early event during secondary degeneration and containment is likely to be required in order to prevent further damage to the nerve.

ORAL-10-03

INTERRUPTING THE INFLAMMATORY CYCLE IN CHRONIC DISEASES

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There is strong evidence that inflammation exacerbates, maintains and accelerates a number of chronic diseases. Regardless of genetic predisposition or external stimulus, these neurodegenerative, cardiovascular and metabolic disorders, once triggered, share common features including sustained inflammatory cell activation and vascular disruption leading to widespread circulation of inflammatory cytokines, making them effectively persistent “wounds”. There is also increasing evidence that the presence of one disease can cause activation of another apparently unrelated disease, leading to multiple disorders via activation of an immune response that „fast forwards disease progression. We propose that a characteristic of these diseases is unwanted gap junction protein expression. In a number of central nervous system injury models including brain, optic nerve and spinal cord, we have observed astrocytosis, blood vessel disruption, neutrophil invasion and activation of the microglia macrophage phenotype. Treatment with connexin43 specific antisense oligodeoxynucleotides or peptidomimetics reduces the inflammatory response. Furthermore, in excised human epileptic mesial temporal lobe (n=2), Alzheimer’s (n=5) and Parkinson’s (n=5) disease brain tissue, we have found that astrocytosis and connexin43 upregulation correlate with the disease progression. Treatment of such conditions with reagents that disrupt or block gap junction channel function may, as we have shown in acute animal wound models, interrupt the inflammatory cycle, enable recovery of vascular integrity and reduce disease progression.

ORAL-10-04

PEROXIREDOXIN IV IS A NEURONAL PROTEIN AND ASSOCIATED WITH LEWY BODY FORMATION IN DEMENTIA WITH LEWY BODIES AND PARKINSON’S DISEASE

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Endoplasmic reticulum (ER) stress results from the accumulation of miss-folded proteins in the ER and is associated with the generation of reactive oxygen species and has been proposed as a mechanism for neuronal degradation in Parkinson’s disease (PD). Peroxiredoxin 4 (P4) is a 31kD enzyme that is reported to be solely confined to the ER where it inactivates hydrogen peroxide and acts as a molecular chaperone. Electrophoresis and Western blotting of two cases of human tissue from control, dementia with Lewy body and PD brain show that P4 is present as a 31kD protein with lesser amounts of a 62 kD dimer in both white and gray matter. Confocal immunofluorescence microscopy with P4 and specific cellular markers show that this enzyme is present in neurons and some microglia. P4 did not appear in all microglia and the pattern of staining was more consistent with P4 having been phagocytosed. P4 did not appear to be present in astrocytes or oligodendrocytes. When P4 was colocalised with α-synuclein as a marker for Lewy bodies a range of interactions were observed. In neurons with α-synuclein accumulation, P4 and α-synuclein were colocalised as a mass of coalescing vesicles. In many concentric Lewy bodies P4 surrounded the Lewy body, was colocalised with the ring of α-synuclein and was also present in the core. In some Lewy bodies P4 did not colocalise with α-synuclein but was closely associated with the periphery. In conclusion, these results show that P4 is another peroxiredoxin involved in neuronal defences against oxidative stress, and since P4 is confined to the ER indicates that the ER is closely associated with Lewy body formation.

ORAL-10-05

TIME TO FATIGUE IS INCREASED IN MOUSE MUSCLE AT 37°C; THE ROLE OF IRON AND ROS

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Studies exploring the rate of fatigue in isolated muscle at 37°C have produced mixed results. In the present study muscle fibre bundles from the mouse foot were used to study the effect of temperature on the rate of muscle fatigue. Provided iron was excluded from the solutions, time to fatigue at 37°C was increased compared to 22°C (125 ± 8 % of 22°C fatigue time, n = 7). In contrast, when iron was present (~1 µM), fatigue was accelerated (68 ± 10 %, n = 6). Iron can increase reactive oxygen species (ROS), which are believed to accelerate fatigue. The addition of 25-100 µM H_2O_2 at 22°C reduced time to fatigue to 80-20% of the control respectively (n = 15). Iron was added to cultured primary skeletal muscle cells to determine if iron could increase ROS production. Neither iron entry nor ROS production were detected in non-contracting muscle cells ($n \geq 6$). The addition of 8-hydroxyquinoline, which facilitates iron entry, to iron-ascorbic acid solutions caused a rapid rise in intracellular iron and ROS ($n \geq 9$). Our results indicate that time to fatigue *in-vitro* is increased at 37°C relative to 22°C, but the addition of ROS can accelerate fatigue. An increase in muscle iron can accelerate ROS production, which may be important during or following exercise and in haemochromatosis, disuse atrophy and sarcopenia.

ORAL-10-06

ANTI-EPILEPTIC EFFECTS OF TRIHEPTANOIN FEEDING IN CHRONIC EPILEPSY MOUSE MODELS

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The Citric Acid Cycle (CAC) is critical for oxidative metabolism. Also, CAC intermediates are the precursor of neurotransmitters. We hypothesized that impaired CAC activity leads to decreases in ATP levels and potentially seizures. Anaplerosis is the metabolic replenishment of CAC catalytic intermediates. Here, we determined the anti-epileptic effects of feeding ad libitum a diet with 35% calories from triheptanoin, the triglyceride of the anaplerotic C7 fatty acid heptanoate, in CF1 mice. Triheptanoin feeding had no effect on seizure thresholds in 3 acute seizure mouse models. Here, we tested its effect in 2 chronic seizure models. 1) Mice that had developed SE or not ("no SE") in response to pilocarpine injection were fed control or triheptanoin diet for 3 weeks. SE mice on the triheptanoin diet showed statistically significant increases in the brain levels of the anaplerotic CAC precursors, propionyl- and methylmalonyl-CoA ($p<0.01$, n=8-10 mice per group). To assess seizure susceptibility in the chronic stage of the pilocarpine model, seizure thresholds to pentylenetetrazole (PTZ) infusion (i.v.) were determined. SE mice were more sensitive than "no SE" mice to PTZ-induced seizures. In 2 experiments, triheptanoin feeding significantly increased the tonic PTZ seizure thresholds in SE mice ($p<0.05$, n=10-15 mice per diet group), indicating anti-epileptic activity in "epileptic" mice. 2) Mice on either diet were kindled by corneal electroshock after local anaesthesia of their corneas. In 3 independent experiments, triheptanoin feeding produced a statistically significant delay in kindling ($p<0.05$, n=15-25 mice). In summary, triheptanoin was repeatedly anti-epileptic in 2 chronic mouse seizure models and may be anaplerotic in the epileptic brain. It remains to be determined to which extent triheptanoin can be used to treat human seizure disorders.

ORAL-10-07

A GENETIC EPILEPSY RAT MODEL DISPLAYS ENDOPHENOTYPES OF PSYCHOSIS

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The incidence of psychosis is increased in people with epilepsy, including idiopathic generalized epilepsies. To study the biological basis for this co-morbidity, we compared GAERS, a genetic rat model of absence epilepsy, to non-epileptic control rats (NEC). Mature, 14-week old GAERS (n=6-10) showed significantly enhanced amphetamine-induced locomotor hyperactivity – a feature also present in young (6-week old) GAERS prior to epilepsy onset. Prepulse inhibition and its disruption by psychotropic drugs did not differ between strains, although GAERS displayed significantly elevated startle responses at both epileptic and pre-epileptic ages. The frontoparietal cortex of GAERS displayed a twofold increase in the power of gamma (30-80Hz) oscillations ($P<0.05$), a proposed neurophysiological correlate of psychosis. Radioligand binding autoradiography demonstrated significantly reduced densities of dopamine transporters in the caudate nucleus and nucleus accumbens core and of dopamine D2 receptors in the caudate nucleus. GAERS provide an opportunity to study the neurodevelopmental, genetic and therapeutic aspects of psychiatric comorbidities associated with epilepsy.

ORAL-10-08

THE EFFECT OF AMYGDALA KINDLING ON NEURONAL FIRING PATTERNS IN THE THALAMUS: IMPLICATIONS FOR THE RESISTANCE TO KINDLING OF GAERS

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Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are resistant to the progression of amygdaloid kindling. We hypothesized that the later (convulsive) stages of amygdala kindling involves acquired alterations in thalamic neuronal firing properties, and that baseline perturbations in GAERS renders them resistant to this change. Extracellular single neuron recordings were performed in-vivo under neurolept anesthesia. The interictal firing patterns in the thalamic reticular nucleus (TRN) was similar between non-stimulated GAERS (n=10 cells, 5 rats) and NEC rats (n=6 cells, 3 rats) for all parameters examined (mean firing frequency, % burst firing, mean number of action potentials (APs) per burst, maximum number of AP/burst and intraburst firing frequency). In kindled NEC rats (n=10 cells, 6 rats) the TRN firing demonstrated a bursting, lower firing frequency pattern which was not seen in stimulated GAERS (n=18 cells, 5 rats). For the VB cells there were differences between non-stimulated GAERS (n= 10 cells, 5 rats) and NECs (n=8 cells, 5 rats) in the mean firing frequency (14.3 vs 3.8 $p<0.01$) and mean APs/burst (2.3 vs 2.7 $p<0.05$) interictally. VB cells in kindled NECs (n=20 cells, 9 rats) had a lower firing frequency and more burst firing than stimulated-GAERS (n=11 cells, 6 rats). During a seizure in kindled NEC rats the firing pattern in TRN neurons was affected early in the seizure, with rhythmic synchronized burst firing (sometimes precede by a brief suppression) (n=18 cells, 5 rats). However, in stimulated GAERS the TRN firing engaged late in the seizure or not at all (n=10 cells, 6 rats). The results indicate that kindling induces a slower and burst firing pattern in thalamus which may play a mechanistic role in the synchronized TC firing underlying the secondary generalization of the limbic seizures. This is not seen in GAERS, consistent with their resistance to developing secondary convulsive seizures.

ORAL-11-01

SOMATOPIC REPRESENTATION OF MUSCLE PAIN IN THE HUMAN INSULAR CORTEX: FMRI EVIDENCE OF INTRA-LIMB SOMATOTOPY

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Introduction: Using functional magnetic resonance imaging (fMRI) we previously documented somatotopy of muscle and cutaneous pain in the human insular cortex by comparing the spatial representations of noxious inputs from the arm and leg (Henderson et al., 2007). Here we tested the hypothesis that a somatotopic representation can be demonstrated for noxious inputs from different muscles in the one limb. **Methods:** Changes in Blood Oxygen Level Dependent (BOLD) signal intensity were measured using a 3T scanner (Siemens) during bolus intramuscular injections (0.5 ml) of 5% hypertonic saline into the left shoulder (deltoid), forearm (flexor carpi radialis) and hand (first dorsal interoseous) muscles in 14 subjects. 288 volumes (32 axial slices, TR=3 s, TE=50 ms, flip angle=90 deg, raw voxel size=1.96x1.96x4.4mm thick) were collected. Significant changes in signal intensity (SPM5, random effects, FWE corrected $p<0.001$) were determined on a voxel-by-voxel basis, using a box-car model. **Results:** Subjects reported a local dull ache during intramuscular injections into the shoulder, forearm or hand. Correspondingly, there was a clear separation of the maximally activated clusters within the right dorsal posterior insula, both in the anteroposterior and inferosuperior planes. **Conclusions:** Muscle pain is represented somatotopically within the contralateral dorsal posterior insula, and that this somatotopy can be demonstrated for muscles within one limb. This supports the idea that the posterior insula is involved in stimulus localization of noxious inputs. Henderson LA, Gandevia SC & Macefield VG, Somatotopic organization of the processing of muscle and cutaneous pain in the left and right insula cortex: a single-trial fMRI study *Pain* 2007 128: 20-30.

ORAL-11-02

SCIATIC NERVE INJURY CAUSES DISTINCT CHANGES IN DIFFERENT CLASSES OF NOCICEPTORS

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Members of the glial cell line-derived neurotrophic factor (GDNF) family (GDNF, neurturin, artemin) show efficacy in rat neuropathic pain models and promote regeneration following nerve injury. Their respective receptors (GFRα1, GFRα2 and GFRα3) are expressed by dorsal root ganglion (DRG) neurons and reveal unique patterns in spinal projections, strongly suggesting distinct functions. To determine the effect of peripheral (sciatic) nerve injury on GFRs in DRG neurons we utilised two injury models, chronic constriction (n=6) and transection (n=16), which differ in severity and development of nociceptive behaviours. Each observation was made in at least 5 animals on tissues removed from adult male rats (after 7d) and processed for immunohistochemistry and image analysis. We found the two injury types had similar effects, but each GFR responded differently. Following injury, GFRα1 was upregulated throughout most of the dorsal horn extending rostrally to L1. This corresponded with increased GFRα1 expression in DRG neurons and a larger proportion of myelinated neurons expressing GFRα1. Injury also caused an expansion of L3-5 dorsal horn innervated by GFRα3 fibres, which corresponded with increased GFRα3 expression in DRG neurons, and a larger number of CGRP and myelinated neurons expressing GFRα3. In contrast, GFRα2 expression decreased in L3-5 cord and was downregulated in DRG neurons after injury. Injury had no effect on peptidergic sensory fibres. This study has revealed distinct effects of peripheral nerve injury on different classes of DRG neurons, suggesting complex changes in spinal connectivity and altered responses to neurotrophic factors. These need to be considered in the future development of pro-regenerative therapies or treatments for neuropathic pain.

ORAL-11-03

IDENTIFICATION OF VAGAL MECHANO-NOCICEPTOR ENDINGS IN THE GUINEA PIG OESOPHAGUS

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In the guinea pig oesophagus, low threshold mechanoreceptors have peripheral mechanotransduction sites corresponding to “intraganglionic laminar endings” in myenteric ganglia (“IGLEs”), while the endings of non-saturating vagal mechano-nociceptors remain to be identified. Extracellular recordings combined with biotinamide dye fills were made from fine vagal nerve trunks innervating small segments of guinea pig oesophagus, to which mechanical stimuli were applied. Distension by increasing loads (0-400mN), activated low threshold units in a saturating manner, peaking at 48±14Hz at 300mN (averaged over 5s, n=5) whereas mechano-nociceptors fired more slowly in a non-saturating fashion with firing rate of 12±2.4Hz at 300mN load (n=14, P=0.003). Low threshold afferents had large amplitude action potentials (177±20μV) of short duration (449±40ms), whereas non-saturating units had significantly smaller amplitudes and longer durations (64±10μV, P=0.0013, and 787±105ms, P=0.019, n=7). Probing with von Frey hairs (0.1-10mN) activated both low threshold units and non-saturating mechano-nociceptors; but low threshold units had faster instantaneous firing frequencies across the range of von Frey hairs, saturating at 5-10mN (n=3, 4 respectively). Receptive fields of the non-saturating units tended to be larger and aligned with muscle bundles whereas low threshold units had small punctate receptive fields (IGLEs). Biotinamide fills revealed intramuscular varicose axons significantly associated with receptive fields of non-saturating mechano-nociceptors. Our results confirm the presence of non-saturating vagal mechano-nociceptors in the guinea pig oesophagus, which encode distension into the noxious range. These sensory nerves have mechanotransductive endings corresponding to distinctive intramuscular branching arrays of axons; quite different to the IGLEs of low threshold mechanoreceptors.

ORAL-11-04

VIBRATION-EVOKED ALLODYNIA MEDIATED BY UNMYELINATED TACTILE AFFERENTS IN HUMAN HAIRY SKIN

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We recently showed a differential contribution of cutaneous and deep mechanoreceptors to tactile-modulation of muscle pain. Allodynia was evoked by activation of cutaneous afferents; blockade of cutaneous afferents (intradermal anaesthesia) abolished allodynia, revealing an underlying hypoalgesia. However, it remains unclear whether allodynia results from activation of a single class of cutaneous afferent or the convergence of inputs from multiple classes. **Methods:** Detailed psychophysical observations were made in 31 healthy subjects. Sustained muscle pain was induced by infusing hypertonic saline (HS: 5%) into tibialis anterior muscle (TA). Sinusoidal vibration (200Hz-200μm) was applied to the hairy skin overlying TA. Pain ratings were recorded using a Visual Analog Scale (VAS). In order to test the contributions of unmyelinated and myelinated cutaneous afferents to allodynia, compression block (sciatic nerve) and low-dose intradermal anaesthesia (Xylocaine 0.25%) were used. **Results:** Prior to the induction of muscle pain, all subjects reported vibration as non-painful (VAS=0). During muscle pain (VAS 4-6), 14 subjects consistently reported a vibration-evoked increase in pain (allodynia) that was significant, reproducible over time and persisted following the blockade of myelinated afferents (compression block). In contrast, blockade of unmyelinated afferents (low-dose intradermal anaesthesia) abolished allodynia. Allodynia was preserved in the adjacent non-anaesthetised skin. Once the HS-induced pain disappeared, all subjects described vibration as non-painful. **Conclusions:** These results demonstrate that unmyelinated tactile (C) afferents in hairy skin mediate vibration-evoked allodynia. Moreover, the balance of peripheral inputs that determines tactile-modulation of muscle pain can be altered by the preferential activation of unmyelinated cutaneous afferents, predisposing individual vibration-evoked responses toward allodynia.

ORAL-11-05

BRAIN RESPONSES TO NOXIOUS THUMBNAIL PRESSURE ARE REDUCED DURING THE APPLICATION OF A HETEROTOPIC NOXIOUS CONDITIONING STIMULUS

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Purpose: The reduction of pain intensity evoked by a phasic test stimulus during the concurrent application of a heterotopic noxious conditioning stimulus has been well documented, yet the neural correlates associated with this phenomenon are yet to be elucidated. This study measured changes in regional cerebral blood flow (rCBF) associated with endogenous pain modulation. **Method:** Arterial spin labeling (ASL) data were acquired on a 3T Siemens scanner from 27 healthy volunteers during four conditions: (a) rest, (b) intermittent noxious thumbnail pressure (TP), (c) tonic noxious cold stimulation of the foot (CCS), and (d) concurrent TP+CCS. Participants rated the pain intensity of TP stimuli immediately after stimulus offset during conditions (b) and (d). Cold pain and temperature ratings were provided at the same frequency during CCS and rest scans, respectively. General linear modeling was performed to identify changes in rCBF associated with TP, CCS, and their interaction. **Results:** Subjective pain ratings for TP significantly decreased during concurrent CCS (p<0.05). In addition, pain ratings exhibited temporal summation during TP scans, but remained stable during TP+CCS. Decreased rCBF associated with concurrent TP+CCS was observed in several pain processing regions including the insula, anterior midcingulate, and somatosensory cortices (p<0.05). Regions more strongly activated by TP+CCS compared with either condition alone included the anterior cingulate and ventrolateral prefrontal cortex (p<0.05). **Conclusion:** Decreased rCBF observed during concurrent TP+CCS corresponds with the decreased pain ratings for TP stimuli, consistent with an endogenous analgesic effect. The positive interaction between TP+CCS observed in opiodergic regions of the brain indicates the involvement of supramedullary regions in endogenous pain modulation.

ORAL-11-06

RECTAL MECHANORECEPTORS IN WILD TYPE AND LETHAL SPOTTED MUTANT MICE THAT LACK A VISCEROMOTOR PAIN REFLEX TO RECTAL DISTENSION

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Preliminary recordings from lethal spotted (ls/ls) mutant mice, which lack myenteric ganglia in the terminal rectum, showed that the visceromotor responses (VMRs) to noxious levels of rectal distension were absent, or substantially reduced. In this study, we have compared the properties of stretch-sensitive rectal afferents in wild type and ls/ls mice. Methods: In anaesthetized mice EMG recordings were made from the transverse oblique abdominal muscles during rectal distensions (up to 100 mmHg) to activate VMRs. Extracellular recordings were made from rectal nerves in flat sheet preparations of whole colorectum in wild type and ls/ls mice. Results: In contrast to wild type mice, noxious distension of the aganglionic rectum in ls/ls mice failed to trigger VMRs. In the same animals, however, VMRs could be reliably activated by pinching of the tail, hind limb or whiskers (n=6). Stretch-tension responses to ramp distension (50% stretch, 3μM nicardipine) were not different in both types of mice (n=5). Low threshold muscular or muscular-mucosal stretch-sensitive afferents were recorded in both types of mice. However, in wild type mice, there were significantly more stretch-sensitive afferents in each single nerve trunk (1.85 ± 0.21, n=34) when compared to ls/ls mice (1.14 ± 0.38, n=14, P<0.05). The responses of muscular afferents to von Frey probing of receptive field were reduced in ls/ls mice (n=6) compared with wild type (n=7, P<0.05). Conclusions: Differences exist in the properties and proportion of low threshold stretch-sensitive afferents between wild type and ls/ls mice, however, further investigation is needed to determine whether this is responsible for the lack of VMRs in ls/ls mice.

ORAL-11-07

MICRORNA-143 IN MURINE SENSORY DRG NEURONS

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MicroRNAs (miRNAs) are small non-coding RNAs which repress translation or induce degradation of target mRNAs, eg, during differentiation and synaptogenesis. Since one miRNA can target hundreds of different mRNAs, we investigated if miRNAs can act as master regulators of processes activated after nerve injury. The presence, expression profile and function of miRNAs in sensory neurons are unknown. We used miRNA-arrays, qRT-PCR and In-Situ-Hybridisation (ISH) of murine DRG neurons in situ and in vitro to detect miRNA-143 (n = 5). For gain of function and loss of function experiments primary cultured neurons were transfected with mimics or inhibitors of miRNA-143 and subsequently neurochemical characteristics (multiple labelling immunohistochemistry) were analysed (n = 5). Nociceptive I-B4 positive neurons were enriched using microbeads (n = 3). MiRNA arrays showed significantly reduced expression of miR-143 in primary cultured DRG neurons compared with naïve DRGs. qRT-PCR validated the array data and ISH showed the presence of miR-143 in most sensory neurons in situ and in vitro. miR-143 expression was decreased after 1d in culture but recovered to control levels after 5 days. IB-4+ neurons showed a higher miR-143 expression compared with the non-IB4 fraction but inhibitors and mimics modulated the regrowth of fibres from IB4-/Nf200+ neurons. MiR-143 is highly expressed in sensory neurons in situ and in vitro. Nerve damage mimicked by sensory neuron isolation reduced the expression of miR-143. Reduction of miR-143 in culture increased neurite growth in Nf200+ neurons, a process similar to the sprouting of Nf200+ fibres in the spinal cord after nerve damage nerve damage in vivo. This suggests involvement of miR-143 in the response of neurons to nerve damage.

ORAL-11-08

α1-ADRENERGIC RECEPTOR EXPRESSSION ON SENSORY AFFERENTS IN NORMAL SKIN

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This study addresses the hypothesis that crosstalk between sympathetic neurons and primary nociceptive afferents, as a mechanism underlying neuropathic pain, is mediated by expression of α1 adrenergic receptors (α1AR) on sensory fibres. Male Wistar rats (n=6, 9-12 weeks old) were anaesthetised and perfused fixed with Zamboni's fixative. Skin and dorsal root ganglia (DRG) were subsequently processed for immunohistochemistry. Specific adnexa of the skin showed strong α1-AR immunoreactivity including eccrine sweat glands, hair follicles, specific epidermal layers, sebaceous glands, blood vessels, skeletal muscle and structures resembling both nerve bundles and fibres. To confirm α1-AR expression on nerve fibres and to identify the fibre type(s), multiple labelling with antibodies directed against α1-AR and specific neuronal markers was used, including PGP9.5 as a pan-neuronal marker, tyrosine hydroxylase (TH: sympathetic neurones); isolectin B4 (IB4: non-peptidergic sensory neurones), calcitonin gene related peptide (CGRP: peptidergic sensory neurones), transient receptor potential vanilloid receptor 1 (TRPV1: C-, A-delta nociceptive sensory neurones) or myelin basic protein (MBP). Both myelinated and unmyelinated sensory neurones were shown to express α1-AR, and sub-populations of both CGRP and TRPV1-immunoreactive fibres were also double labelled. Immunostaining in DRG confirmed expression of α1-AR within a specific sub-population of CGRP-immunoreactive nerves, whereas the majority of TRPV1-labelled DRG co-expressed the α1-AR. These studies imply a normal physiological role of α1-ARs in the function of C/A-delta afferent sensory fibres, and have important clinical implication relative to the generation of hyperalgaesia in animal models of neuropathic pain and inflammation.

ORAL-12-01

TYROSINE HYDROXYLASE ACTIVITY USED TO DETERMINE ACTIVE CATECHOLAMINERGIC CIRCUITS IN MESOLIMBIC PATHWAYS RESPONDING TO STRESSORS

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To investigate the role midbrain dopaminergic cell groups have in response to autonomic and metabolic stressors, we have investigated tyrosine hydroxylase (TH), the rate limiting enzyme of catecholamine biosynthesis. Regulation of TH activity is mediated by phosphorylation of serine (ser) residues 19, 31 and 40. We have explored four regions (ventral tegmental area (VTA), substantia nigra/retrorubral field (SN), the nucleus accumbens (NAc), medial prefrontal cortex (mPFC)) to determine the response to hypotension (hydralazine 10mg/kg ip) and glucoprivation (2-DG 400mg/kg ip). The degree of phosphorylation and the amount of TH protein were determined by western blotting and compared to vehicle injected animals (n=6: all groups). In combined SN/VTA tissue hydralazine evoked an increase in THpser40 at 5, 20 (>1.8 fold) and 60min, an increase in pserTH19 at 5 and 20 (>4 fold) but not at 60min with no effect at THpser31 or in total TH levels. All further data were collected 20 min post stimulus. In the VTA, hydralazine increased pser40 (>1.6 fold) and pser19 (>3.1 fold) with no effect at pser31 whereas decreases were seen in all ser residues in the SN. Two sites of projection of the dopaminergic cell groups were explored. No effects were seen in NAc whereas in mPFC only pser31 was significantly elevated. These data indicate hypotension increases the activity of TH containing neurons or inputs in the VTA, releases catecholamine in the SN reducing TH activity, increases the TH activity and therefore the release of catecholamine in mPFC but not in the NAc. A different pattern of activity was seen following glucoprivation.

ORAL-12-02

HUNTINGTIN-ASSOCIATED PROTEIN 1 (HAP-1) IS A NOVEL REGULATOR OF EXOCYTOSIS

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

ORAL-12-03

A HIGH THROUGHPUT METHOD FOR STUDYING SYNAPTIC VESICLE ENDOCYTOSIS

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Synaptic vesicle endocytosis (SVE) is essential in maintaining neurotransmitter release. The GTPase dynamin I is known to play a critical role in regulating SVE. In the past few years our group has identified a number of small molecule inhibitors of dynamin I activity. In order to allow rapid screening of novel compounds for their potency and specificity in blocking dynamin I activity, and hence SVE, we developed a high throughput assay of SVE. We report a simple assay in which synaptosomes are attached to a glass-bottom 96-well plate and their uptake of the styryl dye FM 4-64 in response to depolarisation determined by high-throughput fluorescence microscopy. Synaptosomes are prepared from rat brain and then frozen and stored for up to a month before their use in this assay. We find that several compounds that we have previously reported to inhibit dynamin I GTPase activity mediate dose-dependent blockade of FM 4-64 uptake (n = at least 3 independent experiments). Furthermore, we find that with slight modifications the assay can be used to specifically assay different types of SVE. Clathrin-mediated endocytosis (CME) is the dominant form of SVE at low stimulation intensity, and can be assayed by depolarising synaptosomes using 0.1 mM 4-aminopyridine. By contrast, activity-dependent bulk endocytosis (ADBE) is induced by intense stimulation (80 mM K+), and can be specifically monitored by replacing FM 4-64 with fluorescently labelled dextran. Thus, CME and ADBE can be analysed independently. In summary, we report a high-throughput screening method for SVE that will be highly effective in examining the potency and specificity of inhibitors of SVE.

ORAL-12-04

NDFIP1 PROMOTES UBIQUITYLATION AND NUCLEAR TRANSLOCATION OF PTEN DURING NEURONAL SURVIVAL FOLLOWING BRAIN INJURY

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Ndfip1 (Nedd4-family interacting protein 1), is a neuroprotective protein which is capable of increasing neuronal survival following stress (Howitt *et al.* PNAS. 2009 Sep 8;106(36):15489-94). Ndfip1 is known to associate with the Nedd4 family of ubiquitin ligases, and subsequently aids Nedd4-mediated ubiquitylation of proteins in the neuron. However, the precise mechanism and targets of Ndfip1's action remain unknown. In the present study, we demonstrate using co-immunoprecipitation experiments that Ndfip1 binds to PTEN (phosphatase and tensin homolog, a potent tumor suppressor and multifunctional signalling protein) and this interaction resulted in the ubiquitylation of PTEN. To examine the cellular consequences of PTEN ubiquitylation, Ndfip1 was overexpressed in SY5Y cells, and this showed nuclear localization of PTEN. Control cells showed only cytoplasmic localization of PTEN. Since PTEN activity in the cytoplasm has previously been shown to be inversely correlated to neuronal survival, we investigated whether or not neuron survival in a mouse model of traumatic brain injury might be correlated with PTEN localisation. Following injury, dying neurons may be identified by TUNEL staining. Surviving neurons invariably showed over-expression of Ndfip1. While neurons with normal Ndfip1 levels showed cytoplasmic PTEN, neurons that over-expressed Ndfip1 showed nuclear localisation of PTEN. Together, these results strongly suggest that neuroprotection by Ndfip1 is effected by PTEN binding and ubiquitylation, and PTEN modification by ubiqutin leads to nuclear localization, promoting neuron survival effects.

ORAL-12-05

MYD88, CHANGING THE OUTCOME OF STROKE THOUGH COMMUNICATION

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Recent Toll-like receptor (TLR) literature has focused on the role of TLRs in neuropathologies like stroke. However, the many neural cell types involved in stroke express multiple TLRs suggesting a complex and integrated TLR response after neural injury. To gain a better understanding of the role of TLRs in the response to ischemia this study used MyD88 knockout (KO) mice, neurons and glia to understand the interplay between MyD88 dependant and independent signalling. This study comprised two models of stroke, firstly an in vivo stroke model using middle cerebral artery occlusion and secondly an in vitro model of oxygen glucose deprivation (OGD), enabling the investigation of specific cell types. The in vivo studies (n=6) showed that a MyD88 deficiency leads to a decreased infarct size. Surprisingly, we found no difference between wild type (WT) and KO survival in both glia and neurons following 4 hours of OGD (n=9). However, when neurons and glia are co-cultured only KO neurons showed increased survival when incubated with either WT or KO glia (n=6). Interestingly, the increased survival of the KO neurons correlate with an earlier increase in the activation of ERK and a change in the phosphorylation profile of JNK from phospho-p54 to phospho-p46 isoform. WT glia were unable to activate either ERK or JNK in the presence of KO neurons. This investigation has shown that in vitro activation of MyD88 dependant pathways can lead to an increase in cellular death however, in vivo MyD88 dependant signalling can lead to a decrease in damage. This contradictory outcome suggests an important role for MyD88 in the invading cells that regulate the inflammation and injury following stroke.

ORAL-12-06

CALCIUM RELEASE FROM INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS INFLUENCES CARDIAC PACEMAKER FUNCTION IN MOUSE SINO-ATRIAL NODE

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It has been found that Inositol 1,4,5-trisphosphate receptors (IP_3Rs), which function as inositol 1,4,5-trisphosphate (IP_3)-gated Ca^{2+} channels, are expressed in working cardiac myocytes. It has been suggested that IP_3Rs are involved in the generation of cardiac arrhythmias. However, there is little direct evidence whether IP_3Rs expression in adult mammalian sino-atrial node (SAN), the origin site of generating rhythm in the heart. In current studies, we quantified the level of the expression of IP_3Rs in the SANs by using a new cell direct qPCR technique. We find that both centre and peripheral SANs expression of IP_3Rs. We also studied the effect of IP_3R agonist, such as endothelin-1, IP_3-butyryloxymethyl ester (IP_3-BM), and antagonist 2-aminoethoxy diphenylborate (2-APB), on intracellular Ca^{2+} of spontaneously firing sinoatrial node preparations. In the presence of 10 nM endothelin-1, the resting $[Ca^{2+}]_i$ was increased by 36 ± 13 % (n = 5; P < 0.05) and the firing rate was increased by 20 ± 8 % (P < 0.05). The results were similar when IP_3-BM was used. IP_3R antagonist 2-APB reduced intracellular Ca^{2+} and slowed the firing rate. However, such effects were only seen in wild type but not in IP_3R2 knock out mice. The localisation of IP_3R2s and IP_3 induced Ca^{2+} sparks also further support that that IP_3Rs are involved in cardiac peacemaking through the release of Ca^{2+} from the intracellular Ca^{2+} stores that are near subsarcolemmal membrane.

ORAL-12-07

THE UBIQUITYLATION OF DMT1 BY NDFIP1 REGULATES THE LEVEL OF METAL IONS WITHIN THE BRAIN

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The control of metal iron transport within the body is critical for a number of physiological processes. Within the brain regulation of metals is critical as aberrant metal accumulation has been linked to neurological diseases such as Parkinson's and Alzheimer's. The divalent metal transporter 1 (DMT1) plays a central role in the regulation of iron and other metals within the cell, hence failure of DMT1 regulation is linked to human brain pathology. Recently we have discovered that DMT1 is regulated by Ndfip1, an adaptor protein that recruits E3 ligases to ubiquitinate target proteins (Howitt *et al*. PNAS. 2009, 106(36)). Using human neurons we show that Ndfip1 is upregulated and binds to DMT1 in response to metal exposure. This interaction results in the ubiquitylation and degradation of DMT1, resulting in reduced metal entry into the cell. Induction of Ndfip1 expression protects neurons from metal toxicity and removal of Ndfip1 by shRNAi results in hypersensitivity to metals. We identify Nedd4-2 as the E3 ligase recruited by Ndfip1 for the ubiquitylation of DMT1 within neurons. Comparison of brains from Ndfip1-/- with Ndfip1+/+ mice exposed to iron reveals that Ndfip1-/- brains accumulate iron within neurons. Together, this evidence suggests a critical role for Ndfip1 in regulating metal transport in neurons and provides a mechanism that could be targeted in a number of brain pathologies.

ORAL-12-08

ROLE OF AUTOPHAGY IN PROGRAMMED CELL DEATH IN PRIMARY CORTICAL NEURONS UNDER OXIDATIVE STRESS

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Neuronal cells can undergo a diverse range of death responses. We have recently shown programmed cell death (PCD) occurs as PCD-Type I (apoptosis) following staurosporine (STS) insult and PCD-Type III (programmed necrosis) under hydrogen peroxide (H_2O_2) insult in primary cortical neurons (C57/Black 6J mice). However, another form of death may occur involving autophagy (PCD-Type II) after exposure to H_2O_2. In lysates of cultured neurons treated for 24 h with STS or H_2O_2, LC3-I to LC3-II conversion increased 3-fold, indicative of autophagy. In cells expressing GFP-LC3 increased formation of fluorescent puncta confirmed autophagy in about half of cells exposed to either insult. In both cases puncta formation was blocked by the autophagic inhibitor 3-methyladenine (3-MA). Cell death monitored by uptake of propidium iodide (PI) was substantially blocked by 3-MA during H_2O_2 but not STS treatment. Although this suggests that autophagic death (PCD-Type II) occurs during H_2O_2 treatment, the situation with STS is less clear. In preliminary experiments, knockdown by siRNA of the autophagic proteins Beclin-1 and Atg7 similarly suppressed cell death during both STS and H_2O_2 treatment. We conclude that autophagic death is induced in cortical neurons by H_2O_2 treatment. This type of death proceeds alongside the programmed necrosis that occurs under severe oxidative stress.

ORAL-13-01

PROLIFERATION OF NEURONS AND NON-NEURONAL CELLS IN THE DEVELOPING MOUSE STELLATE GANGLION

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Mouse sympathetic ganglia appear around E9.5 with differentiated neurons present at E10.5 and the first glial cells at E11.5. From E10.5 onwards, the number of cells in the ganglion increases dramatically. We have used immunoreactivity to Sox10, to mark uncommitted neural crest cells and glia, and tyrosine hydroxylase (TH), to mark sympathetic neurons in E10.5 to E18.5 stellate ganglia (n=4 in each case). We have also estimated cell division rates with bromodeoxyuridine (BrdU) and s-phase length with BrdU and ethyldeoxyuridine(EdU). At E10.5, 99% of all cells are positive for sox10 expression, with half of this population also expressing TH; only 1% of cells express only TH. By E11.5, 19% of cells express sox10-only, 6% express both sox10 and TH and 76% of cells express TH-only. The proliferation rate of both TH-IR and Sox-IR cells was highest around E12.5, when around 50% of both neuronal and non-neuronal cells in the ganglion contained BrdU after a two hour pulse. We have also confirmed that S-phase lengths for neuronal and non-neuronal cells are similar during this period. Proliferation of non-neuronal cells overtook that of neurons on E16.5, when neuronal division had dropped to a low rate. Between E11.5 and E14.5, the growth of the ganglion is largely due to the division of existing neurons. The increasing disparity between the number of neurons and non-neuronal cells during this period does not depend on differences in proliferation rate or cell cycle length, but solely on the relative starting numbers of neurons versus non-neuronal cells, which is established on E10.5 when most, but not all, of the neural crest precursor cells differentiate into neurons.

ORAL-13-02

NEOGENIN CONTROLS NEURAL TUBE CLOSURE BY REGULATING CELL POLARITY

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Neogenin is a bidirectional axon guidance receptor, promoting chemoattraction in response to Netrin1 and chemorepulsion in response to RGMa. Our laboratory has recently shown that Neogenin is required for neural tube formation in Xenopus (n=306) and zebrafish (n=799) embryos (Mawdsley et al, 2004; Kee et al, 2008). Using a morpholino antisense strategy we have demonstrated that knockdown of Neogenin results in loss of neuroepithelial morphology in the mature neural tube and a failure in lumen formation. Neogenin is required for establishing the morphology of deep layer cells in the Xenopus neural plate. Loss of Neogenin severely disrupts the microtubule network within the deep layer cells suggesting that Neogenin-dependent microtubule organization within the deep cells is essential for radial intercalation with the overlying superficial cell layer, thereby driving neural fold elevation. Analysis of zebrafish morphants during early neurulation stages revealed that this phenotype resulted from the inability of neural plate cells to undergo epithelialization at the neural keel to rod stage when wildtype cells are forming adherens junctions and establishing apicobasal polarity. Immunostaining for ZO-1, a cytoplasmic component of adherens junctions in neuroepithelia, showed that ZO-1 was localized adjacent to the apical surface of cells at the midline of the neural rod in control embryos but not in Neogenin morphants. Instead, ZO-1 was found to be distributed uniformly around the plasma membrane of morphant cells. Therefore we propose that Neogenin is required to establish the apicobasal polarity of neural plate cells as they undergo epithelialization. Mawdsley D, Cooper HM, et al. (2004) Dev Biol. 269: 302-315. Kee N, Wilson N, De Vries M, Bradford D, Key B, Cooper HM. (2008) J Neurosci 28:12643-12653.

ORAL-13-03

COMPLEMENT FACTOR C5A: A NOVEL MEDIATOR OF FOLATE-DEFICIENT NEURAL TUBE CLOSURE

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Traditionally, the complement system is known as a zymogen cascade that triggers the innate immune response to foreign surfaces. However, our laboratory has recently demonstrated novel roles for complement components in the development of the mammalian nervous system. This study demonstrates mRNA and protein localisation of complement 5a (C5a) anaphylatoxin receptors, CD88 and C5L2, to the apical surface of the cephalic neuroepithelium at the point of neurulation (embryonic days 8.5-10.5) (n>3/group). At the same time points, mRNA of the precursor to C5a, C5, was expressed throughout the neuroepithelium (n=3). We hypothesised that the expression of complement factors during this period of embryogenesis may be due to a role in neural tube closure. Significantly, the loss of CD88 signaling in the mouse, through either genetic deletion or pharmacological blockade, induced a very high incidence (40-60%) of neural tube defects (NTD) in foetuses of folate-deficient dams. These represented a broad array of NTD expression, whereas in folate-sufficent embryos, complement expression was restricted to cephalic localisation. This apparent anomaly was further investigated through examination of embryos from folate-deficient dams, which demonstrated a major shift in CD88 mRNA localisation to the dorsal ectoderm (n>3/group). These data suggest that expression of CD88 in the dorsal ectoderm, under folate-deficient conditions, may somehow alleviate NTD pathologies. Our results demonstrate for the first time, the spatial and temporal expression of complement factors in the murine developing neural tube, and the effects of maternal dietary deficiency.

ORAL-13-04

NOREPINEPHRINE DIRECTLY ACTIVATES ADULT HIPPOCAMPAL PRECURSORS VIA β3 ADRENERGIC RECEPTORS

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Hippocampal neurogenesis, an important form of cellular plasticity in the adult brain, is greatly influenced by neural activity. Among the neurotransmitters that are widely implicated in regulating this process are serotonin and norepinephrine, levels of which are modulated by stress, depression and clinical antidepressants. However, their role in regulating hippocampal neural precursor activity is not well understood. Here we used the neurosphere assay to demonstrate a two-fold increase in adult hippocampal precursor activity in the presence of norepinephrine but not serotonin (n>3; $p<0.001$). Moreover, we show that norepinephrine directly activates a self-renewing and multipotent population of stem and precursor cells. Using selective pharmacological blockers of adrenergic receptors, we provide evidence that β3 adrenergic receptors, which are preferentially expressed on a Hes5-expressing precursor population in the subgranular zone (SGZ) of the hippocampus, mediate this norepinephrine-dependent activation ($p<0.001$). Furthermore, in a novel *ex vivo* 'slice-sphere' assay that maintains an intact neurogenic niche, we demonstrate that antidepressants that selectively block the reuptake of norepinephrine, but not serotonin, robustly increase hippocampal precursor activity via β adrenergic receptors. Finally, we show that *in vivo* administration of a selective and potent β3 adrenergic receptor agonist in mice significantly increases the number of proliferating cells in the SGZ (n>5), suggesting that the activation of neurogenic precursors via β3 adrenergic receptors could be a potent mechanism to increase neuronal production, providing a putative target for the development of novel antidepressants.

ORAL-13-05

PTF1A INDUCES INHIBITORY NEURONS WHOSE SUBTYPE DEPENDS ON THE EXCITATORY LINEAGES FROM WHICH THEY ARISE IN THE DEVELOPING RETINA

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During neurogenesis of the central nervous system (CNS), a multitude of different neuronal types arise from multipotent progenitor cells. Differental gene expression helps to co-ordinate these fates. The pancreas transcription factor 1a (Ptf1a) has been implicated in the determination of inhibitory fates throughout different CNS areas. Using a transgenic *ptf1a*:GFP line, we show that Ptf1a is expressed transiently after the terminal division of progenitors in differentiating inhibitory neurons of the retina (3 types of horizontal cells and 28 types of amacrine cells). The role of Ptf1a in the generation of these neurons was studied in loss and gain of function experiments (n > 60 embryos). Ptf1a is necessary for inhibitory fates, as morpholino knockdown resulted in respecification of cells into excitatory cell types (photoreceptors, bipolar and ganglion cells). Conversely, gain of function experiments showed that Ptf1a is sufficient to respecify excitatory fates to inhibitory ones. Intriguingly, time-lapse (n = 16) and lineage studies (n = 129 embryos) using transgenic lines reporting previously described fate determination gene expression of excitatory cell types, revealed that *ptf1a* turns on in cells from different parallel excitatory fates. The original lineage in which *ptf1a* expression turns on is a predictor of the subtype fate of the inhibitory neurons, suggesting factors that influence subtype specification are restricted to particular lineages and that the combination of Ptf1a and these factors determine subtype identity. We also show that the proportion of cells turning on *ptf1a* expression can be influenced by local feedback signals (n > 100 embryos), suggesting that both intrinsic and extrinsic factors influence the generation of inhibitory neurons.

ORAL-13-06

FGF8 IS REQUIRED FOR EMBRYONIC DEVELOPMENT OF THE MOUSE COMMISSURAL PLATE

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Malformation of the forebrain commissures (corpus callosum (CC), hippocampal commissure (HC) and anterior commissure (AC)) is associated with numerous cognitive deficits, and may occur due to incorrect patterning at the point of midline crossing, called the commissural plate (CoP). **Purpose**: To determine the molecular patterning and origins of the CoP. **Methods**: Diffusion tensor magnetic resonance imaging (DTI) in embryonic mice (n=3) revealed that commissures crossed the CoP at an oblique coronal angle. Anatomical delineation of CoP into the dorsal Massa Commisuralis (MC) and ventral Area Septalis (SA) was made according to work in human fetal brain (Rakic and Yakovlev 1968). **Results**: The MC could be divided into two molecular regions: (1) EMX1+ and NFIA+, and (2) NFIA+ and ZIC2+. The SA could be further divided into two regions based on the expression pattern of SIX3. Fate mapping using an IREScre transgenic mouse driven by the telencephalic morphogen *Fgf8*, showed *Fgf8* expressing cells throughout the developing CoP. Conditional knockdown of *Fgf8* at approximate embryonic day (E) 10.5 by *Emx1*Cre-loxP recombination resulted in agenesis of the CoP and failure of CC and HC crossing, but not AC crossing. Conditional knockdown of *Fgf8* at the later embryonic age of E11.5 following *Nestin*Cre-loxP recombination resulted in CC and some HC midline crossing failure without severe CoP agenesis. In **conclusion**, a narrow temporal window exists during embryonic development that is crucial for formation of the CoP. Disruption of *Fgf8* expression during this event results in malformation of multiple commissures.

ORAL-13-07

BRAIN-DERIVED NEUROTROPHIC FACTOR SIGNALS THROUGH THE TRKB-MAPK SIGNALLING PATHWAY IN OLIGODENDROCYTES TO REGULATE CENTRAL NERVOUS SYSTEM MYELINATION

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Myelination in the central nervous system (CNS) requires dynamic and complex signals between neurons and oligodendrocytes. The molecular mechanisms in particular the signals that control CNS myelination are poorly understood. By utilizing in vitro myelination assay, co-culturing the dorsal root ganglia neurons and oligodendrocyte precursor cells, and compartmentalized co-cultures, we found that exogenous Brain-Derived Neurotrophic Factor (BDNF) significantly enhances myelin formation via acting directly upon oligodendrocytes. The expression of the BDNF receptor, TrkB, increases throughout oligodendendocyte differentiation and myelination in vitro, and correlates with increased myelin protein expression. Furthermore, phosphorylation of full length TrkB (TrkB-FL) increases with BDNF-induced myelination, and blocking its activity in oligodendrocytes markedly reduced myelination, and importantly also inhibited the promyelinating effect of BDNF. We then screened downstream signalling pathways and found that the activation of MAPK/Erk signalling pathway paralleled BDNF-induced myelination in vitro. The inhibition of the MAPK/Erk signalling pathway significantly blocked BDNF's pro-myelinating effects. To unequivocally confirm the role of MAPK/Erk signalling in oligodendrocytes, we used mutant constructs that are known to activate (constitutively-active MEK) and block (dominant-negative MEK) MAPK signalling. Our data suggest that activation of MAPK/Erk signalling in oligodendrocytes is sufficient to mimic the pro-myelinating influence of BDNF, whereas blocking the MAPK signalling in oligodendrocytes substantially inhibits the basal level of myelination and importantly also blocked the BDNF's effect. Together, these data indicate that BDNF promotes CNS myelination via directly activating the TrkB-FL receptors and the downstream MAPK/Erk signalling pathway within oligodendrocytes. Importantly, our data suggest that MAPK signalling through the Erk proteins within oligodendrocytes regulates CNS myelination.

ORAL-13-08

DO LEVELS OF NEUROGENIC FACTORS IN THE HUMAN ADULT SUBVENTRICULAR ZONE CHANGE WITH AGE?

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Neurogenesis, the birth of new neurons, is regulated by numerous pro- and anti-neurogenic factors in the subventricular zone (SVZ). Levels of the putative anti-neurogenic factors interleukin-1β (IL-1β), interleukin-6 (IL-6) and $p16^{INK4a}$ increase with age in neurogenic areas of non-human adult animals (1,2). Correspondingly, levels of the putative pro-neurogenic factors insulin-like growth factor-1 (IGF-1), glial-derived neurotrophic factor (GDNF), basic fibroblast growth factor (FGF_2), and transforming growth factor-α (TGF-α) decrease with age (3). These changes are associated with a decrease in the rate of neurogenesis with age (3). The current project employed enzyme-linked immunosorbent assays to investigate changes in levels of neurogenic factors in the healthy human SVZ throughout the adult lifespan (18–104 years). Levels of IL-6 and the mitogen epidermal growth factor (EGF) were found to significantly decrease with age ($n=14$; $p<0.01$). Levels of IL-1β, $p16^{INK4a}$, IGF-1, GDNF, FGF_2 and TGF-α did not change with age. These data suggest that regulation of neurogenesis in the human brain may differ to that in other species. In future work, these data will be directly correlated with levels of neurogenesis in the SVZ in the same human brains to further understand the control of human adult neurogenesis. (1) J Neurosci Res (2002) 68:337-43. (2) J Neuroimmunol (1999) 93:139-48. (3) Aging Cell (2008) 7:569-89.

ORAL-14-01

COMPLEX INFLUENCE OF AT_{1A} RECEPTORS ON CARDIOVASCULAR REACTIVITY TO NATURAL BEHAVIOURS IN MICE

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Our recent studies suggest that AT_{1A} receptor knockout ($AT_{1A}^{-/-}$) and $AT_{1A}^{+/+}$ mice display regular ultradian bouts of behavioural activities and that blood pressure (BP) rises associated with these natural bouts are similar between strains. In this study, we examined behavioral substrates of ultradian BP variation in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice at low stress conditions (home cages in the animal house) and during mild sub-chronic stress (home cages relocated to the laboratory). The stereotyped behaviours including exploring, grooming, sniffing and nesting, and associated BP rises, were examined using synchronized video and telemetry recording. Relocation had a limited effect on ultradian behavioral and cardiovascular variation in $AT_{1A}^{+/+}$ mice. Their behavioural activities remained clustered together and were separated by long non-disrupted rest periods. Conversely, ultradian behavioural patterns were disrupted in $AT_{1A}^{-/-}$ mice, with individual behaviours distributed more evenly over time, and resting periods frequently interrupted by single short-lasting behavioural events. In all mice, during transition from resting to active behaviour, BP started to rise before the actual behaviour was exhibited. These BP rises were sympathetically mediated as they were abolished by α_1-adrenergic blockade with prazosin (1 µg/g, i.p.). Likewise, prazosin blocked pressor response to shaker stress in 'home-based' AT1A-/- mice (+2±2 mmHg, n=5). Unexpectedly, prazosin only attenuated pressor response to shaker in 'relocated' AT_{1A}-/- mice (+22±4 mmHg, n=4), although in these animals pressor response to phenylephrine was reduced by ~5-fold. These data suggest that AT_{1A} receptors may play a more complex role, as stress regulators, than previously thought. In particular, they may switch neuroeffector mechanisms of defence reaction from sympathetic to neuroendocrine mode, perhaps indirectly, by affecting behavioural or anxiety state.

ORAL-14-02

REDUCTION IN CARDIOVASCULAR STRESS REACTIVITY IN AT1A RECEPTOR KNOCKOUT MICE IS STIMULUS INTENSITY - DEPENDENT

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Our recent studies suggest that angiotensin AT_{1A} receptor knockout ($AT_{1A}^{-/-}$) mice have attenuated blood pressure (BP) responses to a number of intense aversive stimuli including restraint, footshock and contextual fear conditioning. Conversely, BP responses to less intense aversive stimuli, such as novelty exposure or brief handling, appear to be unaltered in $AT_{1A}^{-/-}$ mice. In the present study, we examined whether this selective effect of AT_{1A} receptor knockout relates to the intensity of aversive stimulation. $AT_{1A}^{-/-}$ (n=7) and $AT_{1A}^{+/+}$ (n=7) mice were implanted with BP telemetry devices and subjected to 5-min shaker stress of different intensities (40, 80 and 160 rpm). Resting BP was lower in $AT_{1A}^{-/-}$ than $AT_{1A}^{+/+}$ mice (83±2 and 100±2 mmHg, respectively). Shaker stress increased BP in an intensity-dependent manner in $AT_{1A}^{+/+}$ mice (+21±3, +25±3 and +33±2 mmHg for 40, 80 and 160 rpm, respectively). The BP increases during low- and mid- intensity shaker were not altered in $AT_{1A}^{-/-}$ mice (+21±3 and +26±4 mmHg, respectively). Conversely, the pressor response to high-intensity shaker was substantially reduced in $AT_{1A}^{-/-}$ mice (+25±3 mmHg). This reduction could not be ascribed to attenuated vascular reactivity to intense sympathetic stimulation, because intraperitoneal administration of the α_1-adrenoreceptor agonist phenylephrine (0.1-5 µg/g) dose-dependently increased BP in both strains (up to 50 mmHg), and these increases were similar at all doses tested. These data suggest that AT_{1A} receptors are essential for the full expression of the pressor response to high aversive, but not low aversive stimuli in mice. This selectivity appears to relate primarily to alterations in central stress processing, rather than in vascular reactivity to sympathetic stimulation.

ORAL-14-03

MAMMALIAN DIFFERENCES IN CHOLINOCEPTOR CONTROL OF CORONARY CIRCULATION

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How cholinoceptor activity normally controls coronary flow in man is unknown. Functional data from isolated tissues to whole mammals vary qualitatively and quantitatively. The most consistent data come from the dog where acetylcholine (ACh) dilates the right (R), circumflex (Cx) and anterior descending (AD) coronary beds. To compare *in vivo* cholinoceptor control between species, stimulus-response curves for cholinoceptor activation were described in sheep and compared simultaneously (pulsed Doppler flowmetry) in R, Cx and AD using brief right atrial infusions of ACh, and in the same sheep (isoflurane anaesthesia), electrical stimulation (ES) of the cut, peripheral end of the left vagus nerve. The heart was paced at 150 b/min. Infused ACh caused differential vasodilatation in the 3 beds. In the R, efficacy was 3-fold (*P<0.001*) with marginally greater potency (*P=0.05*) than in the left-sided beds, where efficacy in each was 2.5-fold (*P<0.05*). The responses were abolished by methscopolamine. ES of the vagus caused differential vasoconstriction as frequency rose in all coronary beds. Cx conductance fell to maximum of 83%, in AD to 88%, and in R to 91% (all *P<0.001*; *Pdiff between Cx and AD/R, 0.001*). Maximal vasoconstriction occurred at 10.5Hz in R, 12 Hz in Cx and 16 Hz in AD. The effects were blocked with methscopolamine. Thus while intravascular ACh causes in vivo vasodilatation in sheep coronary beds as in dogs, ES of vagus in sheep causes vasoconstriction i.e. the opposite effects to those in dogs. Our hypothesis is that survival is consistent with evolutionary differences in resting and reflex cholinoceptor control of coronary circulation across mammalian species.

ORAL-14-04

ENDOTHELIAL CELL HYPERPOLARIZATION AND DYSFUNCTION IN DIABETES

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Endothelial dysfunction is a major risk factor for the vascular complications of diabetes mellitus. Endothelium-derived hyperpolarizing factor (EDHF) is an important vasodilator in small arteries and arterioles and its actions are impaired in diabetes. We investigated the mechanisms contributing to EDHF dysfunction in resistance arteries of the streptozotocin (STZ)-induced diabetic rat. Diabetes was induced in 8 week old male Wistar rats by injection of 60mg/kg STZ in citrate buffer into the tail vein (*n*=20) and control rats received citrate buffer only (*n*=20). Eight weeks after injection small mesenteric artery function was examined in arteries mounted on a wire myograph or in arteries that were cut longitudinally and secured in a chamber for the recording of endothelial and smooth muscle cell membrane potentials using intracellular glass microelectrodes. EDHF-mediated smooth muscle hyperpolarization and relaxation are underpinned by the opening of intermediate (I)- and small (S)-conductance calcium-activated K^+ (K_{Ca}) channels in the endothelial cells. We found that the ability of the endothelial cells to generate hyperpolarization was impaired in diabetes, with maximum endothelial cell hyperpolarization reduced by 60% (*P*<0.0001), and this accounts for the halving of EDHF-mediated smooth muscle hyperpolarization and relaxation. Sequential application of apamin and charybdotoxin to block S/IK_{Ca} activity revealed that the contribution of both channel types to smooth muscle hyperpolarization and relaxation were similarly impaired. Using an ATP-sensitive K^+ channel opener to evoke smooth muscle hyperpolarization we found that the functional patency of the myoendothelial gap junctions was unaltered in diabetes. In conclusion, impairment of EDHF-mediated smooth muscle relaxation in diabetes is due to the reduced ability of endothelial cells to generate hyperpolarization and not due to disruption of transmission pathways between the endothelium and the smooth muscle.

ORAL-14-05

RELAXIN INDUCES DIFFERENTIAL ARTERIOLAR DILATIONS AND GAP JUNCTIONALLY MEDIATED UPSTREAM ARTERIOLAR DILATIONS

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Recently, we demonstrated the hormone relaxin causes vasodilation in the terminal microvasculature *in vivo* and identified mechanisms (nitric oxide, potassium channels, protein kinase A, phosphoinositide 3-kinase) that mediate this response. We have extended this study to elucidate whether relaxin equally vasodilates different branches of the terminal microcirculation in the blood-perfused, anaesthetized, hamster cremaster preparation. Relaxin (10^{-10}M) was applied by micropipette directly onto transverse, branch or module inflow (MI) arterioles and the local application site was observed (n = 8 in each case). For all experiments, n=8 and statistical significance was accepted at $P \leq 0.05$. Relaxin caused a significant, transient vasodilation in transverse (TA) and branch but not MI arterioles. In order to determine if capillaries respond to relaxin, we applied relaxin (10^{-10}M) to capillaries and observed the upstream MI for possible vasodilation. Relaxin application significantly vasodilated upstream MI arterioles indicating capillaries are responsive to relaxin. To explore this transmitted upstream vasodilation further, we applied relaxin to a TA and observed ~1000µm upstream; significant vasodilation was observed in response to relaxin. Since gap junctions have been implicated in transmitting vasodilation and to investigate the mechanisms by which relaxin may stimulate upstream vasodilation, we placed one of two gap junction un-couplers [18-β-glycyrrhetinic acid ($40x10^{-6}$M) or halothane (0.07%)] midway between the local and upstream observation sites and tested the effect of relaxin. Both gap junction un-couplers significantly inhibited the transmitted vasodilation. Taken together, this study is the first to report differential vasodilatory responses to relaxin in the terminal microcirculation, the first to demonstrate relaxin's ability to transmit vasodilation, and the first to implicate gap junctions in this response.

ORAL-14-06

NONLINEAR RELATIONSHIP BETWEEN HYPERPOLARISATION AND RELAXATION ENABLES LONG DISTANCE PROPAGATION OF VASODILATION IN VIVO

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Spread of vasodilation from arterioles to feed arteries enables large increases in blood flow due to the rapid spread of hyperpolarization along the vessels. However, dilations encompass larger distances than can be explained by passive spread. We hypothesized that the unattenuated spread of vasodilation results from a nonlinear relationship between hyperpolarisation and relaxation. Membrane potential and diameter were recorded simultaneously after blockade of nitric oxide synthase and cyclooxygenase in the cremaster microcirculation in vivo. Superfusion of acetylcholine (1µM, 10µM), evoked concentration-dependent hyperpolarisation (-11±1mV, -23±2mV, from-28±1mV) but maximal relaxation (90±2%, 96±2%, n=7). Inhibition of voltage-dependent calcium channels with nifedipine (1µM) also evoked maximal relaxation (97±2%) with submaximal hyperpolarisation (-10±2mV, n=6). Hyperpolarisation beyond -38mV was always accompanied by maximal relaxation. Conduction of dilation was studied following ionophoresis of acetylcholine. Locally induced hyperpolarisations (-17.5±4mV) decayed with distance (1500µm: -7±1mV) while dilation remained intact (105±16% of local response, n=7). Selective destruction of the endothelium (light dye treatment) prevented conduction of dilation without impairment of the local response. Using a computational model of the vessel wall, we could correctly predict the spread of vasodilation by applying a voltage threshold for maximal relaxation. We conclude that long distance spread of local dilations is facilitated by spread of supramaximal hyperpolarisations through the endothelium, due to a saturating relationship between hyperpolarisation and dilation that minimizes the impact of electrotonic decay. Our data suggests that changes to vasodilatory control will result rapidly from functional deficits in potassium channels or shifts in the voltage sensitivity of calcium channels.

ORAL-14-07

A PATHWAY LINKING CARDIOVASCULAR NEURONS ON EACH SIDE OF THE BRAINSTEM

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Bulbospinal barosensitive neurons in the rostral ventrolateral medulla (RVLM) set sympathetic tone and control blood pressure. Little is known about what determines their tonic firing or the rhythmic bursting of sympathetic nerves. Here we hypothesise that sympathetic premotor neurons form an intrinsic component of the tone generating network. Responses to contralateral RVLM stimulation (electrical or glutamate microinjection, 50 mM) or inhibition (muscimol or isoguvacine, 10 mM) were examined in 23 bulbospinal barosensitive RVLM neurons recorded in urethane-anaesthetised (1-1.3 g/kg), vagotomised Sprague Dawley rats. Neurons were strongly excited (N=9), inhibited (N=6) or unaffected (N=1) by contralateral RVLM stimulation. Contralateral RVLM inhibition increased firing in all cases (P<0.05, N=5) and reduced the rhythmic oscillation of these neurons (P<0.05, n=5). Electrical stimulation evoked antidromic spikes in 3/21 neurons. We examined retrograde transport of red Retrobeads(tm) unilaterally injected into the RVLM of five ketamine (75 mg/kg)/medetomidine (0.5 mg/kg)-anaesthetised rats. In some cases green Retrobeads identified bulbospinal neurons. Many contralaterally projecting RVLM neurons were identified. The density of labelling increased from virtually none at the facial nucleus (cpVII) to 41±16 neurons/section 800µm caudal to cpVII, decreasing in more caudal sections. In situ hybridization revealed 27.6±4.7% of contralaterally projecting neurons were enkephalinergic, compared to 16.6±2.5% containing GABA and 3.5±0.5% containing adrenaline. A small number were double labelled for tracer transported from the spinal cord. We conclude that many RVLM neurons, including some sympathetic premotor neurons, project to the contralateral RVLM, and that the tonic activity of this projection influences cardiovascular neurons. The mutual interactions between sympathetic premotor neurons described here may contribute to the oscillation of sympathetic nerve activity.

ORAL-14-08

DIFFERENT NEUROPEPTIDES DISTINGUISH SUBPOPULATIONS OF ADRENALLY PROJECTING SYMPATHETIC PREGANGLIONIC NEURONS

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Noradrenergic and adrenergic chromaffin cells are innervated by different populations of sympathetic preganglionic neuron (SPN) projecting from the spinal cord. How these SPN populations differ functionally has not been determined although chemical coding has been implicated. The neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and enkephalin have been proposed to mediate catecholamine secretion from the adrenal medulla based on immunohistochemical localization of terminals on chromaffin cells. Sensory innervation may be involved in release of catecholamines into the circulation. Our aims were to determine the distribution and proportion of (1) SPN that contain preproPACAP (PPP) or preproenkephalin (PPE) mRNA, (2) SPN projecting to the adrenal medulla (AM-SPN) that contain PPP or PPE mRNA and (3) sensory neurons in the dorsal root ganglia (DRG) projecting to the adrenal medulla (AM-DRG) that contain PPP or PPE mRNA. Following anaesthesia and perfusion m) of male Sprague Dawley ratsµ(4%PFA/0.1MPB) spinal cord (T4-T10) sections (40 were processed for in situ hybridization (ISH) for PPP and PPE combined with immunohistochemistry (IHC). Few SPN (vesicular acetylcholine transporter immunoreactive) were PPE+ (4±2%, n=4) whilst the majority were PPP+ (80±3%, n=3). In contrast, 52±15% of AM-SPN (cholera toxin B (CTB) immunoreactive, after CTB injections into the adrenal medulla) were PPE+ (n=5), whilst 97±5% were PPP+ (n=4). Few AM-DRG were PPE+ (0.7±0.7%, whereas 74.3±12.2% were PPP+. Thus AM-SPN use PPP as a co-transmitter and 50% of these SPN are also enkephalinergic. Enkephalin may provide a neurochemical basis for differential control of sympathetic outflow to either adrenalin (75%) or noradrenaline (25%) containing chromaffin cells. Furthermore, the adrenal medulla receives a minor innervation from sensory neurons largely containing PPP.

ORAL-15-01

EFFERENT ACTIVATION MODULATES HYPERACTIVITY IN GUINEA PIG INFERIOR COLLICULUS AFTER ACOUSTIC TRAUMA

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Tinnitus, a phantom hearing sensation, is often associated with a mild hearing loss. Animal and human data suggest that tinnitus is accompanied by hyperactivity in central auditory pathways. We have recently shown in a guinea pig model that spontaneous hyperactivity in inferior colliculus is present after mild hearing loss caused by acoustic trauma, and that at short recovery times this hyperactivity is dependent on cochlear neural output (Mulders and Robertson, Hear Res. 256:85-92, 2009). Efferent systems projecting to the cochlea (olivocochlear systems) are known to suppress cochlear neural output and we therefore investigated the capacity of these systems to alter hyperactivity. Sixteen anaesthetized guinea pigs were exposed to a loud tone (1 hr, 10 kHz, 124 dB). Two weeks after recovery, single neuron recordings in inferior colliculus were made and increased levels of spontaneous activity were confirmed. Electrical stimulation of the olivocochlear efferent system was then applied and its effects on cochlear neural output and on highly spontaneous neurons in inferior colliculus (n=73) were assessed. Stimulation of the efferent system did suppress the spontaneous hyperactivity in inferior colliculus. Interestingly, in some instances the central suppression was much longer lasting than reported previously for primary afferents. These findings are in agreement with our earlier study that hyperactivity can be modulated by altering cochlear neural output. More research is needed to investigate whether the increased central effects of olivocochlear efferent stimulation are due to central intrinsic circuitry or to co-activation of central efferent collaterals to the cochlear nucleus.

ORAL-15-02

CHRONIC NEUROTROPHIN INFUSION AND ELECTRICAL STIMULATION IN THE DEAF COCHLEA: IMPLICATIONS FOR COCHLEAR IMPLANT SPATIAL SELECTIVITY

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The application of exogenous neurotrophins to the cochlear fluid prevents the degeneration of spiral ganglion neurons (SGN) following the loss of cochlear hair cells. The SGN peripheral nerve fibers also resprout in an abnormal disorganised manner following neurotrophin treatment. This study aimed to investigate the extent of disruption of auditory nerve cochleotopic organisation with regards to the spatial selectivity of electrical stimulation by a cochlear implant. Two weeks after ototoxic deafening, adult guinea pigs (n=22) were given intracochlear neurotrophins or artificial perilymph via an osmotic pump. Half of each group also received chronic intracochlear electrical stimulation (ICES) from a banded electrode array and clinical speech processor. Following a four week treatment period multi-unit spike clusters were recorded across the inferior colliculus in response to ICES on different bipolar electrode pairs to determine the sharpness of spatial tuning. Chronic ICES resulted in significantly broader spatial tuning (Two-way ANOVA, $p<0.03$) across different stimulation sites and over a range of intensities up to 3.5dB above threshold. Neurotrophin treatment did not have a significant effect on tuning curve width ($p>0.05$). Therefore, neurotrophin treatment does not reduce the spatial selectivity of cochlear implant electrode arrays with designs based on current clinical models.

ORAL-15-03

INHIBITION OF ADENOSINE KINASE IN THE COCHLEA DELAYS THE ONSET OF HEARING LOSS IN AGING MICE

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The present study was undertaken to determine the role of adenosine signalling in the development of age-related hearing loss (presbyacusis). Adenosine is a constitutive cell metabolite with a putative role in tissue protection and regeneration. Adenosine kinase (AdK) is the key enzyme regulating intracellular and extracellular adenosine concentrations. AdK down-regulation is emerging as a potent neuroprotective strategy in brain injury induced by stroke and epilepsy. In this study, 3 months old C57BL/6 mice, known to exhibit the early onset presbyacusis, were treated with a selective AdK inhibitor ABT-702 (1.5 mg/kg i.p.) twice a week for the period of 6 months. Hearing thresholds of these mice were evaluated using auditory brainstem responses (ABR) once a month until the age of 9 months. The second group of older C57BL/6 mice (6 months) were treated with ABT-702 for 3 months. At the age of 9 months, both groups treated with ABT-702 showed lower ABR threshold shifts (10-15 dB for auditory clicks and pure tone frequencies) compared to control animals receiving the vehicle solution only. Functional studies were supported by increased survival of hair cells in the organ of Corti of ABT-treated mice. This study thus provides the first evidence that the manipulation of adenosine signalling in the cochlea can mitigate age-related hearing loss. We postulate that the inhibition of AdK can increase cochlear resistance to oxidative stress by increasing adenosine levels in cochlear fluids. Pharmacological inhibition of AdK thus represents a novel otoprotective strategy to stem presbyacusis.

ORAL-15-04

TIME-COURSE OF TONOTOPIC CHANGES IN THE AUDITORY SYSTEM FOLLOWING NOISE-INDUCED HEARING LOSS

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Unilateral noise-induced hearing loss results in changes throughout the auditory system. Moreover, a growing body of evidence suggests that hearing loss and its accompanying neuronal changes are involved in tinnitus. Understanding the development of the neuronal changes following noise-induced hearing loss will provide insight into plastic reorganisation in the auditory system and the neural basis of tinnitus. We examined neuronal changes at three different levels of the auditory pathway and at different time-periods up to 6 months following exposure to a damaging narrow band noise. Male Long Evans rats aged 3-4 months (n = 16) were unilaterally exposed to a 115 dB SPL 16 kHz 1/10th octave bandpass noise for 1-hour. We investigated frequency tuning and spontaneous activity of neurons in cochlear nucleus, inferior colliculus and auditory cortex by simultaneously recording from each of these structures using 32 or 64 channel electrodes. Six unexposed rats served as controls. Hearing was assessed before and at different time-points following the noise trauma procedure using auditory brainstem response audiograms. Frequency tuning curves were obtained using tone pips (1-44 kHz, 50 ms duration, 0-80 dB SPL, 1 Hz presentation rate). At 30 days following the noise treatment the majority of multi-unit clusters in auditory cortex had two peaks in their frequency tuning curves (9-12 kHz and 30-35 kHz), which bordered the spectral range of the noise-trauma stimulus. Similar changes were evident 6-7 months after noise trauma. Less pronounced tonotopic changes were observed in the inferior colliculus. The only observable change in the cochlear nucleus was an absence of neuronal activity in response to ~16 kHz stimulation.

ORAL-15-05

LOCALISATION OF THE STEM CELL COMPARTMENT WITHIN ADULT MOUSE TASTE BUDS

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Adult taste buds are maintained by the lifelong proliferation of epithelial stem and progenitor cells, the identities of which have remained elusive. It has been proposed that these cells alternatively reside within the taste bud (intragemmal) or in the surrounding epithelium (perigemmal). Here, we apply three different *in vivo* approaches enabling single cell resolution of proliferative history to identify putative stem and progenitor cells associated with adult mouse taste buds. Experiments were performed across the circadian peak in oral epithelial proliferation (04:00 am), a time period in which mitotic activity in taste buds has not yet been detailed. Using double label pulse-chase experiments, we show that defined intragemmal and perigemmal cell types undergo rapid, sequential cell divisions and thus represent potential progenitor cells. Strikingly, mitotic activity was observed in intragemmal taste cells previously thought to be postmitotic (labeled cells occur in 21% of palatal taste buds after one hour BrdU exposure; n=58 taste buds). Intragemmal basal cells showed expression of the transcription factor p63, required for maintaining the self-renewal potential of various epithelial stem cell types. Candidate taste stem cells were identified using the label-retaining cell approach to localise slow-cycling cells. Label-retaining cells occurred solely within basal intragemmal cell populations (0.07 ± 0.01 cells/taste bud; $p<0.05$; n=436 taste buds). Together, these results indicate that both stem- and progenitor-like cells reside within the mammalian taste bud.

ORAL-15-06

CORTICOTHALAMIC INTERACTIONS IN THE WHISKER-BARREL PATHWAY: EVIDENCE FROM BILATERAL WHISKER STIMULATION

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The rat whisker-touch is an ideal model system for studying sensory information processing due to its functional efficiency and well-characterised structural organisation: different stages of the pathway maintain the arrangement of whisker pad in distinct neuronal clusters known as barreloids (thalamus) and barrels (cortex). Here, we investigate the transformation of signal from thalamus to cortex by studying how stimuli presented simultaneously to ipsi- and contra-lateral whiskers interact. We recorded spiking activity and local field potentials (LFP) in ventral posterior medial (VPM) nucleus of the thalamus and barrel cortex (BC) of anaesthetised rats (n=18) while vibrations of different intensities were applied to ipsi- and contra-lateral whiskers. Two rats were successfully trained in a behavioural paradigm to discriminate between such bilateral stimuli, and four sinusoidal vibrations (0Hz-0.0mm, 30Hz-0.02mm, 60Hz-0.03mm, 90Hz-0.05mm) were selected. BC neurons (n=105) responded with a positive peak (4-30ms) followed by a suppression (50-100ms) below spontaneous activity. In contrast, VPM neurons (n=152) were divided into two distinct groups; some (n=98) gave an early positive activity (2-40ms), while others (n=54) elicited a late response suppression (20-60ms) below their spontaneous activity. As expected, VPM and BC activities predominantly encoded the contra-lateral stimulus intensity (or mean speed) with respective response onset latencies of 4ms and 6ms. However, in both areas, spiking activity and LFP still showed a slight (but significant) modulation in response to ipsi-lateral whisker stimulation. Due to its feedback nature, this modulation occurred initially in BC neurons (12ms), then in the VPM neurons with positive response profile (20ms) and finally in those VPM neurons with suppressed response (44ms). The distinct activity profile of thalamic neurons and the timing of their modulation, suggest that selective feedback channels shape the response properties of VPM neurons.

ORAL-15-07

MAPPING RETINAL DEGENERATION IN AGED RD1-FTL MICE

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Retinitis pigmentosa (RP) refers to a family of inherited photoreceptor degenerations resulting in blindness. Our research focuses on further understanding the disease process of RP particularly during mid to late stages when total loss of photoreceptors has occurred and significant remodelling of inner retinal neurons has taken place. Double mutant transgenic mice, *Rd1-FTL*, contain a mutation in the β subunit of phosphodiesterase 6 leading to RP and an axon-targeted β-galactosidase (β-gal) reporter system which is under the regulation of the c-fos gene. In *Rd1-FTL* mice we observed an increase in β-gal expression during photoreceptor degeneration (P10-26) followed by a complete loss of expression (P90). Surprisingly, following P120 strong upregulation of β-gal re-occurred in the central retina, despite the loss of all photoreceptors. To investigate whether this β-gal expression was associated with remodelling in this area, retinae from mice between P30 to P330 were processed as wholemounts or cryostat sections and immunocytochemically stained for β-gal. Some tissue was also resin embedded and processed for post-embedding amino acid immunocytochemistry. Upregulation of β-gal in the central retina was observed in a confined area lateral to the optic disc from P120 (n=3) that increased in size with age and spread to the entire central region (>P150). This labelling was still present following P330. Co-localization with cell markers revealed ganglion and amacrine cells expressing β-gal in this region. Notably, Müller cells showed a loss of glutamine synthetase immunoreactivity in regions of high β-gal. We propose this significant upregulation of β-gal activity may reflect active cellular signalling that is important for the remodelling of inner retinal neurons.

ORAL-15-08

BIPOLAR AND AMACRINE INPUT TO MIDGET AND PARASOL GANGLION CELLS IN MARMOSET RETINA

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Purpose: To study the density and arrangement of excitatory and inhibitory synaptic input to the dendrites of midget and parasol ganglion cells in marmoset (*Callithrix jacchus*) retina. **Methods:** Ganglion cells were retrogradely labeled (5% dextran, tetramethylrhodamine and biotin, 3000 MW "microruby") from the lateral geniculate nucleus and subsequently photo-filled (Dacey et al., 2003). Presumed bipolar cell synapses (excitatory input) were immunocytochemically identified using antibodies against the GluR4 subunit of the AMPA receptor. Presumed amacrine cell synapses (inhibitory input) were identified using antibodies against the 93kD protein gephyrin. **Results:** Twenty midget ganglion cells (15 OFF, 5 ON; eccentricity 0.29 to 1.53 mm) and thirty-two parasol cells (15 OFF, 17 ON; eccentricity 0.20 to 2.54 mm) were analyzed. All cells had comparable average densities of GluR4 IR puncta (about 5 per 100 μm^2 dendritic surface area), and of gephyrin IR puncta (about 6 to 7 per 100 μm^2 dendritic surface area). The density of GluR4 and gephyrin IR puncta colocalised with the cell membrane was correlated linearly (correlation coefficients: 0.28 midget cells and 0.77 parasol cells). The inhibitory input was greater than the excitatory input (paired t test; $P < 0.05$) with a ratio of approximately 55% amacrine to 45% bipolar input. Approximately 18% of gephyrin IR puncta were located within 1 µm of a GluR4 IR punctum. **Conclusions:** Comparable mechanisms appear to govern the distribution of excitatory and inhibitory inputs onto the dendrites of midget and parasol ganglion cells. Approximately one-fifth of amacrine synapses to ganglion cells are likely to be involved in feed-forward synapses at dyads.

ORAL-16-01

DEVELOPMENTAL VITAMIN D DEFICIENCY (DVD) AND BRAIN DOPAMINE ONTOGENY

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Developmental vitamin D (DVD) deficiency is a candidate risk factor for schizophrenia. For the past 7 years our group has been developing a rodent model of DVD deficiency. We have previously extensively described alterations in both spontaneous and psychomimetic induced locomotion in this model. Most recently we have been using this model to explore the role of vitamin D in the development of dopaminergic systems. Vitamin D deficiency is induced in female Sprague-Dawley rats by dietary restriction. Females are then mated with vitamin D normal males and the pregnant females are maintained on their respective diets during this period. At birth all maternal animals are placed on a vitamin D normal diet. The period of DVD-deficiency is therefore restricted to the gestational period only. Resultant DVD-deficient progeny were examined either as embryos, as neonates or as adults. Our findings indicate dopamine signalling is disturbed in this model. a) the superior colliculus (the proto-basal ganglia) is the site where the receptor for vitamin D is first expressed in foetal rat brain (Embryonic day E12); b) mRNA for both Nurr-1 (a nuclear transcription regulator important in dopamine neuron development) and tyrosine hydroxylase (a marker of dopamine cell maturity) are both reduced in the embryonic DVD-deficient mesencephalon by E15; c) Catechol-O-methyl transferase (a major metabolic enzyme for dopamine) was reduced in the DVD deficient neonatal rat brain; d) the dopamine metabolic profile in these brains reflected this enzymatic change. e) As adults, dopamine transporter density and/or affinity were altered in DVD deficient adult female offspring whilst Catechol-O-methyl transferase and dopamine cell number were reduced in DVD deficient male offspring (all $P<0.05$ $n \geq 8$). The developmental absence of vitamin D affects dopamine neuron ontogeny. We believe that alterations in dopamine metabolism and/or release mediate the behavioural sensitivity to psychomimetics displayed in this model.

ORAL-16-02

CHANGES IN PARVALBUMIN IMMUNOREACTIVITY IN THE HIPPOCAMPUS OF THE RAT AFTER NEONATAL LIPOPOLYSACCHARIDE ADMINISTRATION: MODELLING THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

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Events occurring within the intrauterine or perinatal environment can produce subtle structural injury and neurochemical deficits that result in behavioural dysfunction only after adolescence. These events have been linked to an increased risk for schizophrenia. The present study aimed to determine whether neonatal infection produced long-term disruptions in behaviour and pathology that might provide a parallel with that observed in schizophrenia. Rats were administered lipopolysaccharide (LPS; 500µmg/kg i.p.) on postnatal day 7 and 9. LPS administration produced no early impairment in task performance at day 35, however at day 70 LPS animals spent significantly less time exploring the novel object than control animals. Analysis of brains showed a reduction in expression of parvalbumin immunoreactive neurons in the hippocampus of LPS animals with significant reductions selectively localised to the CA1-CA3 region, and not the dentate gyrus. No changes were observed in prefrontal cortex. These results show that neonatal LPS results in pathophysiological brain changes in hippocampal CA1-CA3 subregions. We suggest that the developmental dependence of the observed LPS-induced reduction in parvalbumin neurons seen in adult rats may serve as a model to explore the biological link between postnatal hippocampal development and adult behaviour and lead to an understanding of the development of the pathophysiology of schizophrenia.

ORAL-16-03

PROMOTER SPECIFIC ALTERATIONS OF BDNF MRNA IN PEOPLE WITH SCHIZOPHRENIA

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The cause of the reduction in brain-derived neurotrophic factor (BDNF) expression in the dorsolateral prefrontal cortex (DLPFC) in the brains of people with schizophrenia is unknown. Control of BDNF production is accomplished by regulation of 4 major BDNF promoters (I-, II-, IV- and VI-IX), so we set out to determine which promoter(s) is deficient in the disease. Utilizing postmortem human brain tissue from two independent cohorts of normal vs schizophrenics matched for age, pH, PMI and RIN, the Sydney (n=37/37) and the NIH (n=34/34) cohorts, we assayed BDNF alternate transcript expression by quantitative real-time PCR and protein expression by western blotting. We found reduced BDNF protein (14kDa) (-23%; $p<0.01$) in schizophrenia, replicating our earlier study (Weickert et al. 2003). We found significant reductions in BDNF II-IX mRNA (-19%; $p<0.03$) in the Sydney cohort and confirmed this reduction (-34%; $p<0.02$) using the NIH cohort. In 3 different brain regions (DLPFC, parietal cortex and hippocampus), we found that multiple BDNF transcripts were significantly up-regulated in schizophrenia cases treated with antidepressants. As reductions in BDNF transcripts may be masked by antidepressants, we removed schizophrenic cases with recorded use of antidepressants and found significant reductions in BDNF IV-IX and VI-IX expression in the parietal cortex and hippocampus ($p<0.05$) in schizophrenia. Our findings suggest that impairments in BDNF expression are fairly widespread in schizophrenia, but that treatment with antidepressants may partially reverse these deficits.

ORAL-16-04

ADOLESCENT NEUREGULIN 1 MUTANT MICE ARE LESS SUSCEPTIBLE TO THE EFFECTS OF THC ON OBJECT RECOGNITION MEMORY AND SOCIAL INTERACTION

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Objective: Cannabis consumption is associated with increased risk of developing schizophrenia in susceptible individuals. Adolescence is a particular time of vulnerability to the detrimental effects of cannabis on cognition and behaviour. We investigated the behavioural profile of the primary THC in a battery of schizophrenia-relevant psychotomimetic cannabis constituent tests in adolescent heterozygous transmembrane domain *Neuregulin 1* mutant (*Nrg1* HET) mice. *Neuregulin 1* is a schizophrenia susceptibility gene, and *Nrg1* HET mice display age-dependent hyperactivity. **Methods**: Four week old male *Nrg1* HET mice and their wild type-like littermates received vehicle or THC (10 mg/kg i.p.) for 21 days. On the first day of treatment and throughout chronic treatment, behavioural testing took place to assess locomotor activity (open field test), anxiety-like behaviour (light-dark test), sensorimotor gating (prepulse inhibition), working memory (novel object recognition) and social interaction. **Results**: The hyperlocomotor phenotype of the *Nrg1* HET mice emerged in vehicle-treated mice by the third week of chronic treatment (6.5 weeks of age), and was present after cessation of THC treatment (7.5 weeks of age). *Nrg1* HET and wild type-like controls were equally sensitive to the locomotor suppressant effects of acute and chronic THC. THC decreased the startle response, but there were no main effects of treatment or genotype on prepulse inhibition. THC decreased the percentage of time spent exploring a novel object in wild type-like, but not *Nrg1* HET mice. In the social interaction test, THC decreased the time spent nosing and the frequency of anogenital sniffing in wild type-like, but not *Nrg1* HET mice. **Conclusion**:Male *Nrg1* HET mice exposed to THC during early adolescence appear to be less sensitive to some of its behavioural effects than wild type-like controls. These data represent the first report of the behavioural phenotype of *Nrg1* HET mice during adolescence and also one of the few reports of THC effects in adolescence in mice with a schizophrenia-relevant mutation.

ORAL-16-05

MOLECULAR ANALYSIS OF GTF2IRD1: A GENE IMPLICATED IN THE WILLIAMS-BEUREN SYNDROME COGNITIVE AND BEHAVIOURAL PROFILE

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Williams-Beuren syndrome (WBS) results from a hemizygous microdeletion within chromosome 7q11.23 involving 28 genes. WBS is characterised by specific physical, neurological and cognitive defects. The cognitive and behavioural features are collectively called the Williams syndrome cognitive profile (WSCP) and provide compelling evidence of a genetic basis for aspects of human cognition and behaviour. Genotype/phenotype correlations in patients with smaller deletions have mapped the most prominent features of the WSCP to a pair of evolutionarily-related genes, GTF2IRD1 and GTF2I. We generated Gtf2ird1 knockout/LacZ knockin mouse lines to map expression in the brain and examine the consequences of gene inactivation. These mice show behavioural features reminiscent of the WSCP. To understand the underlying biochemistry, we used phylogenetic footprinting analysis of the GTF2IRD1 upstream region and found a conserved sequence containing a cluster of canonical GTF2IRD1 binding sites. GTF2IRD1 binds to this region with high affinity and negatively regulates its own transcription. Binding is contingent upon the presence of multiple DNA recognition sites. This has enabled us to predict GTF2IRD1's potential range of target genes in the brain. In addition, microarray analyses of pooled RNA samples were each obtained from three specific regions of wild type and KO brains: cerebral cortex, hippocampus and olfactory bulb (n=7). Pairwise comparison within each brain region revealed a total of 32 genes that are dysregulated by at least 2-fold in the KO mice.

ORAL-16-06

DIFFUSE TRAUMATIC AXONAL INJURY INDUCES SENSORIMOTOR DEFICIT, MEMORY LOSS AND HIPPOCAMPAL AXONAL HYPEREXCITABILITY

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Introduction: Traumatic brain injury (TBI) often results in cognitive deficit which is thought to be due, in part, to impaired synaptic activity and axonal function. This study investigated the effects of traumatic axonal injury (TAI, a model of diffuse TBI) on cognitive, and axonal and synaptic functions using sensorimotor and memory assessments and hippocampal electrophysiology. **Methods:** TAI (n=5) was induced by dropping a weight of 450g from 2 meters while sham received surgery only (n=5). Each animal underwent sensorimotor assessments (rotarod, beam-walking and adhesive tape removal from the front paws) before and after injury. Memory test was performed on day 6 post-trauma. Electrophysiological recordings of long term potentiation (LTP), paired pulse facilitation (PPF) and input/output (I/O) were obtained from hippocampal slices at day 7 post-trauma. **Results:** Sensorimotor assessments showed that TAI animals have severe neurologic deficits after TAI; run lower speed on the rotarod (TAI: 11.3±3.2-21.0±4.5rpm; shams 26.8±1.3-29.5±3.2rpm, $P<0.05$); took longer to remove adhesive tapes (TAI: 1.12±0.40min, sham: 0.14±0.03min at day 1, $P<0.05$), and had difficulties in balancing and walking on the 2cm beam. TAI animals lost their memory (discrimination index=0.635±0.123) as compared with sham controls (discrimination index=0.735±0.07, $P<0.05$). TAI and sham animals had similar LTP and PPF curves. However, TAI had significant increased in I/O curve signals (1.97±0.34mV) when compared with sham (0.90±0.35mV, $P<0.05$). **Conclusions:** These findings suggest that diffuse brain injury induces sensorimotor deficits and loss of short-term memory. Post- and pre-synaptic functions (LTP, PPF) were normal, while an increase in I/O suggested axonal hyperexcitability that may cause axonal dysfunction and memory impairment.

ORAL-16-07

THE AMYLOID PRECURSOR PROTEIN IS NEUROPROTECTIVE FOLLOWING MILD TRAUMATIC BRAIN INJURY

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The amyloid precursor protein (APP) is known to increase following traumatic brain injury (TBI), with a number of investigators assuming that increased APP may be deleterious to outcome due to the production of neurotoxic Aβ. Conversely, this upregulation may be beneficial as cleavage of APP via the alternative non-amyloidogenic pathway produces the soluble α form of APP (sAPPα), which is known to have many neuroprotective and neurotrophic functions. Indeed a previous study showed that treatment with sAPPα following a diffuse injury in rats reduced apoptotic cell death and axonal injury with a corresponding improvement in motor outcome. However, it is not yet known whether endogenous APP plays a similar beneficial role following TBI. In order to investigate this the functional and histological outcome of APP knockout mice was compared to that of APP wildtype mice following a mild diffuse injury. Following injury APP knockout mice showed impaired spatial memory, with a significantly increased latency ($p<0.005$) to locate a previously learned escape hole in the Barnes Maze. They also took significantly longer to learn a new location when the escape hole was moved ($p<0.05$). In contrast injured APP wildtype mice performed no differently to sham animals. The cognitive deficits seen in the APP knockout mice were correlated with a significant increase in dark cell change, an indicator of neuronal cell stress, in the CA region of the hippocampus ($p<0.05$), the dentate gyrus ($p<0.005$) and the cortex ($p<0.0005$) at day 3 post-injury when compared to injured APP wildtype mice. These findings indicate that the upregulation of APP seen following injury is a protective response.

ORAL-16-08

SPASTIC PARALYSIS PRECEDES OVERT DEMYELINATION IN A TRANSGENIC MODEL OF INDUCIBLE OLIGODENDROCYTE APOPTOSIS

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Oligodendrocyte (OL) apoptosis is among the earliest neurohistopathological features of multiple sclerosis (MS). To model OL apoptosis in vivo, we have generated transgenic mice in which OLs are rendered selectively sensitive to Diphtheria toxin (DT)-mediated apoptosis. MBP-DTR transgenic mice expressing Diphtheria toxin receptor (DTR) under the regulatory control of the truncated mouse myelin basic protein (MBP) promoter exhibit OL-specific DTR expression in the CNS. Administration of 200ng DT to MBP-DTR mice resulted in the development of a progressive neurological phenotype characterised by spastic paralysis of the hind limbs. Histopathological analysis of DT-challenged MBP-DTR mice at the peak of clinical disease revealed a 35% reduction in the density of CC1-immunoreactive OLs relative to DT-challenged wild type mice (n=4 per genotype). Despite loss of OL cell bodies, myelin integrity as assessed by RIP immunohistochemistry was unaffected. Although myelin appeared to be grossly normal in clinically affected DT-challenged MBP-DTR mice, the presence of β-amyloid precursor protein-immunoreactive axonal swellings suggests that axonal protein trafficking was disrupted in some neurons. Furthermore, a marked inflammatory response was indicated by the presence of numerous activated Iba1-immunoreactive microglia, particularly in grey matter. Our results suggest that neuronal pathology following OL apoptosis precedes demyelination and implies that microglia could be pre-phagocytic at this time-point. We are currently examining myelin integrity at the ultrastructural level and characterising changes in the expression of ion channels and adhesion proteins at paranodal and nodal regions of the axo-glial junction. Ongoing analyses using the MBP-DTR model will provide a unique opportunity to characterise the molecular and cellular consequences of OL apoptosis, a hallmark of newly-forming MS lesions, and for defining new strategies to facilitate neuroprotection and repair.

ORAL-17-01

WNT5A REGULATES MIDBRAIN DOPAMINERGIC AXON GROWTH AND GUIDANCE

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During development, precise temporal and spatial gradients are responsible for guiding axons to their appropriate targets. Within the developing ventral midbrain (VM) the cues that guide dopaminergic (DA) axons to their forebrain targets remain poorly understood. In recent years, Wnt morphogens have been identified as axon guidance molecules. Furthermore, a number of Wnts are expressed in the VM during the development of DA neurons and have been associated with DA neurogenesis. Here, we illustrate a temporo-spatial expression of Wnt5a that overlaps with the development of VM DA axon projections. Using VM primary cultures, enriched in DA neurons, we show that Wnt5a significantly promotes neurite growth and alters DA neuron morphology, at a time when axons are exiting the VM. Later in development, when DA axons are approaching their striatal target, Wnt5a causes neurite retraction. Antagonism experiments reveal that these effects are mediated via the atypical receptor tyrosine kinase, Ryk resulting in downstream activation of the non-canonical Wnt pathway, and more specifically via the GTPase, Rac1, of the planar cell polarity pathway. Further, VM explants co-cultured with Wnt5a-over-expressing cell aggregates reveal that Wnt5a repels DA neurites. The importance of Wnt5a in DA axon morphogenesis was verified in Wnt5a (-/-) mice (n>5), where fasciculation of the medial forebrain bundles was disrupted as well as the density of DA neurites and striatal terminals. These results identify a novel role Wnt5a in DA axon morphogenesis, findings that could have important implications for understanding a number of DA related neurological disorders such as Parkinson's disease.

ORAL-17-02

DEFINING THE ROLE OF THE OLIVO-COCHLEAR SYSTEM IN THE DEVELOPMENT OF THE AUDITORY SYSTEM IN RATS AND MICE

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Neural circuits that process sensory information require electrical activity during development to promote maturation and refine synapses. Early spontaneous (sensory-independent) activity occurs in discrete bursts of action potentials separated by long periods of silence. Studies in the visual system indicate that bursting activity originates in the retina, pointing to an important role of sensory organs in activity-dependent neuronal development. How is bursting activity generated in the auditory system? This is relevant since neuronal feedback to the sensory organ (the cochlea) occurs via the olivo-cochlear system, an inhibitory cholinergic pathway located in the ventral brainstem. To begin addressing this question we performed electrophysiological recordings in the ventral brainstem of anesthetized neonate rats (n = 69 between P0-11). We found that central auditory neurons fire action potentials in a precise sequence of mini-bursts prior to the onset of hearing in rats. This stereotyped pattern is similar to that recorded in primary auditory neurons in cochlear explants (N. Tritsch and D. Bergles, personal communication), indicating that olivo-cochlear feedback is not necessary to generate bursting activity. To determine the cellular mechanisms involved in generating bursting activity, we obtained whole cell recordings from identified auditory neurons in vivo, where we observed that firing was also driven by EPSPs (n=6/6, ages P1-6). Interestingly, the proportion of bursting units increased with age, and after P4 only bursting units were observed. We hypothesize that changes in the pattern of spontaneous activity are modulated by the olivo-cochlear system. To test the hypothesis we are performing similar electrophysiological experiments in α9 nicotinic receptor knockout animals, where olivo-cochlear function is compromised. This mouse model is under current examination for expression of activity-dependent markers in auditory neurons.

ORAL-17-03

CHANGES IN PRE- AND POSTSYNAPTIC PROTEIN COMPONENTS OF AFFERENT NEURONS IN THE COCHLEA DURING SYNAPSE REMODELING

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The afferent innervation in the developing cochlea undergoes dramatic remodeling prior to the onset of hearing. Our recent data have demonstrated that the primary auditory neurones exhibit precisely timed neurite outgrowth, neurite refinement and neurite retraction to establish the mature innervation pattern. However, the mechanisms driving this process of synapse remodeling remain elusive. One theory is that the molecular makeup of the afferent synapse may determine synapse elimination or survival. Our hypothesis is that the fate of the neurites might be associated with synaptic strength, as determined by the ribbons and glutamate receptor expression. We have investigated the expression of synaptic ribbons and glutamate receptor subunits in the cochlear afferent synapses during development using confocal immunofluorescence microscopy. We found that the number of the ribbons in outer hair cells increased from P0 to P3 then decreased at P6, correlating with the transient innervation of type I fibers to outer hair cells between P0 and P3. In addition, the number of ribbon synapses in inner hair cells was also reduced from P6 to P12, and this reduced density was sustained in the adult. The stable expression of the GluR2/3 and GluR4 glutamate receptor subunits was found on the nerve fibers opposed to the ribbon labeling under inner hair cells. Transient glutamate receptor (GluR2/3 and GluR4) expression was detected under outer hair cells between P0 and P3. Correlation of the changes in expression of synaptic ribbons and the post-synaptic glutamate receptors during synapse remodeling with the type I and type II innervation patterns during development (Huang et al., 2007) may provide insight into how afferent synaptic fate is determined and how this defines to the adult innervation pattern in the cochlea.

ORAL-17-04

THE FUNCTIONAL ROLES OF RGMa DOMAINS WITHIN THE EMBRYONIC VERTEBRATE NERVOUS SYSTEM DEVELOPMENT

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Repulsive guidance molecule A (RGMa) is a glycosylphosphatidylinositol-anchored plasma membrane protein that has been implicated in a variety of developmental processes including chemorepulsive axon guidance, cell death and neuronal differentiation. To characterise the role of wild-type RGMa in the early embryonic vertebrate nervous system development we have overexpressed RGMa in a ubiquitous or mosaic manner in *Xenopus* embryos. Our results show that RGMa is responsible for an early embryonic cell death, pioneering axon guidance and ectopic neuronal differentiation *in vivo* (n≥50, ≥3 repeats). To examine the roles of specific functional domain in RGMa two deletion mutants were generated: ΔvWF-RGMa and ΔRGD-RGMa. ΔvWF-RGMa and ΔRGD-RGMa were overexpressed in *Xenopus* embryos (for each experiment, n≥50, ≥3 repeats) and the penetrance of neurodevelopmental phenotypes were analysed. Control embryos were injected with wild-type RGMa. The resultant phenotypes suggest that the partial von Willebrand factor type D mediates cell death during embryogenesis while other domains are responsible for neural tube defects. This approach has begun to reveal for the first time the functional roles of RGMa domains *in vivo*. The receptor mediating neural tube defects remains to be determined using anti-sense morpholinos against Neogenin.

ORAL-17-05

SEMAPHORIN 3A-INDUCED GROWTH CONE COLLAPSE IN ADULT RAT PELVIC GANGLION NEURONS IS MEDIATED BY CYCLIC NUCLEOTIDES AND DIMINISHED BY NEUROTROPHIC FACTORS

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Semaphorins constitute a family of guidance molecules that repel axons from inappropriate targets during development, but relatively little is known about their action in adult neurons. The cyclic nucleotides, cAMP and cGMP, are strongly implicated in setting the neuronal growth cone response to guidance molecules and neurotrophic factors. Inadvertent injury to autonomic pelvic ganglion neurons during pelvic surgical procedures is common and functional deficits may be exacerbated by inefficient or inappropriate regeneration. Adult rat pelvic ganglion neurons were cultured for two days. All experiments were conducted on at least 3 cultures obtained from separate rats. The secreted Semaphorin member, Sema3A (100 ng/ml), was bath applied for the final 30 mins and caused growth cone collapse in both noradrenergic (tyrosine hydroxylase-immunoreactive, TH) and cholinergic (nitric oxide synthase-immunoreactive, NOS) neurons. In NOS-neurons, Sema3A-induced collapse was mediated by cAMP production. Activation of adenylyl cyclase (AC) caused collapse whilst AC inhibition prevented the actions of Sema3A. Conversely, Sema3A-induced collapse was mediated by cGMP production in TH-neurons. Activation of either AC or soluble guanylyl cyclase (sGC) caused filopodial retraction in TH-neurons, but only sGC inhibition prevented Sema3A-collapse. Treatment with neurotrophic factors relevant to each neuron class also affected growth cone dynamics; both nerve growth factor and artemin reduced basal growth cone collapse levels in TH-neurons, and artemin also blocked Sema3A-induced collapse. Similarly, neurturin blocked Sema3A-induced collapse in NOS-neurons. This study provides novel insights into the plasticity of adult pelvic autonomic neurons and their growth responses, which may be amenable to modulation to promote functional regeneration following injury.

ORAL-17-06

CYCLIC NUCLEOTIDE-DEPENDENT SWITCHING OF MAMMALIAN AXON GUIDANCE DEPENDS ON GRADIENT STEEPNESS

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Guidance of axons to their targets during development often requires them to respond to gradients of specific cues. However, both the concentration and gradient steepness of such cues are likely to change as the distance from the target changes. Previous *in vitro* assays investigating guidance in response to steep gradients over short distances have shown that protein kinase A (PKA) levels can determine whether an attractive or repulsive response to a given guidance cue is observed. However, the question remains of whether this mechanism is utilized in response to all gradient conditions. Here, we have directly compared the guidance responses of rat superior cervical ganglion (SCG) axons to nerve growth factor (NGF) presented in steep gradients over short distances, and shallow gradients over long distances, with and without PKA modulation. Using the steep gradients of the growth cone turning assay, attraction of SCG growth cones was converted to repulsion through inhibition of PKA activity ($p = 0.005$, Kolmogorov-Smirnov test). We also found that the chemotactic sensitivity curves for attraction and repulsion to NGF in steep gradient conditions are of similar shape, both peaking when 10 μM NGF is used in the pipette. Surprisingly however, when SCGs were presented with shallow gradients in a 3D gel for two days, PKA inhibition reduced the attractive response but did not switch it to repulsion. Thus, the mechanisms determining axonal guidance responses may differ depending on the steepness of the gradients they encounter.

ORAL-17-07

IMPORTANCE OF TEN-M MOLECULES IN THE DEVELOPMENT OF THE MOUSE VISUAL PATHWAY

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We have previously demonstrated that the transmembrane glycoprotein Ten-m3 plays a critical role in the formation of the binocular visual pathway. Here we have investigated potential roles for other members of the Ten-m family in this process. *In situ* hybridisation showed that Ten-m2 and Ten-m4 are both expressed in the dLGN from P0, and are present in visual cortex. Ten-m1 was not observed in the dLGN. Anterograde tracing of retinal projections using cholera toxin B conjugated to red or green fluorescent dyes showed that ipsilateral retinal projections from retina occupy a smaller area of the dLGN in Ten-m2 knockouts (n=10) than in WTs (n=12). This difference was significant within the rostral half of dLGN ($p<0.05$, Multivariate ANOVA). Conversely, in Ten-m4 KOs (n=12), ipsilateral retinal projections occupied significantly more of the dLGN than WTs, but this difference was limited to caudal dLGN ($p<0.05$, Multivariate ANOVA). To isolate the source of these defects, retrograde tracing was performed using injections of wheat-germ agglutinin (WGA) conjugated to horse-radish peroxidase (HRP) into the dLGN of adult WT (n=5) and KO mice. In Ten-m2 KOs (n=5), there was a significant decrease in the number of ipsilaterally-projecting retinal ganglion cells (RGCs) and their area of origin ($p<0.05$, Student's t-test). Retrogradely-labelled cells were absent specifically from ventral retina. In Ten-m4 KOs (n=6), the number and area of origin of ipsilaterally-projecting RGCs was increased into dorsotemporal retina ($p<0.05$, Student's t-test). Our data suggest that Ten-m2 and Ten-m4 exert opposing influences on the specification and/or guidance of ipsilateral retinal projections at the midline.

ORAL-17-08

THE MOTILITY OF OLFACTORY ENSHEATHING CELLS: A HITCHHIKER'S GUIDE TO OLFACTORY AXON MIGRATION

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During development and during regeneration in the adult, olfactory ensheathing cells (OECs) are intimately associated with axons of olfactory sensory neurons along their entire trajectory. It is assumed that OECs are essential for axon growth but little is known about how OECs and axons interact and respond to each other. We hypothesised that the motility of OECs directs the migration of olfactory axons. To address this we used timelapse imaging of fluorescently labelled primary cultures of olfactory sensory neurons and OECs to determine the mechanisms of olfactory axon extension and OEC interactions. We reveal here for the first time that the extension of pioneer olfactory axons is largely dependent on the motility of the underlying OECs. This intimate association is in part initiated and mediated by lamellipodial waves along the shaft of OEC processes. Moreover, as axons remain adhered to axons at all times, perturbation of OEC movement via GDNF and inhibitors of the JNK and SRC kinases significantly altered axon motility (n=20). We also reveal that inhibition of NCAM significantly disrupted OEC cell-cell recognition (n=10) resulting in increased OEC migration, while OEC-axon adhesion was maintained. Laser ablation of OECs, or substitution of OECs by Schwann cells, inhibited axon outgrowth. These results demonstrate that olfactory sensory axon outgrowth is dependent on cell-cell contact with OECs. Rather than merely providing support for axon growth the glia of the olfactory system strongly regulate the migration of olfactory axons.

ORAL-18-01

THE ROLE OF AN ENDOGENOUS INHIBITOR OF CALCINEURIN IN THE REGULATION OF GLUCOSE HOMEOSTASIS AND ISLET FUNCTION

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Calcineurin (CaN) is a protein phosphatase important in the regulation of transcription and protein phosphorylation. The CaN/NFAT transcription pathway regulates pancreatic β-cell growth and insulin secretion. We are investigating the effect of a gene known to endogenously inhibit CaN and its possible role in the pathogenesis of type 2 diabetes. Expression of our gene of interest increased 2.5 fold ($p<0.05$) when islets were exposed in vitro to 16.7 mM glucose for 6 days. Transgenic mice (Tg) with a universal over-expression of this gene were used for this study. In Tg islets, genes such as those mutated in hereditary forms of monogenic type 2 diabetes (MODY) and others that regulate β-cell survival, proliferation and insulin production are downregulated. In vivo studies show that Tg mice develop diabetes characterized by increased fasting blood glucose levels of 5.8 ± 0.3 mmol/L at 60 days old compared to 4.2 ± 0.2 mmol/L in wild-type mice (n=9, $p<0.05$) and this hyperglycemia worsens with age. Immunohistochemical analysis of pancreatic islets reveals that Tg mice develop a 70% reduction in islet area at 100 days (n=4) while no change is observed at 40 days. Tg mice also show poorer glucose tolerance, these changes are not due to differences in body weight or insulin resistance. Our findings highlight a novel role of this endogenous inhibitor of calcineurin in regulating glucose homeostasis, expression of major β-cell regulatory genes and islet growth. As this gene is also up-regulated by chronic hyperglycemia, our findings suggest that it may be involved in the β-cell failure and hypoinsulinemia characteristic of type 2 diabetes.

ORAL-18-02

GLUCOPRIVATION INCREASES TYROSINE HYDROXYLASE PHOSPHORYLATION AND THE RELEASE OF CATECHOLAMINES FROM RAT ADRENALS

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In these studies we aimed to investigate for the first time the phosphorylation of tyrosine hydroxylase (TH) and the release of catecholamines from the adrenal glands of rats treated with the glucoprivic agent 2-deoxyglucose (2DG). Animals received a single intraperitoneal injection of 2DG (400mg/kg), or saline and then were sacrificed 10 min, 20 min, 60 min or 24 h after the injection. Adrenals were rapidly removed and TH phosphorylation at Ser40, Ser31, Ser19 and TH protein were analysed by western blotting (n=6 for all groups). Plasma adrenaline and noradrenalin levels were measured by HPLC analysis. TH phosphorylation at Ser19 was not significantly changed in response to 2DG at any time. TH phosphorylation at Ser31 was significantly increased by 2DG at 20 min (3.8 fold, $p<0.001$) and reached maximum at 60 min (9 fold, $p<0.001$). TH phosphorylation at Ser40 reached maximum at 20 min (2 fold, <0.001) and declined to 1.5 fold at 60 min ($p<0.05$). TH protein was significantly increased by 2DG only at 24 h (2.3 fold, $p<0.001$). Plasma adrenaline and noradrenalin concentrations were significantly increased by 2DG at 20 min (2.8 fold, $p<0.01$ and 1.7 fold, $p<0.05$ respectively), but not at 24 h. These findings suggest that 1) TH is activated 20 min after administering 2DG to maintain capacity of the adrenal gland to replenish the catecholamines which were released; 2) TH protein synthesis is increased 24 h after administering 2DG in order to increase capacity of the adrenal gland for the catecholamine synthesis in response to later stresses.

ORAL-18-03

HIGH-FAT FEEDING ALTERS THE CARDIOVASCULAR ROLE OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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Increased sympathetic nerve activity is associated with obese-related hypertension, but the underlying central neural mechanisms are not clear. We examined the role of the hypothalamic paraventricular nucleus in the regulation of sympathetic nerve activity in rats fed either a normal chow (controls) or a high fat diet (36% fat) over 12 weeks. The effects on blood pressure, heart rate and lumbar sympathetic nerve activity induced by microinjection of the GABA(A) receptor agonist, muscimol, or the antagonist, bicuculline, were monitored in anesthetized rats. Compared with chow-fed rats, rats fed a high fat diet were not significantly different in body weight but had a significant 80% increase in epididymal fat mass, significantly elevated fasting blood glucose and a significantly impaired glucose tolerance. Resting blood pressure and heart rate were not significantly different in rats fed a high fat or normal chow diet. Muscimol microinjected into the paraventricular nucleus of rats fed a high fat diet elicited a greater reduction of blood pressure and lumbar sympathetic nerve activity compared to controls (blood pressure, -14 ± 6 vs. -7 ± 2 mmHg; lumbar sympathetic nerve activity, -35 ± 6% vs. -10 ± 9%; high fat fed vs control, respectively). Microinjection of bicuculline into the paraventricular nucleus increased blood pressure and lumbar sympathetic nerve activity but the responses were similar in high fat fed and control rats. In conclusion, high fat feeding may alter the influence of the paraventricular nucleus on cardiovascular variables prior to the development of hypertension and obesity.

ORAL-18-04

NEUROMETABOLOMIC ANALYSIS OF THE GABAERGIC SYSTEM IN GUINEA PIG CORTICAL SLICES

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γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, and acts through interaction with three types of specific GABA receptors (A, B and C). We have investigated the multiparametric response of guinea pig cortical slices (n = 230) to different concentrations of GABA, and pharmacological modulators of GABA receptors in the presence of ^{13}C pyruvate as an energy source. Global metabolic profiles were measured using ^{1}H and ^{13}CNMR spectroscopy, and data sets were subject to principal components analysis (PCA) and projections to latent structures (PLS). Multivariate statistical analysis revealed distinct clusters of metabolic responses that did not correlate with GABA receptor subtypes, but with affinity for GABA. This model offers unique insights into the modes of action of GABAergic ligands.

ORAL-18-05

ADDITIVE ACTIONS OF THE CANNABINOID AND NEUROPEPTIDE Y SYSTEMS ON ADIPOSITY AND LIPID OXIDATION

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Lei Zhang[1], Nicola J Lee[1], Ronaldo F Enriquez[2], Bernhard Stehrer[1], Ernie Yulyaningsih[1], Shu Lin[1], Yan-Chuan Shi[1], Herbert Herzog[1], Amanda Sainsbury[1], [1]Neuroscience Program, [2] Bone and Mineral Program Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, Sydney, NSW 2010, AUSTRALIA The Cannabinoid and NPY systems are critical regulators of energy homeostasis. Thus, we studied the effects of the CB1 antagonist Rimonabant on energy balance, body composition and bone physiology in wild type (WT) and NPY knockout ($NPY^{-/-}$) mice. Rimonabant was administered orally and voluntarily at 10mg/kg body weight twice per day for 3 weeks. Data were collected from 7-10 mice per group. We show that mice with dual blockade of CB1 and NPY signalling (Rimonabant-treated $NPY^{-/-}$ mice) exhibited greater reductions in body weight and adiposity than mice with single blockade of either system alone (Rimonabant-treated WT or vehicle-treated $NPY^{-/-}$ mice). Importantly, these changes occurred without loss of lean tissue mass or bone mass. The mechanism for the synergistic reduction in adiposity by dual blockade of CB1 and NPY involves an additive increase in lipid oxidation, without any effects on food intake, energy expenditure or physical activity. In addition, our results reveal a coordinated involvement of the CB1 and NPY systems in the regulation of different aspects of energy homeostasis. Indeed, we show that CB1 and NPY signalling may regulate feeding and substrate oxidation via independent mechanisms, whereas effects of CB1 on the hypothalamo-pituitary-adrenal axis may require the presence of NPY signalling. Taken together, these findings open new avenues for more effective treatment of obesity via dual pharmacological manipulations of the CB1 and NPY systems.

ORAL-18-06

PANCREATIC POLYPEPTIDE REDUCES FOOD INTAKE VIA ARCUATE Y4 RECEPTORS AND THE MELANOCORTIN SYSTEM IN MICE

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Pancreatic polypeptide (PP) is a gut hormone released from the pancreas in response to ingestion of food and known to inhibit food intake in the absence of conditioned taste aversion or nausea. However, the central mechanism behind this process is unknown. In this study, we demonstrate that in response to i.p. injection of PP in wildtype but not in Y4 receptor knockout mice, immunostaining for the neuronal activation marker c-Fos is induced specifically in neurons of the nucleus tractus solitarus and the area postrema in the brainstem, notably in cells also showing immunostaining for tyrosine hydroxylase. Importantly, strong c-Fos activation is also detected in the arcuate nucleus of the hypothalamus (ARC), particularly in neurons that co-express alpha melanocyte stimulating hormone (α-MSH), the anorexigenic product of the proopiomelanocortin (POMC) gene. Interestingly, other hypothalamic regions such as the paraventricular nucleus, the ventromedial nucleus and the lateral hypothalamic area also show c-Fos induction after PP injection. In addition to c-Fos activation, PP injection up-regulates POMC mRNA expression in the ARC as detected by *in situ* hybridization. These effects are a direct consequence of local Y4 signaling, since hypothalamus-specific conditional Y4 receptor knockout abolishes PP-induced ARC c-Fos activation and blocks the PP-induced increase in POMC mRNA expression. Additionally, the hypophagic effect of i.p. PP seen in wild type mice is completely absent in melanocortin 4 receptor knockout mice. In summary, these findings show that PP reduces food intake predominantly via stimulation of the anorexigenic α-MSH signaling pathway, and that this effect is mediated by direct action on local Y4 receptors within the ARC, highlighting a potential novel avenue for the treatment of obesity.

ORAL-18-07

AGE-RELATED CHANGES IN NAD+ METABOLISM IN THE BRAIN OF AGED FEMALE WISTAR RATS

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Normal aging is characterised by elevated levels of oxidative stress and a global decrease in cell density in the brain and other tissues. DNA damage and reduced energy (ATP) production are hallmarks of this degenerative process. Nicotinamide adenine dinucleotide (NAD+) has recently attained prominence as a link between energy production, resistance to stress and longevity. NAD+ not only serves as a redox carrier but also as a substrate for the NAD-dependent poly(ADP-ribose) polymerase (PARP), an important DNA nick sensor, and the Sirtuins, a family of transcription regulators that play crucial roles in cellular resistance to stress and increased lifespan. We examined age associated effects on intracellular NAD+ metabolism in selected brain regions in female wistar rats. Our results are the first to show a significant decline in intracellular NAD+ levels and NAD:NADH ratio after 12 and 24 months of age compared to young 3 month old rats, in parallel with an increase in lipid peroxidation, and increased formation of protein carbonyls and decline in total antioxidant capacity in the cortex, brainstem, hippocampus and cerebellum. We also found elevated levels of phosphorylated H2AX levels, a measure of DNA damage, in these same brain regions. Reduced mitochondrial complex I activity was also observed in aged rats, suggesting an accumulation of NADH and enhanced potential for production of free radicals by Fenton chemistry. A strong positive correlation was found between oxidative stress, PARP activity, ADP-ribose polymers and NAD+ depletion suggesting a role for NAD+ as a longevity assurance factor. While decreased Sirt1 activity was observed in response to NAD+ depletion, our observed moderate over-expression of Sirt1 may be a response to retard aging and confer resistance to oxidative stress in the brain.

ORAL-18-08

IFN-γ MODULATES THE PROLIFERATION AND DIFFERENTIATION POTENTIAL OF HUMAN AND MOUSE STEM CELLS INCLUDING NEURAL STEM CELLS VIA ACTIVATION OF INDOLEAMINE 2,3 DIOXYGENASE (IDO)

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The kynurenine pathway of tryptophan metabolism has been classically linked to antimicrobial activity, control of cell proliferation and modulation of innate and adaptive immune systems. However, the role of IDO in stem cell biology and the exact nature of the underlying mechanisms controlling stem cell proliferation and differentiation are completely unknown. We showed that human and mouse mesenchymal and neural stem cells (MSCs and NSCs) express the complete, functional KP enzymatic machinery, including IDO1 and its recently identified paralogue IDO2, and that the expression of the KP enzymes is highly regulated by both type I and II interferons i.e. IFN-β and IFN-γ respectively. We also report a differential transcriptional modulation of KP components in stem cells between the type of interferon, cell types and species. Our findings provide evidence that IFN-γ has a significant anti-proliferative effect in long-term MSC and NSC cultures. Finally, specific IFN-γ- and IFN-β-induced IDO activation in mouse and human MSCs and NSCs caused significant changes at the transcriptional and protein level of neural markers after differentiation induction procedures. Our findings support a direct molecular link between tryptophan metabolism and adult neurogenesis, thus offering entirely novel therapeutic opportunities.