ORAL SESSIONS

ORAL-01-01

DEPHOSPHORYLATION OF CAMKII AT T253 CONTROLS PROGRESSION THROUGH METAPHASE

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PURPOSE: CaMKII (calcium/calmodulin-stimulated protein kinase II) is a protein kinase that regulates a range of biological functions, and we have recently shown that it is involved in controlling the cell cycle. The biological properties of CaMKII are regulated by multi-site phosphorylation and targeting via interactions with specific proteins. **METHODS:** SHSY5Y (neuroblastoma) and MDA-MB-231 (breast cancer) cell lines were stably and inducibly transfected with wild type CaMKII (WT), T286D-CaMKII (mimicking phosphorylation at T286), or T253D-CaMKII (mimicking phosphorylation at T253), and morphology and growth rate of these cells was examined (n=3). Changes in endogenous CaMKII phosphorylation at various stages of the cell cycle was examined by immunoblotting (n=4). **RESULTS:** Transfection with WT or T286D-CaMKII approximately doubled the growth rate without any alteration in cell morphology. By contrast, transfection with T253D-CaMKII dramatically reduced cell growth and altered cell morphology. To identify the mechanism behind this T253D-CaMKII mediated block in proliferation, we examined changes in endogenous CaMKII phosphorylation at various stages of the cell cycle. We found that while there is no change in total CaMKII expression or T286 phosphorylation, there is a marked decrease in T253 phosphorylation during mitosis. We have also shown that pharmacological inhibition or molecular knockdown of PP2A in non-transfected cells inhibits dephosphorylation of CaMKII at T253, but not at T286 (n=3). Furthermore, if this dephosphorylation is prevented by overexpression of phosphomimic T253D-CaMKII, cells become arrested in metaphase (n=3). **CONCLUSION:** These results strongly suggest that dephosphorylation of CaMKII at T253 is involved in controlling the progression of the cell cycle through the metaphase-anaphase transition.

ORAL-01-02

REDUCED PROLIFERATION IN THE ADULT MOUSE SUBVENTRICULAR ZONE INCREASES SURVIVAL OF OLFACTORY BULB INTERNEURONS

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Purpose: Neurogenesis in the adult brain is largely restricted to the subependymal zone (SVZ) of the lateral ventricle, olfactory bulb (OB) and subgranular zone of the dentate gyrus. We examined, in mice, whether survival of adult-born cells is regulated by the rate of precursor proliferation in the SVZ. **Methods:** Precursor proliferation was decreased by depleting the SVZ of dopamine, after lesioning the substantia nigra with 6-hydroxydopamine. Subsequently, we examined the effect of reduced SVZ proliferation on the generation, migration and survival of neuroblasts and mature adult-born cells in the SVZ, rostral migratory stream (RMS) and OB. Proliferation was measured by injecting 5-bromo-2-deoxyuridine (BrdU) 2 hours prior to death, and by immunoreactivity against Ki67. **Results:** BrdU positive (+) and Ki67-immunoreactive (ir) proliferating cells in the SVZ were reduced by 47% and 36%, respectively, 7 days after DA depletion, and were lower than control after 42 days (29% and 31%, respectively). 7 and 42 days after the 6-OHDA injection, doublecortin-ir neuroblasts in the SVZ and RMS were unchanged from control, as were the number of neuronal nuclei-ir cell bodies in the OB. However, 15 days after administering 6-OHDA, BrdU+ cells in the SVZ increased 70%, with most being neuroblasts that co-labeled doublecortin-like immunoreactivity. Also, 42 days after injecting 6-OHDA, we found an 82% increase in co-labeled BrdU+/γ-aminobutyric acid-ir cell bodies in the granular cell layer, while double-labeled BrdU+/tyrosine hydroxylase-ir cell bodies in the glomerular layer increased 148%. **Conclusion:** We conclude that the number of interneurons in the OB following a reduction of proliferation in the SVZ is maintained through an increased survival of adult-born precursor cells, neuroblasts and interneurons in the SVZ, RMS and OB.

ORAL-01-03

ADRENERGIC RECEPTORS REGULATE A DISTINCT PRECURSOR POPULATION IN THE ADULT HIPPOCAMPUS

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Purpose: New neurons that are continuously generated from the resident populations of stem and precursor cells in the adult hippocampus play a pivotal role in the functional circuitry regulating memory, mood and cognition. Previously, we have shown that norepinephrine can directly activate a stem and precursor pool in the adult hippocampus. In this study, we sought to determine the contribution of various adrenergic receptors (ARs) in regulating this precursor pool. **Methods:** We examined the effects of pharmacological agents that selectively target various adrenergic receptors on hippocampal precursor activity using the neurosphere assay. **Results:** Stimulation of β-ARs with a selective agonist isoproterenol significantly increased the total number of neurospheres and the proportion of very large neurospheres indicating activation of a stem and precursor cell pool ($n>4$, $p<0.05$). While we found no significant change in the total neurosphere numbers in the presence of either the selective β1/β2-AR agonist dobutamine or the selective β2-AR agonist salbutamol ($n=3$, $p>0.05$), the effect of norepinephrine was mimicked by three independent and selective β3 AR agonists, BRL37344 (1 μM: 157.8±12.9%, $p<0.05$), L755,507 (100 nM: 174.9±10.1%, 1 μM: 180.2±0.4%, $p<0.001$) and ZD7114 (100 nM: 182.1±3.0%, 1 μM: 167.9±3.7%, $p<0.001$). Furthermore, systemic administration of BRL37344 *in vivo* was sufficient to activate this latent precursor pool. We found that α1-ARs had no effect ($n>5$, $p>0.05$), however, α2-ARs agonist inhibited the proliferation of hippocampal precursors ($n>5$; $p<0.05$). **Conclusion:** Together, these findings suggest that while β3-ARs play a key role in activating the latent precursor pool, stimulation of α2-ARs inhibit precursor proliferation, thereby maintaining the hippocampal precursor pool homeostasis.

ORAL-01-04

EPHA4 EXERTS AN INHIBITORY EFFECT ON NEURAL PRECURSOR ACTIVITY IN THE ADULT HIPPOCAMPUS

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Purpose: EphA4 has been demonstrated previously to regulate neurogenesis in the sub-ventricular zone and cortex, and is highly expressed in the adult mouse hippocampus. We therefore aimed to determine whether EphA4 had an effect on neural precursor activity in the hippocampus, as well as to investigate the mechanism through which it may act. **Methods:** We have used the hippocampal neurosphere assay to test neural precursor activity *in vitro*. In addition, we have used neuronal cultures alongside western blotting to determine EphA4 expression in mature neurons. **Results:** While we observed that EphA4 was not expressed by neural precursors, hippocampal cells from EphA4$^{-/-}$ and EphA4 kinase-dead transgenic mouse models gave rise to an increase of 198 ± 79% and 154 ± 58%, respectively, in precursor activity compared to wild-type controls. Blocking EphA4 with EphA4-Fc also led to an increase precursor activity of 89.5 ± 19.4%. Cellular depolarization with KCl has previously been demonstrated to lead to a 2-fold increase in neural precursor activity *in vitro*, however EphA4$^{-/-}$ and EphA4 kinase-dead hippocampal cells did not respond to depolarization with an additional increase in precursor activity. To determine whether this was due to EphA4 availability, we observed protein levels following depolarization and found that there was 20% less EphA4 present with treatment. Additionally, effects of KCl on adult hippocampal precursors were eliminated in the absence of EphA4-positive cells. **Conclusions:** These results indicate that EphA4 inhibits neural precursor activity in the adult mouse hippocampus and may mediate the regulation of synaptic-activated neurogenesis.

ORAL-01-05

GENESIS AND DEVELOPMENT OF DOPAMINERGIC INTERNEURONS IN THE ADULT BRAIN

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Background. The demonstration of adult brain neurogenesis, strengthens the rationale for regenerative therapies for neurodegenerative disorders. The subventricular zone (SVZ) is used extensively in the study of neural stem cells, neurogenesis, migration and differentiation. The SVZ neuroblasts, recapitulate a population of dopaminergic neurons which synapse with olfactory receptor neurons within the olfactory bulb (OB), possibly the only population of dopaminergic interneurons, generated throughout adult life. **Aims.** To investigate a pathophysiological model to determine modulation of cell proliferation in the SVZ. To examine the directed differentiation of SVZ neuroblasts into the dopaminergic phenotype. **Methods.** We focally ablate the olfactory epithelium (OE) over an extended time period (14 days) in adult mice (n=4). The thymidine analogue EdU is used to tag newly generated cells, to quantify them and together with a panel of key marker proteins, define their differentiation. **Results.** The extended lesioning of the olfactory receptor neurons caused a decrease in TH immunoreative neurons in the olfactory bulb. It caused an increase in cell proliferation in a discrete region of the SVZ. We also demonstrate an upregulation of key marker genes. We demonstrate an increase in recruitment of the dopamine cell phenotype into the periglomerular cell population in the OB following focal lesioning of the olfactory sensory neurons. **Conclusions.** These data suggest the olfactory sensory innervations modulates the dopaminergic periglomerular interneurons in the olfactory bulb and neurogenesis in the SVZ. The demonstration of the modulation of dopaminergic neurogenesis in the adult brain is of significance for Parkinson's disease therapy.

ORAL-01-06

THE ROLE OF NEOGENIN IN ADULT NEURONAL DIFFERENTIATION

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Purpose: It is now well established that the slowly dividing neural stem cells in the subventricular zone of the adult brain generate dividing neuronal precursors, which subsequently give rise to neuroblasts. Newly born neuroblasts migrate along the rostral migratory stream towards the olfactory bulb (OB) and integrate into the granule cell and glomerular cells layers. This process is ongoing throughout adult life. Neogenin is expressed by neural stem and progenitor cells in the adult mouse brain. *Neogenin* loss-of-function mice have smaller OBs and preliminary data show a severe loss of OB calretinin-positive granule cells. The purpose of this study is to test the hypothesis that neogenin plays an active role of in neural specification and differentiation during adult neurogenesis. **Methods:** *Neogenin* loss-of-function (*neo* $^{gt/gt}$) and wild type brains were processed for immunohistochemical staining. A stereological analysis was carried out on the OBs of *neo* $^{gt/gt}$ and *neo* $^{+/+}$ mice to investigate the loss of interneuron subtypes (n=3 per phenotype). **Results:** Gross morphological analysis revealed that 66% of the OBs (n=10) of *neo* $^{gt/gt}$ mice exhibited bilaterally smaller olfactory bulbs. An *in vivo* investigation revealed that the number of calretinin-positive cells in the OB were significantly lower ($p<0.001$, Student t test) in the *neo* $^{gt/gt}$ when compared to the wild type mice. There was no change in the calbindin- or tyrosine hydroylase-positive interneuron populations. **Conclusion:** Loss of neogenin affects the number of calretinin-positive interneurons in the adult OB. Therefore neogenin may control the differentiation of these interneurons during adult neurogenesis. Further analysis will address if neogenin is required for the specification of OB interneurons in the SVZ by investigating the expression of specific transcription factors in *neo* $^{gt/gt}$ mice. In addition, the role of neogenin in the differentiation and maturation of granule cells will also be examined.

ORAL-01-07

EFFECTS OF SEX STEROID HORMONES ON THE EXPRESSION OF BDNF AND TRKB IN THE HIPPOCAMPUS DURING ADOLESCENCE IN C57BL/6 MICE

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Purpose: Sex steroid hormones and neurotrophic factors prune and shape the adolescent brain to its mature form. Brain derived neurotrophic factor (BDNF) is implicated in schizophrenia and depression, disorders that tend to emerge during adolescence. We therefore investigated interactions between sex steroid hormones, BDNF and its receptor, TrkB, throughout adolescence. **Methods:** A week by week analysis was conducted from pre-pubescence to adulthood in dorsal (DHP) and ventral (VHP) hippocampus of female and male C57Bl/6 mice. Serum levels of estradiol and testosterone were compared with Western Blot analysis of BDNF and TrkB expression (n=5-6/week). Gonadectomy and sex hormone replacement were done at 5 weeks of age followed by analysis of BDNF and TrkB at 8-9 weeks (n=8 9/treatment). **Results:** Females showed significant age-related changes in BDNF and TrkB phosphorylation with levels peaking at week 6. These changes did not correspond with serum estradiol levels and ovariectomy and estradiol replacement had no effect on BDNF-TrkB signaling. Male mice showed no significant changes in BDNF-TrkB signaling during adolescence despite significant changes in serum testosterone, and no effect of castration was found. However, treatment of castrated males with testosterone or DHT differentially decreased TrkB phosphorylation and expression in the DHP and VHP. This suggests that androgens can regulate TrkB in the male hippocampus but are not essential for maintaining BDNF-TrkB signaling during adolescence. **Conclusion:** These results show differential actions of sex steroid hormones in modulating BDNF-TrkB signaling during adolescence. Our approach may help to identify critical developmental windows for intervention in neurodevelopmental psychiatric disorders.

ORAL-01-08

REGULATION OF RECEPTOR TYROSINE KINASES BY UBIQUITINATION: THE ROLE OF NDFIP1 IN REGULATING TRK RECEPTORS

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Purpose: Receptor tyrosine kinases play a vital role in translating extrinsic signals from neurotrophins into a cell. The Trk family of receptor kinases are required to specify the action of neurotrophic signals, resulting in neuronal survival, differentiation and activity. Once activated Trk receptors are internalized and targeted for degradation. TrkA has been found to be poly-ubiquitinated for degradation by Nedd4-2 ubiquintin ligases, however TrkB and TrkC lack the recognition motif for Nedd4-2 interactions. Here we report that Ndfip1 acts as an adaptor protein that interacts with Trk receptors resulting in the recruitment of Nedd4 ubiquitin ligases and subsequent ubiquitination of the receptors. **Methods:** The interaction between Ndfip1, Nedd4-2 and TrkA, B and C were investigated using mouse primary cortical neurons. TrkA and B interactions with Ndfip1 were investigated further using mutants to study the interaction between the two proteins. Viral infection of BDNF dependent primary neurons with and inducible Ndfip1 construct was investigated in survival assays. **Results:** Ndfip1 was found to interact with all three Trk receptors, and after BDNF treatment were found to be involved in poly-ubiquitination. Mutations in Trk receptors prevented the interaction with Ndfip1, suggesting a direct and specific binding between the proteins. BDNF dependent primary neurons infected with an inducible Ndfip1 construct were found to be susceptible to cell death upon induction of Ndfip1 expression (n=7). **Conclusion:** Trk receptors have been identified to interact with the adaptor protein Ndfip1. This interaction results in the poly-ubiquitin of the receptor through recruitment of the Nedd4-2 ubiquitin ligases. These findings indicate that Ndfip1 is an important determinant in regulating the levels of Trk receptors with important implications for neurotrophin signalling.

ORAL-02-01

CHOLERA TOXIN SIGNIFICANTLY REDUCES COLONIC MIGRATING MOTOR COMPLEXES AND CONSTRICTS THE MOUSE COLON VIA SEPARATE 5-HT$_3$ RECEPTOR DEPENDENT PATHWAYS

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Purpose: While it is well established that cholera toxin (CT) produces a massive hypersecretion across the intestinal mucosa via activation of the enteric nervous system, reports of its effects on intestinal motility have been inconsistent. We investigated the effect of CT on motility in the isolated colon of C57 BL/6 mice. Method: Video imaging was used to construct high resolution spatiotemporal maps of colonic motor patterns in vitro. The full length of the colon was cannulated at each end and mounted horizontally in an organ bath containing physiological saline warmed to 37^{o}C. The proximal end was connected to a reservoir of physiological saline, the distal end to an outflow tube. CT (0.125µg/ml, 1.25 µg/ml, 12.5 µg/ml) was introduced to the lumen after taking control recordings with physiological saline and then CT washed out with physiological saline. Results: CT at all three concentrations significantly and reversibly reduced the number of colonic migrating motor complexes (CMMCs) and the resting colonic diameter ($p < 0.001$, 2 way ANOVA, $n = 5$ in each case). These effects of CT were blocked by granisetron (1 µM, 5-HT$_3$ receptor antagonist) with the number of CMMCs in the presence of both CT and granisetron being indistinguishable from control ($p > 0.05$ in each case). CMMCs were abolished by tetrodotoxin, which constricted the colon, but did not prevent the constriction produced by CT. Conclusions: The results indicate involvement of 5-HT and 5-HT$_3$ receptors in the motility reflex pathways activated by CT, with that regulating CMMCs being neutrally mediated and that regulating resting colonic diameter being independent of neural activity.

ORAL-02-02

ENDOGENOUS SEROTONIN IS NOT REQUIRED FOR THE GENERATION OR PROPAGATION OF PERISTALSIS IN GUINEA-PIG DISTAL COLON

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Purpose: Considerable evidence has been presented to suggest that serotonin is an essential neurotransmitter required for the generation and propagation of colonic peristalsis. This is supported by the fact that antagonists of 5-HT3 and 5-HT4 receptors can abolish colonic peristalsis. It is presumed these antagonists act by suppressing the action of serotonin on 5-HT3 and 5-HT4 receptors. However, this presumption is unproven. **Aim**: To determine whether (1) depletion of endogenous serotonin prevents peristalsis and if not, (2) do antagonists of 5-HT3 and 5-HT4 receptors still inhibit peristalsis in serotonin-depleted preparations. **Methods**: Guinea pigs were pretreated with reserpine for 24 hours in vivo to deplete endogenous serotonin within the enteric nervous system. Depletion was confirmed by mass spectrometry and immunohistochemistry. In reserpinized animals, the distal colon was extracted and the mucosa and submucosal plexus sharp dissected free. Peristalsis was elicited by physiologically relevant distension induced by artificial fecal pellets. **Results**: Mass spectrometry failed to detect serotonin (above nM range) in colons obtained from reserpine-treated animals with their mucosa and submucosa removed. Interestingly, in these preparations robust peristalsis still occurred, with no change in peak propulsive force (11.547+/-0.779gms vs 8.754+/-0.957gms, NS) nor frequency. Ondansetron and SDZ-2024(1µM) significantly slowed the endogenous frequency of peristaltic waves in reserpine-treated preparations (565+/-63secs vs 232+/-54secs, $P<0.05$). **Conclusion**: Endogenous serotonin is not required for the generation and propagation of faecal content in guinea pigs. Inhibition of peristalsis by 5-HT3 and 5-HT4 antagonists is due to mechanisms that do not involve suppression of endogenous serotonin acting on either receptor.

ORAL-02-03

CHARACTERISATION OF COLONIC AFFERENT CENTRAL TERMINALS

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Purpose: Central terminals of colonic afferent neurons and their spinal cord post-synaptic targets are uncharacterised. We aimed to identify colonic afferent central terminals (CACTs), the dorsal horn (DH) neurons they activate in response to noxious colonic distension (CRD), and determine if chronic peripheral hypersensitivity alters these pathways. **Methods:** Fluorescent retrograde tracer was injected into the colon of healthy and TNBS (130µL/ml) post-inflammatory mechanically hypersensitive mice. CRD (80mmHg) was performed seven days later, followed by fixation, thoracolumbar (T10-L1) spinal cord removal and immunohistochemistry. Calcitonin gene related peptide (CGRP), substance P (SP) and isolectin B4 (IB4) identified superficial DH, phosphorylated MAP kinase ERK 1/2 (pERK) identified DH neurons activated by CRD, with calbindin (calb) and GABA immunohistochemistry identifying projection and inhibitory neurons respectively. The density of fluorescent CACTs and the number of pERK-immunoreactive (IR) DH neurons (averaged across 10 sections/spinal segment/mouse/experimental group) were statistically compared between groups (n=4/experimental group) **Results:** CACTs were CGRP-IR and SP-IR, primarily located in DH lamina I (LI), V (LV) and projected in mid and lateral DH collateral pathways. Following CRD, pERK-IR DH neurons were in LI and LV and largely calbindin-IR. Post-inflammation, CACT density was significantly increased ($P<0.0001$) in T12-L1, remaining abundant in LI, yet widespread in deeper laminae. Correspondingly, significantly more DH neurons were pERK-IR following CRD ($P<0.0001$) in T12-L1. Post-inflammation, more pERK-IR neurons were calb-negative ($P<0.05$) and now GABA-IR. **Conclusion:** We identified CACTs and specific DH neurons responsive to noxious CRD. We showed changes in post-inflamed mice, with CACT density and the number of DH neurons activated by CRD increasing. These changes may facilitate aberrant central representation of colonic nociceptive signaling following chronic peripheral ending hypersensitivity.

ORAL-02-04

IN VITRO CHARACTERIZATION OF THE HUMAN COLON: A PIONEERING EFFORT

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Purpose: Colonic motility disorders cause significant societal and financial burdens, and little is know of the abnormalities that underpin it. We have recently published data in which we successfully recorded motor patterns from entire segments of human colon excised for colonic disease or dysfunction. Here we present a proof-of-principle study in which contractile events in excised human colon are recorded with fibre-optic manometry catheter. **Methods**: Entire segments of human colon (descending colon to rectum) were removed from patients with known colonic malignancy and placed in temperature controlled organ baths filled with Krebs. The fibre-optic catheter, was attached to a metal rod positioned above the tissue bath. Lanyards were placed over sensors on the catheter at 2cm intervals. The lanyards were then clipped to the serosa and adjusted to provide suitable tension. Motility was captured for 1 hour before the specimens were sent to pathology. **Results**: A total of 8 (n:8) specimens were studied. In 7 propagating activity was detected and in 6 the activity was present within the first 15 minutes. In 6 of 7 the frequency of propagating events remained constant (0.44 ¬¬± 0.11/min) throughout the recording period. In 1 specimen the frequency of propagating events increase with time from 0.33/min to 0.73/min. In 6 specimens a demonstrable increase in baseline tone occur over 1 hr recording period. **Conclusion**: These preliminary data demonstrate the feasibility of using the fibreoptic cathether to record in vitro human colonic motility patterns. These data have the potential to provide valuable insight into the intrinsic mechanisms controlling colonic motility.

ORAL-02-05

NEURAL AND MYOGENIC MOTOR PATTERNS IN THE ISOLATED RABBIT COLON

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Colonic motility is essential for absorption, formation and excretion of feces. The rabbit colon consists of anatomically distinct regions and their motility is controlled by myogenic and neurogenic mechanisms. Associating these mechanisms with specific motor patterns throughout all regions of the colon has not previously been achieved. Purpose: To investigate the motor patterns in isolated segments of rabbit colon and to establish if these involve myogencic or neurogenic mechanisms. Methods: We investigated spatio-temporal features of motor activity in three isolated segments of the colon: the proximal, mid and distal colon taken from 6 albino rabbits (killed by iv lethabarbital), cannulated and placed in a bath of oxygenated Krebs solution at 37 C. Spatio-temporal maps of changes in diameter were constructed from video recordings (1). Results: The myogenic activity consisted of circular muscle (CM) contractions (ripples) that occurred throughout the colon and propagated in both antegrade (anal) and retrograde (oral) direction. Their frequency was similar in the proximal and distal colon (9.46 ± 0.62 and 11.40 ± 0.79 cpm respectively; n=5) whereas their speed of propagation was significantly higher in the distal colon (6.54± 1.00 mm/s and orally at 8.62± 1.58 mm/s) than in the proximal colon (2.2± 0.2 mm/s aborally and 1.7 ± 0.3 mm/s orally). The neural activity of the proximal colon consisted of slowly (0.1mm.s) propagating colonic migrating motor complexes, which were abolished by hexamethonium (100μM; n=5). In the distal colon, tetrodotoxin-sensitive, thus neurally mediated, but hexamethonium-resistant, peristaltic (anal) and antiperistaltic (oral) contractions were identified (n=5). Conclusions: The distinct patterns of neurogenic and myogenic motor activity recorded from isolated rabbit colon are specific to each anatomically distinct region. The regional specificity motor pattern is likely to facilitate orderly transit of colonic content from semi-liquid to solid composition of feces. References: (1). Hennig et al (1999), J. Physiol., 517, 575-590.

ORAL-02-06

HOME-BASED TRANSCUTANEOUS ELECTRICAL STIMULATION (TES) IMPROVES PAEDIATRIC TREATMENT-RESISTANT SLOW-TRANSIT CONSTIPATION

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Purpose: Physiotherapists regularly use transcutaneous electrical stimulation (TES) for Neuromodulation. Slow-transit constipation (STC) is a severe form of chronic constipation. Previously we showed that transabdominal TES increased defecation and colonic motility in STC children when physiotherapists provided stimulation in clinics and training for home-use. This study tested the effectiveness of home-stimulation overseen by a naïve clinician. Method: A clinician (surgeon) was trained to teach TES to STC children who then self-administered at home using a battery-powered interferential current machine. Bowel diaries, PedsQL4.0 questionnaires and radio-nuclear colonic transit studies were completed before, during and after treatment. Four sticky electrodes were placed, on the trunk with currents crossing. Results: Thirty-eight children (17 female, mean-age 8.4yr, range 3-17yr) self-administered TES for1 hr/day for 3-6 months. Six patients were needed as learning cases for the clinician to acquire skills (data on 32 patients). Three/32 did not return diaries. Group1 (n=13) started with <3 bowel actions (BA)/wk and Group 2 (n=16) with >3BA/wk. Defecation frequency increased in 69% of Group 1 (mean, 1.4 to 3.0/wk, p=0.02). Soiling frequency decreased in 50% of Group 2 (5.4 to 1.9/wk, p=0.04). Seven patients developed urge-initiated defecation (from 13 lacking at start). Abdominal pain decreased in 48% (1.6 episodes/wk to 0.9/wk, p=0.06). Stool consistency improved in 52%. There was significant improvement in quality of life (QOL) scores. Colonic transit improved in 13/25 patients. Conclusion: Home-based TES sped up colonic transit, increased defecation and reduced soiling in >50% of treatment-resistant patients. Six patients were needed to develop clinician expertise. This non-invasive treatment could be tried before surgery.

ORAL-02-07

IMMUNOHISTOCHEMICAL LOCALISATION OF α-SYNUCLEIN IN NEURONS INNERVATING THE GUINEA-PIG RECTUM

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Purpose: α-Synuclein is a major constituent of Lewy bodies and neurites; the pathological hallmarks of Parkinson's disease. At presynaptic terminals, α-synuclein contributes to SNARE-complex re-assembly during repetitive neurotransmitter release. Constipation is a frequent occurrence in Parkinson's disease that may precede motor impairments. Lewy pathology is often identified in colonic biopsies of constipated patients recently diagnosed with Parkinson's disease. We studied which enteric and extrinsic neurons innervating the guinea-pig rectum expressed α-synuclein in their axons. Methods: Co-existence of markers was quantified in preparations immunohistochemically triple-labelled for α-synuclein, VAChT (vesicular acetylcholine transporter) and either SOM (somatostatin), SP (substance P) or VIP (vasoactive intestinal polypeptide) (n=4). In separate preparations, biotinamide was incubated with rectal nerve trunks to reveal extrinsic axons. Preparations were immunohistochemically double-labelled for VAChT and α-synuclein (n=8). Results: α-Synuclein was present in 23.5 ± 0.65% of SOM-IR varicosities; 20 ± 4.32% of SP-IR varicosities and 9 ± 1.3% VIP-IR varicosities (mean ± SEM). However, α-synuclein was localised in significantly more cholinergic varicosities (87.5 ± 3.01% of VAChT-IR, $p < 0.001$). Of SOM-IR, SP-IR and VIP-IR varicosities that lacked VAChT-immunoreactivity, only 0.5 ± 0.29%, 0.25 ± 0.25% and 0% contained α-synuclein-immunoreactivity, respectively. α-Synuclein- and VAChT-immunoreactivity co-existed in 15 ± 1.43% of biotinamide-labelled varicosities on axons of extrinsic origin; only 0.63 ±.26% of biotinamide-labelled extrinsic varicosities contained α-synuclein-immunoreactivity without VAChT-immunoreactivity. Conclusion: In the gut, α-synuclein is preferentially localised in cholinergic terminals and is largely absent from non-cholinergic axons of enteric origin. Similar findings were made for extrinsic innervation of the distal gut via rectal nerves. We speculate that mishandling of α-synuclein in cholinergic neurons may contribute to defective neurotransmission and thus constipation in Parkinson's disease.

ORAL-02-08

THE ROLE OF VASOACTIVE INTESTINAL PEPTIDE IN NEUROGENIC SECRETION IN GUINEA-PIG JEJUNUM

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Purpose: Vasoactive intestinal peptide (VIP) is a potent secretagogue and putative neurotransmitter in the enteric nervous system. However, the actions of VIP and functions served by VIP-receptors ($VPAC_1$ and $VPAC_2$) are not completely understood. We investigated whether VIP stimulates secretion exclusively via a direct action on the mucosal epithelium or also via activation of submucosal neurons, and examined the relative roles of the VIP-receptors in mediating secretion. **Methods**: Guinea-pig mucosa-submucosa preparations were mounted in Ussing chambers to measure short circuit current (I_{sc}) and hence chloride secretion. Drugs were added to the serosal half-chamber to evoke an increase in I_{sc} (VIP) or to potentially block this effect ($VPAC_1$ antagonist, PG97-269, or tetrodotoxin, TTX). The relative expression of mRNA for $VPAC_1$ and $VPAC_2$ receptors in the mucosa and submucosa was determined by quantitative PCR. **Results**: PG97-269 (1 μM) attenuated the response to VIP (50 nM) by 61 ± 8% (n=5; $P<0.05$), but did not block the response suggesting the involvement of another VIP-receptor subtype. TTX (1 μM) decreased the VIP-evoked response by 20 ± 9% (n=6; $P<0.05$), indicating that neuronal activation contributed to a component of the secretory response to exogenous VIP. Quantitative PCR showed that the VIP receptors are differentially distributed, with significantly ($P<0.001$) higher $VPAC_1$ receptor expression in the mucosa (n=8) compared with all other gut layers. In contrast, $VPAC_2$ receptor expression was significantly higher ($P<0.001$) in the submucosa (n=7). **Conclusions**: These results indicate that VIP stimulates secretion primarily through direct activation of $VPAC_1$ receptors in the mucosa. However, a small component of the secretory response is attributable to VIP activating submucosal neurons possibly via $VPAC_2$ receptors.

ORAL-03-01

INTRINSIC MEMBRANE PROPERTIES OF BIPOLAR CELLS IN THE MIDGET AND PARASOL PATHWAYS OF THE PRIMATE RETINA

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Purpose: The midget and parasol ganglion cells represent over 80% of all ganglion cells in the primate retina, yet little is known about the physiological properties of the bipolar cells that provide inputs to these cells. In this study, we investigated the intrinsic membrane properties of bipolar cell types known to provide excitatory inputs to the midget and parasol ganglion cells in the macaque retina. **Methods:** Eyes from adult rhesus macaques were obtained under the Tissue Distribution Program at Oregon National Primate Research Centre. Whole-cell voltage-clamp and current-clamp recordings were made from bipolar cells in a slice preparation. Indirect immunofluorescence immunohistochemistry was used to determine the sub-cellular localization of voltage-gated ion channels including HCN1 and NaV1.1. **Results:** The diffuse cone bipolar cell types, DB3 and DB4, which make inputs to the parasol ganglion cells, exhibited TTX-sensitive, voltage-gated sodium currents (DB4, n=23; DB3 n=8), as well as HCN currents. Under current-clamp, depolarizing current pulses evoked action potentials in both of these cell types. Immunostaining showed the presence of NaV1.1 in the axons of the DB3 and DB4 bipolar cells, whilst HCN1 was observed in the dendrites and axon terminal boutons. In contrast, the flat midget bipolar and invaginating bipolar cells, which provide inputs to the midget ganglion cells, lacked voltage gated sodium or HCN currents. Application of TTX reduced the light-evoked excitatory inputs to ON and OFF parasol ganglion cells, suggesting that bipolar cell action potentials boost glutamate release. **Conclusion:** These data indicate that sodium-dependent action potentials in bipolar cells facilitate excitatory signaling in the magnocellular pathway of the primate retina.

ORAL-03-02

LESIONS OF THE PRIMATE STRIATE CORTEX (V1) DURING INFANCY AND IN ADULTHOOD DIFFERENTIALLY ALTER THE CONNECTIVITY OF THE MIDDLE TEMPORAL (MT) AREA WITH VISUAL THALAMIC NUCLEI

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Purpose: To investigate the thalamic control of visual cortical maturation and how early perturbations alter normal development. **Methods:** Retinothalamocortical connectivity was visualized through intraocular injections of anterograde tracer and cortical injections of retrograde tracer into area MT 12 months after unilateral V1 ablation in postnatal day 14 (PD14, n=3) and adult (n=3) marmoset monkeys (*Callithrix jacchus*) and compared to adult control animals (n=3). cFos immunoreactivity was used to determine activated visual pathways following a stimulation paradigm. Colocalisation of fluorescently labelled area MT relay cells and retinal input was confirmed using the presynaptic marker synaptophysin and statistical analyses of resultant confocal images. **Results:** In the controls it was confirmed that relay cells to ipsilataral area MT were colocalised with labelled retinal ganglion cell terminals in PIm and were activated by light stimulation. Compared to the nonablated adult control and adult V1 ablated animals, neonatal removal of V1 resulted in the sparing of retinal input to PIm and an increase in the size of area MT and contralateral LGN and V1. **Conclusion:** These data provide evidence of a putative pathway involving the pulvinar and area MT that may underpin the improved visual capacity observed in humans following a lesion of V1 early in life (prior to the closing of the critical period) compared with adults.

ORAL-03-03

ANTIDROMIC AND VISUAL EVOKED RESPONSE LATENCY IN MARMOSET LATERAL GENICULATE NUCLEUS

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Purpose: To demonstrate direct connectivity of the koniocellular (KC) visual pathway of the lateral geniculate nucleus (LGN) to the primary visual cortex (V1). We also asked whether the conduction latencies of LGN cells significantly contribute to the visual latencies of the parallel visual pathways. **Methods:** Single electrode, extracellular recordings were made in the LGN of sufentanil-anaesthetised marmosets (*Callithrix jacchus*, n = 3). Area V1 was stimulated using bipolar electrodes with current injection (0-3 mA, 50 µs). Antidromic activation of LGN cells from V1 was confirmed using the collision test and conduction latencies were measured. Visual latencies were measured in the LGN using a brief (200 ms), 60% Michelson contrast, spatially uniform stimulus filling the receptive field centre. Receptive fields were characterised using drifting sinusoidal achromatic and s-cone isolating gratings. **Results:** Thirteen LGN cells were activated antidromically by electrical stimulation in V1: 4 parvocellular (PC); 4 magnocellular (MC); 4 Blue-ON KC; and 1 ON KC cell. The PC cells had latencies 1.96 ± 0.13 ms (mean ± SD); MC cells 1.31 ± 0.25; and Blue-ON KC cells 1.80 ± 0.27. The ON KC cell had a latency of 2.39 ms. Thalamocortical latencies were on average 4% of the visual latency and were positively correlated with visual latencies (r^2 = 0.73). **Conclusions:** Koniocellular cells make direct connections to V1, further substantiating that the koniocellular pathway carries information for visual perception. The conduction latency of thalamocortical fibres contributes very little to the overall latency of visual signals arriving at V1.

ORAL-03-04

AN ACCURATE 3D MODEL OF THE FULL VISUAL FIELD MAP OF THE PRIMARY VISUAL CORTEX (V1) IN THE MARMOSET MONKEY

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Area V1 is organized as a systematic map of the visual field, in which adjacent neurons represent adjacent points of visual space. Performance in visual tasks has been found to depend on the quantitative characteristics of this map. An accurate model is therefore important for understanding perception, and has applications in the design of neural prosthetics. We used computational methods to model the geometry of the V1 map in the marmoset monkey (*Callithrix jacchus*). The receptive fields of 597 neurons were mapped in 2 marmosets anaesthetized with sufentanil (6 µg.kg-1.h-1) and N2O, using electrophysiological recordings. Precise 3D reconstructions of the V1 surface and recording sites were built from histological sections, resulting in the most comprehensive digital maps of V1 in any species. We found that the marmoset V1 is not intrinsically flat, being better described by a model that incorporates intrinsic curvature. Traditional “flat” V1 models, based on the complex log function, require the magnification factor (mm of cortex per degree of visual field) to be greater near the vertical meridians than near the horizontal meridian. Our data show that this is not the case. Furthermore, magnification is lower in the far periphery of the visual field than predicted by these models. The amount of representational anisotropy in marmoset V1 is variable, leading to significant differences in shape between individuals. To accommodate these findings, we extended the model proposed by Rovamo & Virsu (1984) and developed a 3D model that accurately characterizes the shape of the entire V1 map.

ORAL-03-05

THE PERCEPTUAL SPACE OF LOCAL IMAGE STATISTICS

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Purpose. The function of the visual system is best understood in the context of its natural inputs. However, natural scenes have an intricate statistical structure: they contain correlations of low and high orders, and these image statistics covary in a complex way. Thus, it is challenging to move from experiments that measure responses to naturalistic inputs, to computational models that analyze how these responses arise. This motivated us to develop a stimulus library that abstracts the statistics in natural scenes, and enables testing their effects individually and in combination. **Methods.** To reduce the dimensionality of the problem, we focus on binarized images, and, following the findings of Tkacik et al. (2010) concerning informative image statistics, we restrict consideration to configurations that occur in 2x2 neighborhoods. Correlations within these neighborhoods (of all orders) are completely described by 10 image statistics, which can be considered to be coordinates for a perceptual space. We use a set of maximum-entropy constructions to generate synthetic images in which these coordinates vary independently. We use a 4-AFC segmentation task to characterize human visual sensitivity to these local statistics, alone and in combination. **Results.** Sensitivity to first, second, third, and fourth-order image statistics are highly consistent across N=12 subjects (including naïve individuals and experienced observers), and are approximately in the ratio of 1:2:5:4. Pairwise interactions of image statistics are also highly consistent (N=6). A complete characterization of the perceptual metric in all planes of the 10-dimensional image space (N=3) is very close (~5% RMSE) to a Euclidean model. **Conclusions.** Maximum-entropy constructions provide a way to isolate the statistics of natural images, and to study their perceptual impact. Using this approach, we found that the informative statistics of natural images map out a highly conserved and orderly perceptual space.

ORAL-03-06

INTEROCULAR MATCHING OF RECEPTIVE FIELD PROPERTIES OF SINGLE NEURONES IN CAT'S AREA V2

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Most neurones in the striate cortices (V1) of mammals with frontally positioned eyes generate action potentials when appropriate visual stimuli are presented via either eye. The binocular neurones are believed to play an important role in stereoscopic vision and the receptive field (RF) properties revealed by stimuli presented via each eye tend to be very similar. In domestic cats, the parastriate cortex (V2), like area V1, receives its principal thalamic input from the dorsal lateral geniculate nucleus (LGNd). Although virtually all LGNd neurones are monocular, binocular neurones constitute the majority of V2 neurones. **Purpose and Methods:** To examine the degree of interocular matching between the RF properties of binocular neurones recorded from V2 of anaesthetized and immobilized adult domestic cats. The properties were tested using patches of sine-wave-luminance-modulated, high-contrast, drifting gratings. **Results:** 1) In the great majority of neurones (32/36; 89%) the interocular differences in optimal orientations were very small (0 - 10°); 2) In most (24/37; 65%) of the sample, the interocular differences in phase-sensitivities (ratios between F1 component and the mean firing rate - F0, of spike-responses) to optimized gratings were also small (ΔF1/F0 ratios < 0.2); 3) Unlike in V1, in the majority of V2 neurones (22/37) the optimal spatial frequencies for stimuli presented via the dominant eye are very low (< 0.2 cycles/deg). In these cells the interocular matching of optimal spatial and temporal frequencies and direction selectivity indices was poor. **Conclusion:** The poor interocular matching of many RF properties of V2 neurones tuned to low spatial frequencies, suggests that interocular matching of these properties might not be necessary for effective stereoscopic vision.

ORAL-03-07

CHARACTERISATION OF THE GENETIC MUTATION AND RETINAL CHANGES IN A NOVEL, NATURALLY OCCURRING MOUSE MODEL OF ACHROMATOPSIA

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Purpose: Achromatopsia is a congenital disorder that is characterised by total colour blindness, photophobia, low visual acuity and nystagmus. Mutations in the cone transducin gene (GNAT2) are known to lead to this ocular disease. The purpose of this study was to characterise a novel mouse model of achromatopsia. **Methods:** The gnat2 gene was amplified by PCR and the DNA sequence determined for the BalbC-gnat2$^{-/-}$ and wildtype (WT) strains. Rod and cone pathway function were recorded using twin flash electroretinograms (ERGs) *in vivo*, under dark adapted conditions (BalbC-gnat2$^{-/-}$, n=10 and BalbC, n=14). Animals were euthanized and their eyes were collected for histology. **Results:** DNA sequence analysis indicated that there was an A to G point mutation at base pair 517 in Exon 5 of the gnat2 gene. This resulted in an aspartic acid (D) to glycine (G) substitution at amino acid 173 and a total loss of cone transducin protein expression in the outer segments, although cone photoreceptors were still present. The cone ERG response was completely absent in BalbC-gnat2$^{-/-}$ animals, while rod pathway function, including the rod photoreceptor response and the rod post-photoreceptor responses (b-wave and the oscillatory potentials) were similar to WT mice. **Conclusion:** We have characterised the genetic mutation, transducin expression and functional response of a new mouse model of achromatopsia. This mouse may be useful as a model for understanding changes in retinal function and morphology with age in achromatopsia.

ORAL-03-08

HUMAN COLOUR OPPONENCY AS AN ADAPTATION TO OBJECT DETECTION IN CONDITIONS OF PATCHY ILLUMINATION

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Purpose. It has been hypothesized that colour opponency appeared during evolution because it helps us to detect objects in conditions of patchy illumination (Maximov, 2000 Phil. Trans. R. Soc. Lond. B. 355, 1239-1242). In order to facilitate object detection, opponent mechanisms must not be sensitive to variations in light intensity. Two models describing colour opponency have been proposed - 1) linear model and 2) cone ratio model. The linear model predicts that colour opponent mechanisms are generally sensitive to variations of light intensity; the cone ratio model predicts that colour opponent mechanisms are not sensitive to variations of light intensity. Therefore the cone ratio model is consistent with the hypothesis that colour opponency evolved as an adaption for object detection in conditions of patchy illumination, while the linear model is not. **Method**. Thresholds have been measured in three points in the colour space along the [L-M] cardinal direction in three observers. The parameters of the stimuli were adjusted to minimise the sensitivity of luminance mechanisms (Gaussian blob presented on a pedestal). The thresholds in the direction of the minimal sensitivity as predicted by linear (direction 1) and cone-ratio (direction 2) models were compared. **Results.** In the case of the most saturated stimuli, incremental thresholds in the direction 2 were significantly larger than those for direction 1 ($p<0.05$) in two out of three observers. **Conclusion.** Our results are consistent with the predictions of the cone ratio model and, therefore, support the hypothesis that colour opponency appeared as an adaptation for object detection in conditions of patchy illumination.

ORAL-04-01

ADDICTIVE BEHAVIOURS ARE CONTAGIOUS

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Models of instrumental performance and habit development in animals depend on chained sequences of responses (Daw et al., 2005), with a chain typically conceptualized as a leverpress (distal response) to magazine-entry (proximal response) sequence. Animal research shows a transition from goal-directed to habitual performance with an overtrained (OT), but not an undertrained (UT), leverpress (Adams, 1982; Dickinson et al., 1995). This transition is accelerated following amphetamine-sensitization (Nelson & Killcross, 2006), but in neither case is magazine-entry habitual. **Purpose:** Measuring magazine-entry as the proximal response is problematic: magazine-entries represent terminal points of a chain, are confounded by reward proximity, and are potentially Pavlovian. We used a chained sequence of two instrumental responses (leverpress-distal to leverpress-proximal) to ascertain how distal and proximal instrumental responding become habitual. Also of interest is whether d-amphetamine-sensitization facilitates transition to habits within this framework. **Method:** Rats learnt that leverpress-proximal produced reward: this proximal response was either UT or OT. Next they learnt that leverpress-distal followed by leverpress-proximal produced reward: this distal-to-proximal sequence was always UT. Half the rats then received reinforcer devaluation and proximal and distal responses were measured under extinction. In Experiment 1 (n=44), the proximal response was UT or OT. In Experiment 2 (n=42), animals underwent amphetamine-sensitization or control saline-injections prior to acquisition of the UT proximal response. **Results:** Both distal and proximal responding was sensitive to reinforcer devaluation when training involved an UT proximal-response, $p<.05$, but not an OT proximal-response or an UT proximal-response following amphetamine-sensitization: control UT animals remained goal-directed; control OT, and amphetamine-sensitized UT, animals were habitual. **Conclusion:** Novel behaviors that lead to habitual behaviours rapidly become habits themselves. This occurs whether the existing habitual behaviour is acquired through overtraining or following amphetamine-sensitization.

ORAL-04-02

AN INTEROCEPTIVE NICOTINE STIMULUS FUNCTIONS AS A CONDITIONED INHIBITOR IN RATS: IMPLICATIONS FOR MODULATING TOBACCO ADDICTION

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Purpose: Nicotine has been shown capable of signalling sucrose unavailability. The current study further investigates this inhibitory learning property by determining whether nicotine functions as a conditioned inhibitor, suggesting that the nicotine drug state acquires inhibitory properties that modulate subsequent appetitive behaviour. **Method:** In the Retardation-of-Acquisition test, adult male Sprague-Dawley rats received nicotine (n=8) or chlordiazepoxide (n=8) negative feature training. Following acquisition, rats entered nicotine CS training during which nicotine injections (0.4 mg/kg, s.c.) signalled the availability of sucrose and intermixed saline injections signalled an "empty" session. In the Summation test, rats first received either nicotine negative feature training (n=16) or pseudoconditioning (n=16). Subsequently, each group received excitatory training using a white noise CS. At test, the white noise was presented with nicotine. **Results:** The Retardation-of-Acquisition test revealed nicotine negative feature training retarded subsequent acquisition of excitatory conditioned responding to a nicotine CS compared to the chlordiazepoxide control group ($p < 0.05$). The Summation test revealed white noise-evoked conditioned responding was significantly attenuated in the presence of nicotine for the nicotine negative feature group ($p <.05$), but not the pseudoconditioning control group. **Conclusion:** Our data demonstrates that specific learning histories with an interoceptive nicotine stimulus can modulate subsequent appetitive behaviour, revealing the need to extend the conceptualization of how drug stimuli, particularly nicotine in tobacco users, are modulated by various environments.

ORAL-04-03

EFFECT OF ADOLESCENT METHAMPHETAMINE TREATMENT ON LOCOMOTOR BEHAVIOURS IS ATTENUATED IN BRAIN-DERIVED NEUROTROPHIC FACTOR HETEROZYGOUS MICE

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Purpose: Psychosis is prevalent in methamphetamine (METH) users, many of whom transition to develop schizophrenia. Animal models of METH abuse may therefore shed light on the neurobiology of schizophrenia and psychosis. Brain-derived neurotrophic factor (BDNF) has been implicated in schizophrenia pathophysiology and the response to stimulant drugs. The purpose of this study was to determine whether BDNF may play a role in the development of psychosis following METH abuse. **Methods**: Male and female BDNF heterozygous mice (HETs) and wild-type controls (n=8-13 per treatment group) were treated with METH during adolescence (6-9 weeks of age), a critical period of schizophrenia vulnerability. The effect on locomotor activity in response to a challenge dose of amphetamine was examined in adulthood using locomotor photocell cages. **Results**: During adolescence, METH elicited significant locomotor hyperactivity in both genotypes. Two weeks after the end of METH treatment, the locomotor response to a 5 mg/kg amphetamine challenge was enhanced in wild-type mice pre-treated with METH, an effect known as behavioural sensitization. In contrast, in male BDNF HETs this sensitization was absent (genotype x treatment x amphetamine interaction $p=0.025$). Female BDNF HETs also showed altered behavioural sensitization but only in the early stages of the locomotor hyperactivity response (genotype x treatment x amphetamine x time interaction $p=0.001$). **Conclusion**: These results suggest a role of BDNF signalling in the neural adaptations involved in METH-induced behavioural sensitization. These results could be important for our understanding of BDNF involvement and neural plasticity in the development of schizophrenia.

ORAL-04-04

THE INFLUENCE OF MONETARY PUNISHMENT ON COGNITIVE CONTROL IN ABSTINENT COCAINE-USERS

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Purpose: Dependent drug users show a diminished neural response to punishment, in both limbic and cortical regions, though it remains unclear how such changes influence cognitive processes critical to addiction. To assess this relationship, we examined the influence of monetary punishment on inhibitory control and adaptive post-error behaviour in abstinent cocaine dependent (CD) participants. **Method:** 15 recently abstinent CD and 15 matched control participants performed a Go/No-go response inhibition task, which manipulated punishment via monetary fines for failed response inhibition, during collection of fMRI data. **Results:** CD participants showed reduced inhibitory control and significantly less adaptive post-error slowing in response to punishment, when compared to controls. The diminished behavioural punishment sensitivity shown by CD participants was associated with significant hypoactive error-related BOLD responses, in the dorsal ACC, right insula and right prefrontal regions. Specifically, CD participant's error-related response in these regions was not modulated by the presence of punishment, whereas control participants' response showed a significant BOLD increase during punished errors. **Conclusions:** CD participants showed a blunted response to failed control (errors) that was not modulated by punishment. Consistent with previous findings of reduced sensitivity to monetary loss in cocaine users, we further demonstrate that such insensitivity is associated with an inability to increase cognitive control in the face of negative consequences, a core symptom of addiction. The pattern of deficits in the CD group suggests they are not attributable to a lack of motivation, which may have implications for pharmacological or cognitive interventions that attempt to improve such functions.

ORAL-04-05

THE MGLU5 RECEPTOR REGULATES EXTINCTION OF COCAINE-DRIVEN BEHAVIOURS

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PURPOSE:, Extensive evidence exists implicating the metabotropic glutamate 5 (mGlu5) receptor in the rewarding effects of drugs of abuse, as well as drug-seeking and drug-induced plasticity. In the present study, genetic approaches were utilised in order to further explore the role of this receptor in cocaine-driven behaviours. **METHODS:** Mice lacking the mGlu5 receptor (mGlu5-/-) and wild type littermates were subjected to a number of reward-related paradigms. The conditioned rewarding effects of cocaine were evaluated using the conditioned place preference (CPP) paradigm. Extinction of this CPP was then assessed. The reinforcing and incentive motivational properties of cocaine were evaluated utilizing the operant self-administration paradigm. Cocaine self-administration was assessed on both fixed and progressive ratio schedules, followed by cue-induced drug-seeking after a period of 3 weeks abstinence. **RESULTS:** mGlu5 -/- mice were capable of obtaining a CPP to cocaine, suggesting that the mGlu5 receptor is not critical for conditioned rewarding effects of cocaine. In contrast, mGlu5-/- mice exhibited a marked deficit in the extinction of a cocaine-induced CPP. Mice lacking the mGlu5 receptor showed similar operant responding for cocaine as wild type littermates over two different doses of cocaine. However, mGlu5-/- mice displayed enhanced responding on a progressive ratio schedule and cue-induced drug-seeking after abstinence was also exaggerated in mGlu5 -/- mice. **CONCLUSIONS:** Collectively, these findings suggest that while the mGlu5 receptor may not play a critical role in mediating the rewarding effects of cocaine, it is seemingly implicated in the extinction of cocaine-driven behaviours.

ORAL-04-06

GLUCAGON-LIKE PEPTIDE 1 RECEPTOR SIGNALING IN THE LATERAL SEPTUM IS CRUCIAL FOR COCAINE SENSITIZATION IN MICE

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Background: Glucagon-like peptide 1 Receptor (GLP-1R) is a G_s-protein-coupled receptor correlated with phosphorylation of ERK and an increase of Ca^{2+}. The endogenous agonist Glucagon-like peptide 1, which is produced in the gut and in the nucleus of the solitary tract (NTS), regulates appetite and food intake. The exogenous GLP-1R agonist Exendin-4 has been approved for treatment of type-2 diabetes. **Purpose:** As this widely-used drug potentially triggers physiological outputs in brain regions implicated in emotional behaviour we investigate GLP-1R-mediated behaviour in the context of addiction. **Methods and Results:** To determine the expression of GLP-1R in the rodent brain and to reveal the nature of these neurons as well as their anatomical targets, we performed in situ hybridisation studies. Highest GLP-1R-expression was found in the lateral septum (LS), which regulates mood and motivation through connections with the mesocorticolimbic dopamine system. Using retrograde tracing studies we confirm that this brain region is innervated by neurons of the NTS. Systemic application of the receptor agonists triggers neuronal activity in the LS. The loss of GLP-1R signalling in mice (n=12) robustly blunts the behavioural sensitisation to cocaine. This phenotype is restored after adeno-associated virus (AAV)- mediated delivery of GLP-1R cDNA to the LS. **Conclusion:** Our data suggest a pivotal role of GLP-1R signalling in the LS in addiction.

ORAL-04-07

INCREASED NOREPINEPHRINE WITHIN THE BASOLATERAL AMYGDALA IMPROVES EXTINCTION OF REWARD SEEKING

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Purpose: Preventing relapse is a major challenge for the treatment of drug abuse. Exposure to drug-associated environmental stimuli, even following long periods of abstinence, can contribute to craving and relapse to drug use. Reducing the powerful influence of reward-associated stimuli through behavioural extinction may reduce the susceptibility to future recovery of drug-seeking behaviours. **Methods:** The following experiments examined the effects of pharmacological treatments that either increased or blocked noradrenergic activity within the basolateral amygdala (BLA) to test the hypothesis that increased noradrenergic activity in the BLA at the time of extinction training would improve, whereas blockade would impair the extinction of stimulus-reward memories. Rats (n=10-12 per group) were trained that in the presence of a discrete stimulus (e.g. tone), pressing a lever would result in food reward. Responding was then extinguished (i.e. reward was no longer delivered) and prior to the final session, rats received an infusion of saline, norepinephrine or β-2 adrenergic receptor agonists or antagonists into the BLA. Lever-pressing in response to stimulus presentation was tested following a four week delay to assess the longevity of the extinction learning. **Results:** Our results indicate that increasing noradrenergic activity during extinction augments extinction learning resulting in less recovery of responding at test. Further, this effect appears to be mediated, at least in part, by β-2 adrenergic receptors as blocking these receptors abolishes these benefits. **Conclusion:** Our data indicate that engaging the noradrenergic system during extinction can serve to enhance extinction learning whereas disrupting this system can impair extinction learning reflected as greater return of responding in future tests. These results have important implications for models of relapse to drug seeking and the development of extinction-based therapies.

ORAL-04-08

CHRONIC CANNABINOID TREATMENT DURING YOUNG ADULTHOOD INDUCES SEX-SPECIFIC BEHAVIOURAL DEFICITS IN MATERNALLY DEPRIVED RATS

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Introduction: Cannabis is a widely used illicit drug around the world and has been implicated in the development of schizophrenia and depression. Cannabis abuse may trigger disease onset by interacting with early environmental vulnerability factors which target the developing brain. The aim of this project was to study the effect of combined neonatal maternal deprivation and chronic treatment with the cannabinoid CB1 receptor agonist, CP55,940. **Methods:** Wistar rat pups were either separated from their mothers for 3 hours every day from postnatal day 2-14 or left undisturbed. From 8 to 10 weeks of age animals received daily injections of CP55,940 or vehicle. Behavioural changes were assessed at 12 weeks of age and included locomotor activity, Y-maze, plus maze, sucrose preference and PPI (n > 9 in each group). **Results:** CP55,940 significantly reduced locomotor activity on the first treatment day but less so on the last treatment day. Spatial memory in the Y-maze and baseline PPI were not altered. CP55,940 treatment did, however, decrease preference for a sucrose solution in male maternally deprived animals. Furthermore, maternal deprivation and/or CP55,940 treatment reduced time spent on the open arm of the plus maze in males and this was most pronounced after both maternal deprivation and CP55,940 treatment. **Conclusion:** These results suggest that a combination of early stress and cannabinoid receptor stimulation in young adulthood induces altered behaviour in male adult rats. This 'two hit' model could shed new light on the mechanisms by which cannabis abuse is involved in the development of neuropsychiatric disorders.

ORAL-05-01

A FUNDAMENTAL ROLE FOR ALZHEIMER'S DISEASE GENES IN RESPONSE TO OXYGEN LEVELS

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To understand Alzheimer's disease (AD) pathogenesis we must understand the normal biological role of the genes and proteins known to be involved. Examining the regulation and action of these genes/proteins in widely divergent organisms allows us to determine their fundamental, evolutionarily conserved roles. A truncated form of Presenilin protein named "PS2V" (previously described as "aberrant" and observed only in humans) is formed by alternative splicing of *PRESENILIN2* (*PSEN2*) transcripts. PS2V is induced when mitochondria receive insufficient oxygen (i.e. under hypoxia) causing them to produce reactive oxygen species (ROS). PS2V is found at elevated levels in sporadic AD brains and forced expression of PS2V elevates Aβ peptide production in neuroblastoma cells. We have found that: 1) The hypoxia-responsive mechanism of alternative splicing that generates PS2V is highly conserved in most vertebrates including zebrafish (but not mice and rats), 2) PS2V and similar truncations of Presenilin proteins have evolutionarily conserved roles in activation of APP cleavage and Notch signalling, 3) The hypoxia-dependent up-gregulation of *APP*, *PSEN1* & *2* and *BACE1* transcript levels is conserved between mammals and fish. (All *p* values for analysis of adult zebrafish brains were <0.014). Collectively, our results show that the genes involved in Alzheimer's disease pathology respond strongly to decreased oxygen levels and ROS. We discuss the possible involvement of these genes in a mechanism of ROS homeostasis. We show that the recent discovery of the first truly truncating mutation in a *PRESENILIN* gene causing early onset AD (K115Efx10) supports a pathogenic role for PS2V in sporadic AD.

ORAL-05-02

INVESTIGATING THE ROLE OF INHIBITION IN NEUROLOGICAL DISEASE

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Disturbed glutamatergic transmission is a feature of many neurological diseases including Alzhiemers disease (AD). One potential source of disturbed transmission is the altered ratio of excitatory to inhibitory input, however little is known about the vulnerability of interneurons and loss of inhibitory synapses in disease. **Purpose:** To investigate the vulnerability of interneuronal neurites and synapses to degeneration and loss in the vicinity of amyloid plaques relative to changes in excitatory neuron population. **Methods:** To examine morphological alterations to neurites, immunohistochemical analysis of calretinin-positive interneurons and neurofilament-triplet protein expressing pyramidal neurons was performed on tissue sections from two mouse models of AD (TG2576 and APP/PS1, n=5) in addition to human cases (n=12). To examine changes to inhibitory synaptic input on soma close to thioS stained plaques relative to total synaptic input, APP/PS1/YFP mice were immunolabelled with GAD 67 and synaptophysin. **Results:** There was a significantly higher percentage of dystrophic pyramidal neurites in APP/PS1, Tg2576, sporadic and preclinical AD cases (mean ± SEM; 54.6 ± 2.2, 53.2 ± 2.0, 40.3 ± 1.7, 33.4 ± 1.9% respectively, $P < 0.01$) than calretinin neurites (25.6 ± 2.5, 20.5 ± 1.7, 9.6 ± 0.8, 6.7 ± 0.7%) at the plaque edge. There was a significant decrease in synaptophysin puncta on YFP soma near (< 40 µm) plaques (48.2 ± 2.5%, n = 190 soma), relative to distant soma (72.9 ± 3.3%, n = 205 soma, $P < 0.01$). There was no significant difference between GAD67 perisomatic puncta on near (77.0 ± 1.8%, n=120) or distant (85.6 ± 1.6%, n=155) soma. **Conclusion**: These data suggest that interneuron populations may be relatively resistant to amyloid plaque toxicity in AD.

ORAL-05-03

CEREBRAL HAEMORRHAGE IN RAT BRAINS INDUCES HUMAN ALZHEIMER PLAQUE-LIKE PATHOLOGY

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Aims: To test the hypothesis that haemorrhagic lesions to neo- and hippocampal cortex will induce senile-plaque-like pathology. Methods: Needlestick lesions were made in neo- and hippocampal cerebral cortex in Sprague Dawley rats aged 3-5 months. Brains were examined after survival times of 1 to 30d (n= 2 to 4 for each time point) for haemorrhage, cell death, the expression of amyloid precursor protein (APP) and mono- and oligomeric forms of Aβ, gliosis and neuronal and synapse survival using histology, immunohistochemistry and immunoblotting. Human senile plaques were examined with the same techniques. Results: Needlestick injury induced more permanent changes such as haem deposition, cell death, deposition of autofluorescent plaque-like deposits and micro- and macroglial invasion along the needle track, and more transient changes, particularly upregulation of Aβ in neurons and astrocytes flanking the track. Aβ-oligomeric A11 staining was upregulated from 1-5d after lesion at and flanks of lesion. A11 immunoblots confirmed this timecourse. Reactions were similar in hippocampal and neocortex, except that neuronal death was more widespread in the hippocampus. Senile plaques in human neocortex showed features similar to the more permanent changes seen after needlestick injury, including haem deposition, neuronal loss, autofluorescent extracellular core surrounded by deposits and astrocytes. Conclusions: Experimental haemorrhagic injury to Aβ cerebral cortex induced both permanent and transient changes in affected tissue; the more permanent features of the pathology reproduce several features of human senile plaques. The findings support the hypothesis that intracerebral haemorrhage is a factor in the formation of senile plaques in human brain.

ORAL-05-04

TYPE-1 INTERFERON SIGNALLING CONTRIBUTES TO NEURO-INFLAMMATION IN ALZHEIMER'S DISEASE

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Introduction: Neuro-inflammation has recently been implicated in Alzheimer's disease (AD) pathology. The role of pro-inflammatory cytokines in disease progression is yet to be elucidated. Type-1 Interferons (IFNs) are a super-family of pleiotropic cytokines that induce pro-inflammatory gene transcription and are involved in the regulation of the neuro-inflammatory response. Binding of type-1 IFNs to their cognate receptor (IFNAR1) activates the Jak/Stat signalling pathway; significantly implicated as a mediator of the toxicity generated by soluble Aβ1-42 (Wan et al., 2010). **Purpose:** The purpose of this study is to investigate the contribution of type-1 IFN signalling to the neuro-degeneration in AD. **Methods & Results:** To investigate the role type-1 IFNs play in AD, IFNα levels in aged APP/PS1 mouse brains were analysed by ELISA. APP/PS1 brains (9 months) showed a significant 2-fold increase in IFNα protein levels compared to aged matched controls (n=4, $P<0.05$). Western blot showed robust Stat-3 phosphorylation in these APP/PS1 brains (n=4). Elevated levels of type-1 IFN were also confirmed in human tissue. Q-PCR of human AD brain (pre-frontal cortex) homogenates showed significantly increased IFNα (3.84±0.66fold) and IFNβ (4.50±0.86fold) expression compared to control samples; IFNAR levels remained stable (n=9, $P<0.05$). Furthermore, primary cultured neurons from IFNAR1-/- mice, demonstrated neuro-protection against Aβ1-42 (10-15µM, n=9, $P<0.05$). **Conclusion:** This study supports a role for type-1 IFN signalling in the cytokine response and subsequent pathogenesis of AD. A greater understanding of the neuro-inflammatory components in AD is still required, however blocking IFNAR1 may reduce neuro-inflammation and be beneficial in the treatment of AD.

ORAL-05-05

TRKB-SHC IN ALZHEIMER'S DISEASE- EVIDENCE OF ALTERED TRKB PRE-MRNA SPLICING

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Purpose: The mechanism(s) underlying the dysfunction in brain-derived neurotrophic factor (BDNF)/tropomyosin-related kinase B (TrkB) signalling in Alzheimer's disease (AD) are unknown. Truncated isoforms of full-length TrkB (TrkB-TK+) exist and have been shown to compromise BDNF/TrkB-TK+ signalling by acting as dominant negative receptors. A neuron-specific truncated TrkB isoform (TrkB-Shc) has previously been identified but its role in AD is unknown. In this current study, we assess changes in TrkB-Shc expression in AD and delineate how changes in TrkB-Shc expression occur in an AD setting. Methods: We utilized human control/AD (n=6/6) hippocampal brain tissue (Sydney Brain Bank). The neuronal cell-line, SHSY5Y, was used in cell culture assays. Gene expression changes were measured by quantitative real-time PCR (mRNA) and western blotting (protein) in triplicate. Results: We found elevated TrkB-Shc mRNA (t=4.03;df=8;p=0.004) levels in the hippocampus of AD brains. In vitro, increased TrkB-Shc mRNA levels were observed when SHSY5Y cells were treated with amyloid beta (Abeta) (t=2.33;df=15;p=0.03). When cells were transfected with a TrkB minigene, Abeta exposure led to increased splicing of exon 19, which gives rise to increased TrkB-Shc transcripts (t=2.28;df=12;p=0.04). When we assessed gene expression of splicing factors involved in TrkB-Shc/TrkB-TK+ alternative transcript splicing, we found elevated expression of the splicing factor, Srp20 in the AD hippocampus and in cells treated with Abeta. Overexpression of Srp20 in cells increased exon 19 utilization from the TrkB minigene and endogenous TrkB-Shc mRNA levels (t=3.76;df=4;p=0.04). Conclusion: We identify a novel mechanism by which TrkB-Shc transcripts are increased in AD. Our finding of altered splice factor expression in AD has implications for other genes whose expression are altered.

ORAL-05-06

ANALYSIS OF Aβ CELL INTERACTIONS AND UPTAKE IN HIPPOCAMPAL CULTURE

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In Alzheimer's disease, an over-accumulation of amyloid-β (Aβ) protein causes amyloid plaque formation, neurodegeneration and cognitive decline by an unknown mechanism. In late-onset Alzheimer's disease, accumulation of Aβ may be due to defects in the normal clearance of the protein. To better understand how cells in the brain normally degrade Aβ, a comparative analysis of Aβ cell interaction and uptake was undertaken in cultures of primary hippocampal cells. Cultures were prepared from neonatal mice and incubated with fluorescein-labelled human-sequence Aβ for various times (0 - 24 hours) and concentrations (10 nM - 1 μM). The ability of cells to bind and internalise Aβ was assessed by live-cell microscopy and by immunocytochemistry. Aβ was found to be rapidly internalised by microglia (n = 6 experiments). In these cells, Aβ was partially localised with early-endosomal markers EEA1 and Rab5, and with the lysosomal marker LAMP1 (n = 3 experiments). This suggests microglia may traffic Aβ to lysosomes for degradation. Aβ was found to bind rapidly to neurons in a punctate fashion, but Aβ was not internalised by neurons (n > 20 experiments). No co-localisation of Aβ was observed with EEA1, Rab5 or LAMP1 in hippocampal neurons. Optical slicing and live-cell washout experiments revealed that Aβ remained bound to the neuron surface for up to 18 hours of incubation. No binding or internalisation of Aβ was observed in astrocytes. The data suggest that Aβ has a strong affinity for primary hippocampal neurons, but most of the Aβ is not rapidly internalised. Under the same conditions Aβ was internalised by microglia, which may constitute a degradation pathway for Aβ.

ORAL-05-07

SITE SPECIFIC EXCITOTOXICITY: A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Purpose: We have shown that chronic low levels of kainic acid (KA) can cause a distal axonopathy in cultured motor neurons that shows strong similarities to human Amyotrophic lateral sclerosis (ALS) pathology. Furthermore, we have developed a site-specific mouse model of excitotoxicity which in conjunction with these *in vitro* experiments will enable us to investigate the primary site of excitotoxic damage and ALS-like functional decline. **Method:** To examine the time course of distal axonal swelling formation, mature (21 DIV) motor neurons (n=5 cultures) were exposed to 100μm KA for 1 -24 hrs. *In vivo*, a chronic infusion of KA (1-5mM) was delivered to the subarachnoid space of the lumbar region (L4-5) of C57/Bl6 mice using an osmotic mini-pump. The anterograde tracer fluoro ruby (2μM) was concomitantly infused. Animals were processed for immunohistochemistry to determine pathological changes. **Results:** *In vitro*, axonal swellings were rarely present in untreated cultures. At 6 h of KA (100μm) exposure there was a significant ($P < 0.001$) increase in the number of swollen axon distal segments (16.6±3.2 per coverslip) relative to untreated cultures (0.75±0.19). *In vivo*, 28 days after implantation, Fluoro Ruby labelling was present throughout cells within the subarachnoid space (L4-L6) and a small number of cells within the ventral horn, indicating a targeted delivery of KA. Immunolabelling revealed that these cells were motor neurons and also microglia. Exposed microglia had an altered morphology in comparison to control and neuromuscular junction degeneration was also apparent. **Conclusion:** Examining the mechanism underlying distal axonopathy and loss of neuronal function in ALS, and determining the sequence of disease progression, will allow novel potential protective therapies to be developed and specifically directed to the affected neuronal compartment.

ORAL-05-08

NOVEL ACTION OF SURVIVAL MOTOR NEURON IN PROTECTION AGAINST MOTOR NEURON INJURY AND DISEASE

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Purpose Depletion of ubiquitously expressed survival motor neuron (SMN) levels by ~80% causes selective killing of spinal motor neurons in the fatal childhood disorder spinal muscular atrophy (SMA). We recently demonstrated a role for SMN depletion in non-SMA disorders such as amyotrophic lateral sclerosis (ALS) using mutant SOD1 mice and patient tissue. These findings raise the interesting prospect that SMN upregulation may be broadly protective for motor neurons and we sought to test this here using models of acute and chronic motor neuron degeneration. **Methods** Transgenic PrP-SMN mice expressing human SMN driven by the prion promoter were acquired. In the first study, sciatic nerves were unilaterally axotomised or sham-lesioned in neonatal PrP-SMN and wild-type mice (n=5 each). Animals were killed 5 days-post injury and spinal motor neurons counted. In the second part, transgenic SOD1G93A and PrP-SMN mice were crossed. Double transgenic and control genotypes (n=20 each) were examined for weight loss, motor function and survival. Spinal cords (n=6 each) were analysed by motor neuron counts, SMN immunohistochemistry and immunoblotting. **Results** SMN upregulation significantly rescued axotomised motor neuron death in neonatal PrP-SMN mice. SMN overexpression also significantly delayed body weight decline, disease onset and preserved spinal motor neurons ($p<0.05$) in PrP-SMN/SOD1G93A mice. These effects were matched by restoration of nuclear and cytoplasmic SMN levels and SMN-positive Cajal bodies in spinal motor neurons of mice. **Conclusion** We demonstrate a novel and robust action of SMN in protection of motor neurons in models of nerve injury and ALS. In particular, genetic SMN upregulation slows early disease phase and neurodegeneration in mutant SOD1 mice. SMN supplementation or replacement may therefore provide an innovative therapeutic approach to motor neuron degeneration.

ORAL-06-01

METABONOMIC STUDY OF THE DEVELOPMENT OF NEUROCHEMICAL CHANGES IN A MOUSE MODEL OF CANAVAN DISEASE

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Background: Canavan Disease (CD) is a recessive leukodystrophy generally characterized by gross white matter degeneration of the brain, macrocephaly, psychomotor retardation, and premature death. Loss-of-function of the gene encoding the degrading enzyme aspartoacylase (ASPA) results in pathological N-aspartyl acetate (NAA) enrichment in the CD brain. In the ASPA knock out mouse model neurological impairments can be visualized by P14. In this longitudinal study the hypothesis that biochemical alterations precede histological changes has been addressed. The aim of the study was to assess the development of neurochemical alterations in an ASPA KO mouse model. Loss of ASPA activity results in increased NAA and a reduction in lipid levels, however other CD specific metabolic changes have not yet been fully characterized or quantified. **Methods:** Neurological metabonomic phenotypes from wild type, ASPA KO and heterozygous mice at ages ranging from P10 up to 15 months were determined using HR NMR. Myelin lipid disturbances were assessed using a targeted LC-MS/MS metabonomic approach. **Results:** High levels of NAA and myo-inositol in ASPA KO brain were observed at an earlier age compared to the wild type or heterozygous mice. Alterations in myelin lipid constitution were already apparent in younger ASPA KO mice. Other neurological metabolic changes associated with development of CD in these mice included alterations in GABA, glutamine, lactate levels, in addition to lipid and choline metabolism. **Conclusions:** This is the first study to show that neurological biochemical imbalances precede histopathological dysregulation in ASPA KO mice.

ORAL-06-02

LIMITING NOGO-A RECEPTOR 1-DEPENDENT PHOSPHORYLATION OF CRMP-2 REDUCES AXONAL DEGENERATION IN EAE

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Multiple sclerosis (MS) involves demyelination and axonal degeneration of the central nervous system (CNS). The molecular mechanisms of axonal degeneration are relatively unexplored in both MS and its mouse model, experimental autoimmune encephalomyelitis (EAE). **Purpose:** To define the molecular mechanisms which govern neuroinflammatory-mediated axonal degeneration during the disease course of EAE. **Methods:** Female C57Bl/6 mice (aged 10−16 weeks) *ngr1-/-* (n=22) and *ngr1+/+* (n=14) mice were graded daily post-MOG35-55 injection (dpi) for disease progression. Human post-mortem brain and spinal cord tissues were used in the current study and included MS CASES - 4 Chronic-progressive MS cases (67 plaques analysed from brain and spinal cord), 3 Acute-progressive MS cases (3 plaques analysed from brain); OTHER NEUROLOGICAL DISEASES – epilepsy (1 case from temporal lobe), Progressive Multifocal Leukoencephalopathy (PML, 3 cases from brain), Alzheimer's disease (AD, 2 cases from frontal lobe), Meningitis (1 case from frontal lobe); NON-NEUROLOGICAL DISEASES – death by natural causes (10 cases from brain). **Results:** We show that the collapsin response mediator protein 2 (CRMP-2), an important tubulin-associated protein which regulates axonal growth, is phosphorylated and hence inhibited, during the progression of EAE in degenerating axons. The phosphorylated form of CRMP-2 (pThr555CRMP-2) is also localised to spinal cord neurons and axons in chronic-active MS lesions. Specifically, the pThr555CRMP-2 is implicated to be NgR1-dependent, since MOG35-55-induced NgR1 knock-out (ngr1-/-) mice, display a reduced EAE disease progression, without a deregulation of ngr1-/- MOG35-55-reactive lymphocytes and monocytes. The limitation of axonal degeneration/loss in EAE-induced ngr1-/- mice is associated with lower levels of pThr555CRMP-2 in the spinal cord and optic nerve during EAE. Therapeutic administration of the anti-Nogo(623-640) antibody during the course of EAE, associated with an improved clinical outcome, is demonstrated to abrogate the protein levels of pThr555CRMP-2 in the spinal cord and improve pathological outcome. **Conclusion:** We conclude that phosphorylation of CRMP-2, evident in degenerative neurons in chronic-active MS lesions, may be potentiated downstream of NgR1 activation and play a role in axonal degeneration in EAE. This mechanism is inhibited through blockade of Nogo-A/NgR1 interaction and hence this may serve as a viable therapeutic target.

ORAL-06-03

MICROGLIA MODULATE HIPPOCAMPAL NEURAL PRECURSOR CELL ACTIVITY FOLLOWING RUNNING

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Purpose: This study investigated the role of microglia in the regulation of neural precursor cell (NPC) activity under the physiological conditions of exercise and aging. **Methods:** Csf1r-GFP transgenic mice and FACS technology were used to selectively remove, and in some cases subsequently add, microglia from various hippocampal neurosphere preparations (n=4-7 per condition). **Results:** We found that a 37% increase in NPC number observed following running in wild-type animals was abolished when endogenous hippocampal microglia were removed ($p<0.05$). Conversely, when added to preparations from sedentary mice, hippocampal microglia isolated from mice allowed to exercise voluntarily for 2 weeks activated latent NPCs and increased the neurosphere formation frequency by 36% ($p<0.05$). In addition, voluntary wheel running resulted in a significant (16%) increase in protein levels of the chemokine CX3CL1 within the hippocampus. Furthermore, addition of CX3CL1 to neurosphere cultures increased the number of neurospheres by 18%, whereas intraparenchymal infusion of a CX3CR1 blocking antibody (but not control IgG) eliminated the microglia-mediated increase in NPC activity following running ($p<0.05$). We also demonstrated that depletion of microglia from neurosphere preparations of aged (9- or 20-month-old) sedentary mice alleviated the natural age-related reduction in NPC activity ($p<0.05$). The decline in NPC activity within the aging hippocampus correlated with reduction in CX3CL1. However, a 2-week running period successfully elevated the CX3CL1 levels to that of young control animals. **Conclusion:** We provide direct evidence that microglia can exert a dual and seemingly opposing influence over NPC activity within the hippocampus, and that signaling through the CX3CL1-CX3CR1 axis provides critical contribution to this phenomenon.

ORAL-06-04

EXOGENOUS GROWTH FACTOR DELIVERY TO THE DEMYELINATED MOUSE BRAIN MODULATES NUMBERS OF OLIGODENDROCYTES DURING REMYELINATION

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Purpose: A key pathological event in demyelinating diseases of the central nervous system is oligodendrocyte apoptosis, which leads to axons losing their myelin sheaths. Therefore, enhancement of oligodendrocyte regeneration by endogenous progenitor cells is a promising strategy for repair. Bone morphogenic proteins (BMPs) inhibit oligodendrogliogenesis in vitro and are increased in myelin lesions. We have recently found that BMP4 infusion increases oligodendrocyte progenitor cell numbers during cuprizone-induced demyelination, while infusion of Noggin, its endogenous antagonist, increases numbers of mature oligodendrocytes. Here, we report the effects of sequential infusions of BMP4 and Noggin during demyelination on oligodendrogliogenesis and remyelination following 1-week recovery. **Methods**: We used osmotic mini-pumps to infuse vehicle, BMP4 or Noggin in the following combinations: vehicle-vehicle, BMP4-vehicle, vehicle-Noggin and BMP4-Noggin. Lineage specific proteins were detected in tissue sections using immunohistochemistry. Ultrastructure of the corpus callosum was evaluated by electron microscopy. **Results**: Vehicle-Noggin infusion increased the density of Olig2-positive cells and Olig2-CC1 double-positive cells in the corpus callosum compared to vehicle-vehicle, while the density of Olig2-positive cells was reduced in the BMP4-Noggin infused mice compared to vehicle-Noggin ($p<0.05$; n=3,5). The average g ratio of myelinated axons was increased in the vehicle-Noggin infused mice compared to vehicle-vehicle ($p<0.05$; n=3,3). **Conclusion**: These findings indicate that Noggin infusion promotes mature oligodendrocyte regeneration and enhances remyelination. Our results also suggest that BMP4 infusion preceding Noggin infusion does not further enhance repair above what occurs with Noggin infusion alone. We are currently examining the effects of sequential delivery of BMP4 and insulin-like growth factor-1, an oligodendrocyte survival factor, on oligodendrogliogenesis and remyelination.

ORAL-06-05

AAV-MEDIATED GENE DELIVERY TO OLIGODENDROCYTES

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Background: Recombinant adeno-associated viral (rAAV) vectors have become versatile tools for gene transfer to the central nervous system because they are efficient, non-toxic and replication-deficient. While AAV has been used successfully for the treatment of neurodegenerative diseases, the lack of viral vectors with oligodendroglial tropism has precluded gene therapy for leukodystrophies, a group of hereditary disorders caused by oligodendrocyte (OL) dysfunction. **Purpose:** Here we aimed to achieve AAV-mediated transgene expression in OLS using myelin gene promoters. **Methods:** The tropism of different AAV serotypes expressing GFP under the control of the myelin basic protein (MBP) promoter or the chicken β-actin (CBA) promoter was examined in mouse primary OL cultures and after direct brain delivery. **Results:** Mosaic AAV1/2 was identified as the most efficient gene delivery system. AAV-mediated transgene expression driven by the CBA promoter was neuronal. The MBP promoter was sufficient to restrict viral transgene expression to cultured oligodendrocytes. This was confirmed after transfection of the unpackaged AAV constructs suggesting that all cell types are transduced but transgene epression is depending on the tissue specificity of the promoter. AAV-MBP-GFP injection resulted in robust expression of oligodendroglia *in vivo*, and these transduction characteristics were unaffected by the purification method. **Conclusion:** AAV-mediated gene transfer to OLs is possible. Our findings indicate that AAV is the system of choice for the treatment of white matter diseases or whenever safe and efficient gene transfer to OLs is required.

ORAL-06-06

IMPACT OF CHRONIC CAFFEINE EXPOSURE ON WHITE MATTER IN THE VERY IMMATURE BRAIN

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Purpose: Caffeine is widely used to treat apnoea of prematurity. The standard dosing regimen is not always sufficient to abolish apnoea therefore there is pressure on clinicians to administer higher doses. However, the effects of higher doses of caffeine on the very immature brain have not been thoroughly investigated. Here we investigate the impact of chronic high dose caffeine exposure on blood chemistry, body and brain growth and the white matter (WM) in the very immature ovine brain. **Methods:** High dose caffeine (50mg/kg loading; 40mg/kg daily maintenance dose; n=9) or saline (n=9) was administered to the ovine fetus via the maternal circulation from 104 to 118 days of gestation (DG; term = 147 DG). Fetal and maternal blood was sampled to assess blood chemistry and plasma caffeine concentrations. At 119 DG the cerebral hemispheres were immunostained to identify microglia (Iba-1) and astrocytes (GFAP). **Results**: High dose caffeine treatment resulted in an increase ($p<0.05$) in brain weight (11%) and brain to body weight ratio (15%). However, there was no difference ($p>0.05$) in (a) fetal arterial blood chemistry, (b) overall percentage of WM occupied by microglia or (c) overall density of astrocytes in the WM between high dose caffeine-exposed fetuses and controls. **Conclusions:** Chronic high dose caffeine does not adversely affect fetal blood chemistry or result in WM gliosis; oligodendrocytes and myelination are being investigated. It appears that chronic high dose caffeine promotes brain growth however further analyses are required.

ORAL-06-07

RAPID FUNCTIONAL RECOVERY FOLLOWING SCHWANN CELL APOPTOSIS

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Purpose: Schwann cells (SCs) are specialised glial cells in the peripheral nervous system responsible for producing the myelin that ensheaths axons enabling salutatory conduction and providing neuroprotective and trophic support. Ablation of SCs during development has revealed their pivotal role in establishing normal peripheral nerve function. The role of SCs in maintaining axonal integrity and function in adult life remains largely unexplored. **Methods:** We have generated MBP-DTR25 transgenic mice that express the diphtheria toxin receptor (DTR) under the control of the myelin basic protein (MBP) promoter. MBP-DTR25 mice express DTR in SCs and oligodendrocytes rendering both populations selectively sensitive to DT-mediated apoptosis. **Results:** We've shown that twenty-five days following DT challenge (10μg/kg, ip), SCs undergo apoptosis (TUNEL+/S100β+ cells) in the sciatic nerve of MBPDTR25+DT mice but not WT+DT mice ($P<0.05$, n=4 per group). MBPDTR25+DT mice developed hindlimb weakness which peaked within 25 days and rapidly recovered by day 28 post-DT. To elucidate potential mechanisms of rapid clinical recovery, MBP-DTR25+DT and WT+DT mice were administered the thymidine analogue EdU (50mg/kg, ip) four times daily for 3-days prior to perfusion fixation on days-7 and -28 following DT challenge. At day-28, MBP-DTR25+DT mice exhibited a 7-fold increase in proliferative cells compared to WT+DT mice ($P<0.05$, n=8 per group). EdU-positive cells were immunoreactive for Sox-10 and p75 but not S100β or Iba-1, consistent with a Schwann cell precursor (SCP) identity. **Conclusion:** Our data indicate that SC apoptosis elicits a peripheral neuropathy resulting in motor dysfunction followed by rapid recovery that is associated with robust proliferation of SCPs. Further dissection of the pathogenic and regenerative mechanisms will identify the critical mechanisms responsible for rapid functional recovery in this model.

ORAL-06-08

RELAXIN-3 ELICITS ASTROCYTE MIGRATION THROUGH DIVERSE MECHANISMS

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Astrocytes are the most numerous glial cell sub-type in the brain and play an important role in extracellular homeostasis. In response to cerebral injury and hypoxia, astrocytes have been implicated in scar formation and may impede repair and recovery of nervous tissue. The hormone relaxin is a peptide that has been demonstrated to act through diverse signaling pathways to elicit an array of physiological actions that include the modulation of fibroblast migration and extra-cellular matrix (ECM) metabolism to reduce scar formation and fibrosis. Recently, a new variant of relaxin (relaxin-3) has been identified with near exclusive expression in the brain. We sought to determine if relaxin-3 affected astrocyte (fibroblast-like cells in the brain) migration and what signaling mechanisms might be involved in astrocyte migration. First, we determined whether or not relaxin-3 acted on astrocytes maintained in culture through cAMP and calcium-dependent pathways. We then employed a model of cellular migration to determine if relaxin-3 affected the migration of astrocytes and finally explored signaling pathways involved in regulating astrocyte migration. Results indicate that relaxin-3 increases both cAMP and calcium ($P<0.05$) and increases astrocyte migration in a transwell assay. Finally, using selective pharmacological inhibitors of nitric oxide (NO), phosphoinositide 3-kinase (PI3K) and metalloproteinase-3 2and -9, we demonstrated that relaxin-3 mediated the migration of astrocyte through NO, PI3K and matrix metalloproteinase (MMP)-3 and -9. Our data indicate that relaxin-3 affects the migration of astrocytes in vitro by NO, PI3K and MMP-2 and -9 through cAMP and calcium mechanisms. Taken together, these data provide insight into the signaling mechanisms of relaxin-3 in astrocytes and could implicate a role for relaxin-3 in the amelioration of scar formation in the brain as a result of cerebral injury or ischemic challenge.

ORAL-07-01

CENTRALLY ADMINISTERED RESISTIN REDUCES SYMPATHETIC NERVE ACTIVITY TO BROWN ADIPOSE TISSUE VIA ERK1/2

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Purpose: Resistin is an adipokine and its plasma levels are elevated in obesity and diabetes. Resistin is associated with metabolic and cardiovascular disease and it acts in the brain to influence energy homeostasis. Resistin reduces energy intake by reducing food intake and we have shown that resistin reduces the temperature of brown adipose tissue (BAT) suggesting a reduction in energy expenditure and thermogenesis. Since thermogenesis in BAT is regulated by the sympathetic nervous system, in the present study we investigated the effects of centrally administered resistin on sympathetic nerve activity to BAT (BAT SNA). We also investigated the intracellular signaling pathway by examining the role of ERK1/2 in mediating the action of resistin. **Methods:** Overnight-fasted Sprague-Dawley rats were anesthetized (induced with isoflurane gas 2.5%-3% in O_2, and maintained with intravenous urethane, 1-1.4 g/kg initially, followed by supplemental doses of 0.05 g/kg as required). Resistin (7µg) or vehicle was administered into the lateral cerebral ventricle in the presence or absence of the ERK1/2 inhibitor (U0126, 7µg). BAT SNA, body core and BAT temperatures were monitored for 4 hours. **Results:** Compared to the vehicle, resistin significantly reduced BAT SNA by over 50% (n=5) ($P<0.05$). This response was delayed by approximately 150 minutes by U0126 (n=4). **Conclusion:** The findings indicate that resistin reduces thermogenesis by directly reducing BAT SNA via ERK1/2. The decreased thermogenesis elicited by resistin may contribute to metabolic dysfunction observed in obesity and diabetes.

ORAL-07-02

THE EFFECTS OF OXALIPLATIN AND COLORECTAL CANCER ON MOUSE MYENTERIC NEURONS

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Purpose: Diarrhea, nausea and vomiting are common side-effects of anti-cancer chemotherapy. The functions of the bowel are controlled by the enteric neurons that reside within the gut wall. Understanding the effects of the anti-cancer chemotherapy on the enteric nervous system will lead to the development of strategies to prevent gastrointestinal side-effects of chemotherapy. This study investigates the changes that occur in myenteric neurons during the growth of colorectal cancer tumour and anti-cancer chemotherapy. Methods: Oxaliplatin (3 mg/kg-1) was administered via i.p. injections three times a week for 3 weeks. The colorectal cancer model was established by injecting 1x106 C26 cells into the ceacum wall of the mouse. Wholemount preparations from distal colon segments were examined and quantified immunohistochemically in oxaliplatin and sham-injected mice at 3, 7, 14 and 21 days (n=3 per time point) following injections, and in colorectal cancer and sham-operated mice at 3, 7 and 14 days post-surgery. Neuronal cells were labelled with β-Tubulin class III TuJ1 and NOS, while nuclei were labelled with DAPI. Results: Significant increase in the proportion of NOS immunoreactive neurons was evident from as early as 3 days following oxaliplatin injections, and this trend continued at 7-21 days. A significant decrease in the number of neurons in the myenteric plexus also occurred at 14-21 days post-oxaliplatin injections. No significant changes in the myenteric neurons were observed with the growth of colorectal cancer. Conclusion: This study is the first to examine the effects of oxaliplatin and colorectal cancer on the myenteric neurons. Repeated exposure to oxaliplatin causes substantial neuronal loss and an increased proportion of NOS immunoreactive neurons throughout the duration of oxaliplatin administration.

ORAL-07-03

LOW-FREQUENCY PHYSIOLOGICAL ACTIVATION OF THE VESTIBULAR UTRICLE CAUSES ROBUST MODULATION OF SKIN SYMPATHETIC NERVE ACTIVITY IN HUMANS

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Purpose: We have previously shown that sinusoidal galvanic vestibular stimulation (sGVS), a means of selectively modulating vestibular afferent activity, can cause partial entrainment of sympathetic outflow to muscle and skin in human subjects. Given that slow movements of the body, such as those experienced by the sway of tall buildings, can cause motion sickness, the signs of which are linked to an increase in skin sympathetic nerve activity (SSNA), we tested the hypothesis that physiological activation of the vestibular system at low frequencies entrains SSNA. **Methods:** Sympathetic nerve activity was recorded via tungsten microelectrodes inserted into cutaneous fascicles of the common peroneal nerve in 10 human subjects. Subjects were seated on a motorised platform and slow sinusoidal accelerations-decelerations (~4 mG) were applied in the X (anteroposterior) or Y (mediolateral) direction at 0.08 Hz; composite movements in both directions were also applied. **Results:** Subjects either reported feeling a vague sense of movement (with no sense of direction), or no movement at all. Nevertheless, cross-correlation analysis revealed a marked entrainment of SSNA for all types of movements: vestibular modulation was 97±3 % for movements in the X-axis and 91±5 % for displacements in the Y-axis. For each sinusoidal cycle there were two major peaks of modulation - one associated with acceleration as the platform moved forward or to the side, and one associated with acceleration in the opposite direction. **Conclusion:** We interpret these observations as reflecting displacement of the cilia within the utricle during acceleration, which causes a robust vestibulosympathetic reflex.

ORAL-07-04

SIMULTANEOUS AND CO-ORDINATED ACTIVATION OF SYMPATHETIC VASOMOTOR, CARDIAC AND RESPIRATORY OUTPUTS BY NEURONS IN THE MIDBRAIN COLLICULI

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Apart from the midbrain periaqueductal grey (PAG), the superior and inferior colliculi in the midbrain are believed to be part of the central pathways regulating autonomic activity during defensive behaviour. In this study we examined whether these regions regulate respiratory activity in addition to sympathetic vasomotor activity and heart rate. In urethane-anaesthetized rats (n=18), microinjections of bicuculline (50 pmol in 50 nL) into sites within the deep layers of the inferior colliculus and superior colliculus, evoked a highly distinctive response characterized by short intense bursts of renal sympathetic nerve activity (RSNA) and phrenic nerve activity (PNA). Each burst of RSNA had a duration of approximately 400-500 msec, and occurred slightly later (latency of approximately 40 msec) than the corresponding burst of PNA. The bursts of RSNA and PNA were also accompanied by transient but substantial increases in heart rate and arterial pressure. Injections into sites centred 0.5 mm from such sites evoked either no response or a completely different pattern of response. The results indicate that disinhibition of neurons within this localized midbrain region evokes a widespread and intense activation of sympathetic vasomotor and cardiac activity, together with an increase in inspiratory activity. It is possible that this response is generated by a common population of “command neurons”, and the sympathetic-respiratory changes may be part of a generalized escape response that is normally evoked by an acute threatening stimulus.

ORAL-07-05

FUNCTIONAL CONNECTIVITY BETWEEN THE DORSOLATERAL PREFRONTAL CORTEX, VENTROMEDIAL HYPOTHALAMUS AND RVLM IN AWAKE HUMANS

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Purpose: By recording muscle sympathetic nerve activity (MSNA) concurrently with functional magnetic resonance imaging (fMRI) of the human brain, we have been able to identify the cortical and brainstem regions responsible for the generation of spontaneous MSNA. In the present study we aimed to determine the functional connectivity between these regions. Methods: A tungsten microelectrode was inserted into a muscle fascicle of the common peroneal nerve, at the level of the fibular head in 15 subjects. Gradient echo, echo planar fMRI was performed using a 3T scanner (Phillips, Achieva). Two hundred scans were collected continuously in a 4s-ON, 4s-OFF protocol (46 axial slices, TR = 8s, TE = 4s, flip angle = 90 deg, raw voxel size = 1.5x1.5x2.75 mm). Sympathetic burst amplitudes were measured from the RMS-processed mean voltage amplitude during the 1-2 s interval of the 4s-OFF period, so as to account for the location of the hypothalamus in the caudorostral scanning direction. A fixed-effects group analysis (minimum cluster size=10 voxels, $p<0.005$) was performed to identify a volume of interest for use as a 'seeding area'. A connectivity analysis was performed (fixed-effects, minimum cluster size=10 voxels, $p<0.005$) with the ventromedial hypothalamus used as a 'seeding area'. Results: The ventromedial hypothalamus showed positive relationships with the precuneus, dorsolateral prefrontal cortex and bilateral rostroventral lateral medulla. Conclusion: Spontaneous fluctuations in sympathetic nerve activity to muscle covaries with activity in multiple brain regions. Furthermore, this supports the idea that the hypothalamus contributes to the ongoing control of resting blood pressure and may be driving other brain regions involved in autonomic control.

ORAL-07-06

AMYGDALA-PROJECTING SOLITARY TRACT NUCLEUS (NTS) NEURONS ARE PREDOMINATELY HIGHER ORDER

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Purpose: The amygdala receives visceral information via the solitary tract nucleus (NTS). Although previous studies suggest broad interaction between visceral, NTS and other central information, little is known about amygdala-projecting NTS neurons (e.g. 2nd order or higher). Here, we examined interactions between solitary tract (ST) afferents and NTS neurons projecting to the central nucleus of the amygdala (NTS-CeA). **Methods**: To identify NTS-CeA projection neurons, fluorescent retrograde tracers were injected into CeA. Later in 250 μM horizontal slices, we recorded from tracer filled NTS-CeA neurons. **Results**: Graded intensity ST shocks evoked postsynaptic currents (PSC) whose responses became more complex with increasing shock intensity indicating multiple convergent inputs. Analysis of ST-PSC amplitude, synaptic jitter and failure rates identified two populations of neurons. Some NTS-CeA neurons (n=8) received low jitter ST-EPSCs identifying them as 2nd order neurons with direct ST afferent input. However, most NTS-CeA neurons (10 of 18) were higher order exhibiting no low jitter ST-EPSCs, rather, multiple high jitter EPSCs and IPSCs with frequent failures. Higher order neurons received afferent information through highly convergent polysynaptic pathways. **Conclusion**: The identified neuron classes raise interesting questions about differential integration of visceral information to the amygdala in complex behaviours.

ORAL-07-07

GASTROINTESTINAL FUNCTION IS ALTERED IN THE NEUROLIGIN 3 MOUSE MODEL OF AUTISM

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Purpose: Gastrointestinal (GI) symptoms and dysfunction are commonly reported in individuals with autism spectrum disorders (ASDs) but whether these are secondary to the central nervous dysfunction or a real symptom of the ASDs is controversial. We investigated colonic motility in the Neuroligin-3 Arg451Cys (NL-3) mouse model of autism to determine whether there is altered colonic function. Methods: Isolated segments of colon from NL-3 mice and wild type littermates were placed in an organ bath, cannulated and luminally perfused with physiological saline. Contractile activity was monitored using video imaging techniques to create spatiotemporal maps as described previously. Colonic migrating motor complexes (CMMCs) were assessed at baseline and in the presence of bath applied antagonists acting on GABA ($GABA_A$ bicuculline 10 μM, gabazine 10 μM, $GABA_B$ CGP 54626 10 μM), and serotonin (tropisetron 10 μM, $5\text{-}HT_3$ and $5\text{-}HT_4$) receptors. Results: Both $GABA_A$ receptor antagonists bicuculline and gabazine reversibly depressed CMMCs in NL3 mice but not in wild types (2-way ANOVA $n > 10$ $p = 0.0001$; $n = 8$ $p < 0.05$, respectively), but the $GABA_B$ receptor antagonist, CGP 54626 ($n = 4$) had no effect. The $5\text{-}HT_3/5\text{-}HT_4$ antagonist tropisetron depressed CMMCs in NL-3 more than wild type ($n > 8$ $p = 0.01$). There were no observed strain differences in control. The effects of the antagonists appear to be on the mechanism initiating the CMMCs because there were no obvious changes in other properties of these stereotyped motor patterns. Conclusions: Colonic motility is altered in NL-3 mice in the presence of GABA and 5-HT receptor antagonists and reveals for the first time that GI symptoms observed in at least one form of ASD originate in the ENS.

ORAL-07-08

CENTRALLY ADMINISTERED RESISTIN INCREASES THE RENAL SYMPATHETIC NERVE ACTIVITY VIA PI3K

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Purpose: The plasma level of resistin, an adipokine, is elevated in obesity and metabolic syndrome and is associated with metabolic and cardiovascular disease. Characteristics of obesity include an increase in sympathetic nerve activity (SNA) to the kidney and skeletal muscle vasculature. Resistin increases lumbar SNA suggesting it may contribute to the elevation in SNA to the skeletal muscle vasculature seen in obese patients. In the present work we investigated (i) the effect of centrally administered resistin on sympathetic nerve activity targeting the kidney (RSNA), a key organ involved in cardiovascular regulation, and (ii) the intracellular signaling pathways mediating this response. **Methods:** Sprague-Dawley rats were fasted overnight and anesthetized (induced by isoflurane gas 2.5%-3% in O2, and maintained with intravenous urethane, 1 to 1.4 g/kg initially, followed by supplemental doses of 0.05 g/kg as required). Resistin (7ug) or vehicle was administered into the lateral cerebral ventricle (ICV) in the presence or absence of the PI3K inhibitor (LY294002, 5ug ICV). The RSNA, mean arterial blood pressure (MAP) and heart rate (HR) were recorded for 4 hours. **Results:** Compared to controls, resistin significantly increased RSNA by approximately 40% ($n=7$) ($p<0.05$). This response was prevented by ICV LY294002 ($n=5$) ($p<0.05$). There was no significant effect of restistin on MAP and HR. **Conclusion:** The findings indicate that resistin can act centrally to increase RSNA via PI3K. Thus, resistin may contribute to the increased RSNA observed in obesity and this may contribute to the increased sodium retention and impaired kidney function observed with excess weight gain.

ORAL-08-01

LIMITED VALIDITY AND PUBLICATION BIAS RESULT IN OVERESTIMATION OF EFFICACY IN EXPERIMENTAL STUDIES

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Purpose: The poor conduct and reporting of animal experiments, due to limited internal and external validity and the presence of publication bias, have been implicated in the discordance observed between the results of animal and human studies. Systematic review and meta-analysis have proven to be useful tools in deriving empirical evidence of the impact of validity on the outcome of animal studies. Methods: We have systematically collected data from over 2500 studies describing 4700 experiments modelling six neurological diseases reporting outcome from over 60,000 animals. We used DerSimonian and Laird random effects meta-analysis to assess the impact of validity on outcome; and used trim-and-fill to assess the impact of publication bias. Results: Assessment of publication bias in 499 focal ischaemia publications using 1300 animals identified that 1 in 6 experiments remain unpublished, which leads to an overstatement of efficacy of at least 30%. Furthermore, only 3% of studies report performing a sample size calculation, and about a third of studies report random allocation to group and blinded assessment of outcome – both associated with overstatements in reported efficacy. In publications reporting the use of transgenic mouse models of Alzheimer's disease only 16% report random allocation to group, 22% report blinded assessment of outcome and no publications performed a sample size calculation. In publications of experimental autoimmune encephalitis (a model of multiple sclerosis) efficacy was substantially overstated in those reporting measures to avoid bias (random allocation to group: 20.6% [95%CI 11.4–29.7] versus 41.6% [36.7–46.5] and blinded assessment of outcome: 29.8% [19.8–39.8] versus 41.0% [36.2-45.8]). Conclusions: Empirical evidence of the impact of potential sources of bias has allowed us to develop good laboratory practice guidelines but also highlight the impact of not publishing experimental results and the value of reviewing evidence before embarking on clinical trials.

ORAL-08-02

HYPOXIC PRE AND POSTCONDITIONING PROVIDE NEUROPROTECTION IN A NEONATAL RAT MODEL OF HYPOXIC-ISCHEMIC BRAIN INJURY

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Previous studies have found that preconditioning with mild hypoxia can protect the brain against hypoxic-ischemic (HI) brain injury and this protective effect is likely to be due to increased expression of the transcription factor hypoxia-inducible factor-1 (HIF-1) and its target genes. More recently, post-injury (or postconditioning) treatment with hypoxia has been shown to provide brain protection in an adult stroke model. Here we have compared the protective effects of hypoxic pre- and postconditioning in a neonatal rat model of HI injury. Sprague-Dawley rat pups (postnatal day 7) were anaesthetised with isofluorane and underwent a unilateral common carotid artery ligation and were then exposed to 3 hours of 8% oxygen. Hypoxic treatments were: either 3 hrs of 8% oxygen performed 24 hrs prior to injury (Preconditioning); or 1 hr of 8% oxygen 24 hrs post-injury, performed once a day for 5 days (Postconditioning); normoxic controls were exposed to room air. Brains were removed 1 week post-injury for histological analysis. Both hypoxic pre (n=12) and postconditioning (n=16) significantly reduced the amount of brain damage when compared with normoxic controls (n=15), as indicated by the 73% and 46% reduction in lesion size ($p<0.05$, ANOVA). To examine neuronal loss, NeuN immunohistochemistry and regional brain area analysis was performed. Preconditioning with hypoxia prevented cortical, hippocampal and striatal neuronal loss, by 79%, 127% and 84% respectively. While, postconditioning reduced cortical neuronal loss by 61%, there was no difference in neuronal loss in striatal or hippocampal regions compared to normoxic controls. Here we have observed for the first time that hypoxic postconditioning can protect the neonatal brain against injury HI.

ORAL-08-03

PRE-DIFFERENTIATION OF HUMAN NEURAL PROGENITOR CELLS ENHANCES NEURONAL REPOPULATION FOLLOWING TRANSPLANTATION WITHIN THE STROKE DAMAGED BRAIN

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There is a compelling need to develop effective treatment strategies for patients affected by stroke. The use of stem cell based therapies to promote brain repair may offer new hope for improving patient recovery after stroke. To this end, we have isolated and characterized human neural progenitor cells (NPCs) that can be differentiated into neural cells. **Purpose:** To investigate the effect (histological and behavioural outcomes) of undifferentiated and pre-differentiated hNPC transplants into the rat brain 7 days post-stroke. **Methods:** The middle cerebral artery was constricted by endothelin-1 (ET-1) in conscious rats, n=15 in total; group 1 receiving undifferentiated hNPCs, group 2 receiving pre-differentiated hNPCs and group 3 receiving vehicle with media alone. Neurological outcome was assessed by neurological deficit score, sticky label test, and cylinder test. 7 days after ET-1 stroke undifferentiated, pre-differentiated hNPC's or vehicle were stereotaxically injected into the rat brain at 8 predetermined sites to target both the striatum and cortex. Brains were harvested 28 days post-transplant. hNPC survival, proliferation and neurite outgrowth were assessed using immunohistochemistry and confocal microscopy. **Results:** Neurological functional assessment revealed spontaneous recovery across all three treatment groups. Undifferentiated as well as pre-differentiated hNPCs survived the grafting procedures and within the stroked affected brain. Immunohistochemical analysis revealed pre-differentiated hNPCs maintained their neuronal phenotype post-transplant as evidenced by human neuron specific enolase (hNSE) or human nuclear antigen (hNA) colabeled with β-III-tubulin. Among the implanted undifferentiated hNPCs, a small percentage was immunostained for hNSE while the majority of cells were double-positive for hNA and glial fibrillary acidic protein indicating differentiation into astrocytes. **Conclusion:** Pre-differentiating hNPCs into neuronal cells prior to transplantation results in a greater number of hNPC-derived neuronal populations within the damaged region of the stroke affected brain. Our findings may shed a light in use of hNPCs to promote restoration of the stroke affected brain.

ORAL-08-04

OVINE MCA OCCLUSION MODEL: 24HR INTRACRANIAL PRESSURE MONITORING STUDY

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Purpose: More than 60 000 Australians suffer a stroke each year, with devastating consequences. Many animal models of cerebral ischaemia, mainly in rodents, have been developed to mimic clinical stroke, although novel treatments characterized in these models have failed to translate to the human condition. Accordingly, we have recently developed an ovine model of MCA occlusion where reperfusion can be achieved. The aim of the present study was to characterise the intracranial pressure (ICP) changes which occur following MCA occlusion in the sheep. **Methods:** Merino sheep (n=15) were subject to either sham surgery or MCA occlusion achieved by either diathermy (permanent) or the application of an aneurysm clip (2h occlusion). Brain tissue oxygenation (Licox®), ICP, blood pressure and blood gases were recorded. Animals were monitored for 24hrs after the induction of stroke and killed by saline perfusion. Brain were removed for infarct volume assessment by tetrazolium chloride (TTC) staining and then processed for histological assessment. **Results:** MCA occlusion produced by either permanent or transient vessel occlusion resulted in significant increases in ICP over the 24hr monitoring period. Histological assessment revealed infarction within the MCA territory, confirmed by TTC assessment of infarct volume. Such changes were most pronounced in the permanent MCA occlusion group. **Conclusions:** The sheep model of MCA occlusion produces changes in ICP and histological abnormalities and lesion volumes consistent with what is observed in clinical stroke. Such findings emphasise the value of this model in pre-clinical development of potential therapeutic agents for the treatment of stroke.

ORAL-08-05

NEURAL INJURY AND FUNCTIONAL RECOVERY FOLLOWING REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION IN A RODENT MODEL OF TRANSIENT FOCAL CEREBRAL ISCHAEMIA

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Purpose: Stroke is a leading cause of disability in Australia. Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive tool that can modulate neural activity. We investigated the effect of rTMS on functional recovery and tissue damage in an established model of transient focal cerebral ischaemia (middle cerebral artery occlusion – MCAo). Animals were treated with an acute rTMS paradigm, in combination with one of two chronic rTMS strategies, which have previously been shown to have beneficial effects. **Method:** Spontaneous Hypertensive rats (n=22) underwent MCAo (90min) and recovery was monitored over 8 days. Eighteen rats received acute rTMS (20Hz) in combination with either i) chronic high frequency (6-9Hz) (n=6), ii) chronic low frequency (1Hz) (n=6) or iii) sham stimulation (n=6). A control group (n=4) received no rTMS. Daily behaviour testing was performed (bilateral paw asymmetry, neurological 5-point scale-of-deficit and open-field). Outcome measures for behavioural tests were taken presurgically, and on days 3 and 8 post-MCAo. Following animal sacrifice on day 8, brain infarct volume was determined. **Results:** Behavioural tests revealed all animals improved over time ($p<0.0001$), but with no significance treatment effect ($p=0.0766$). Infarct size, ≈ 8% of total brain volume, was similar across treatment groups ($p=0.2437$). **Conclusion:** The application of rTMS at these frequencies and intensity (equivalent to 8mT) did not improve functional or histological outcomes in this model. Although no beneficial effects of treatment were observed, it was encouraging for future studies in less severe injury models that no detrimental effects were detected.

ORAL-08-06

OVER-EXPRESSION OF RCAN1/DSCR1 IMPROVES STROKE OUTCOME

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Purpose: Ischaemic stroke occurs following loss of blood flow to an area of the brain resulting in the deprivation of oxygen and glucose. Further damage occurs once blood flow is restored due to an influx of inflammatory and immune cells. RCAN1 was reported to be up regulated after ischaemic stroke in mice. **Methods:** We subjected mice over-expressing RCAN1 to ischaemia and reperfusion (I-R) by MCAO (middle cerebral artery occlusion). **Results:** RCAN1-TG mice had an improved neurological score and performance in a hanging wire grip test compared with WT counterparts ($n = 14$, $P\leq0.01$) and total infarct and oedema volumes were 50% smaller ($P\leq0.05$). Expression of macrophage inflammatory protein-1α (*MIP-1α*), monocyte chemoattractant protein-1 (*MCP-1*), cytokine-induced neutrophil chemoattractant (*CINC*) and cyclooxygenase-2 (*COX-2*) were substantially increased at 6 h and 24 h post-IR in WT mice but significantly smaller increases were evident in RCAN1-TG mice ($P\leq0.05$). RCAN1-TG mice exhibited reduced neutrophil infiltration following ischaemia ($P\leq0.01$) and while there was no difference in the density of microglia between TG and WT mice, more microglia were in a resting state and fewer were in activated or phagocytic states in the RCAN1-TGs ($P\leq0.05$). Neurons isolated from RCAN1-TG mice were more resistant to apoptotic cell death following glucose deprivation compared with WT controls and this was associated with reduced expression of activated caspase-3. Conclusion: RCAN1 over-expression improves functional and histological outcome following stroke. We speculate that the mechanisms underlying this protection involve anti-apototic and anti-inflammatory effects mediated by RCAN1.

ORAL-08-07

CAMKII IS REGULATED DIFFERENTLY IN BRAINS REGIONS WITH DIFFERING SENSITIVITIES TO ISCHAEMIA/EXCITOTOXICITY

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Purpose: Two groups of interacting molecules are important mediators of ischaemia and excitotoxicity-induced cell death : ionotropic glutamate receptors (especially AMPA-R and NMDA-R) and the enzyme, calcium/calmodulin-stimulated protein kinase II (CaMKII). Inhibition of CaMKII activity is neuroprotective in experimental models of ischaemia and excitotoxicity (JBC 285:20675-82). It has been hypothesised that increased expression of CaMKII leads to increased basal phosphorylation of AMPA-R at S831-GluR1 and enhanced sensitivity to excitotoxicity (Stroke 38:3007-15). We tested this hypothesis. **Methods**: Basal and stimulus-induced phosphorylation of CaMKII, the GluR1 subunit of AMPA-R and the NR2B subunit of NMDA-R was measured by western blotting in microslices generated from the striatum and cortex (regions more sensitive and more resistant to ischaemia/excitotoxicity, respectively) of Sprague Dawley rats (n=20). **Results**: We found no correlation between sensitivity to ischaemia/excitotoxicity and level of expression or basal phosphorylation of CaMKII, GluR1 or NR2B. However, CaMKII mediated responses to excitotoxic stimulation were different in striatum and cortex. AMPA or NMDA or glutamate stimulation produced a much greater increase in phosphorylation of CaMKII at T253 in striatum than in cortex but an equal increase in phosphorylation at T286. Phosphorylation by CaMKII of GluR1 at S831, but not NR2B at S1303, was significantly elevated in striatum, but not in cortex, following stimulation with AMPA. Additionally, phosphorylation by CaMKII of NR2B at S1303, but not GluR1 at S831, was significantly elevated in striatum following stimulation with NMDA. **Conclusion**: These results suggest that tissue differences in T253 phosphorylation-mediated targeting of CaMKII are involved in the differential sensitivities of brain regions to ischaemic and excitotoxic insults.

ORAL-08-08

NOREPINEPHRINE OR ISOPROTERENOL TREATMENT MODULATES NEUROGENESIS AFTER A HIPPOCAMPAL STROKE MODEL IN ADULT MOUSE

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Purpose: The hippocampus is vulnerable in various disease states, including stroke, but pools of activatable hippocampal stem/precursor cells represent novel therapeutic targets to repopulate damaged regions. We hypothesise that activating latent stem cells with norepinephrine following stroke will enhance neurogenesis, which may critically contribute towards improved behavioural outcomes. **Methods:** To model stroke, adult female C57Bl/6 mice received a unilateral intrahippocampal injection of vasoconstrictor Endothelin-1. Stem/precursor cell activation was evaluated using the neurosphere assay. To examine activation *in vivo*, starting 7d after ischaemia, animals received daily intraperitoneal injections for 7d of either selective beta-adrenergic receptor agonist isoproterenol, previously shown to activate hippocampal precursors, or saline, followed by BrdU. Animals were sacrificed 14d after the final injection, and immunohistochemistry and stereological cell counts conducted. **Results:** Whilst there was a reduction in doublecortin-positive neurons in the stroke hemisphere of saline-treated animals to 53% of sham hemisphere levels ($n=5$-6/group, $p<0.05$), we found no associated reduction in precursor cell numbers as stroke and sham hemispheres produced similar neurosphere numbers ($n=27$). Moreover, norepinephrine treatment resulted in the production of large neurospheres from both sham and stroke hemispheres, suggesting preservation of latent stem cell pools ($n=13$). While there was no change in BrdU-positive cell numbers in isoproterenol-treated animals, it led to a significant elevation of doublecortin-positive cells (143%) in the stroke hemisphere compared to saline-treated controls ($p<0.05$). Consistently, there was an increase in BrdU-positive cells colocalising with doublecortin ($p=0.0786$). **Conclusion:** This study shows that following hippocampal stroke resident stem/progenitor cells are activateable *in vitro* and *in vivo* with norepinephrine. Future experiments will examine the effect of these increases in neuronal production on hippocampal learning and memory.

ORAL-09-01

GLIAL FIBRILLARY ACIDIC PROTEIN MRNA AND PROTEIN EXPRESSION IN SCHIZOPHRENIA PATIENTS

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Purpose: We have observed increased density of microglia in dorsolateral prefrontal cortex (DLPFC) white matter and altered expression of cytokines in DLPFC grey matter from patients with schizophrenia. Since astroglia may also respond to neuroimmune activation, we asked if changes in astrocytes would be evident in these patients. **Methods:** We studied a brain tissue collection from 37 schizophrenia patients and 37 controls, matched on age, sex, postmortem interval and pH. Using DLPFC grey matter tissue, we measured GFAP mRNA by RT-PCR and GFAP protein by Western blotting. Immunohistochemistry for GFAP was performed using frozen tissue sections. **Results:** Grey matter GFAP mRNA levels correlated positively with age and negatively with tissue pH in both schizophrenia patients and controls. We found a 6% decrease in GFAP mRNA in schizophrenia patients relative to controls ($F(1)3.093$, $p=0.08$). Whilst total DLPFC grey matter GFAP protein correlated positively with age and negatively with tissue pH, there was no significant diagnostic difference in GFAP protein ($F(1)0.102$, $p>0.05$). No diagnostic difference in astrocyte morphology was found in white matter DLPFC (Chi-square(2)0.543, $p>0.05$). Fibrous astrocyte morphology was significantly associated with decreased tissue pH, increased GFAP protein expression and age in schizophrenia samples, whereas astrocyte morphology was dissociated from these variables in control samples. **Conclusions:** Despite our predictions GFAP mRNA was decreased in schizophrenia, but this parallels previous reports. The lack of an increase in GFAP mRNA, GFAP protein or reactive astrocyte-like morphology suggests that the normal astroglia response accompanying neuroimmune activation is absent or attenuated in schizophrenia.

ORAL-09-02

ALTERED GAMMA OSCILLATIONS IN A NEUREGULIN 1 MOUSE MODEL OF SCHIZOPHRENIA

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Disruptions in synchronised oscillatory activity in the brain are an emerging hypothesis for the cause of the diverse pathophysiology seen in schizophrenia, while Neuregulin 1 (NRG1) has emerged as a leading candidate for a schizophrenia 'risk' gene. NRG1 signalling is important for neural development and migration with a particularly significant role in the development of parvalbumin (PV) interneuron inhibitory circuits. Fast-spiking PV interneurons underpin the control of neural networks through GABAergic inhibition of pyramidal cells and the synchronous activity of interneurons generate gamma oscillations (30-100 Hz). Gamma oscillations have been shown to be altered in schizophrenia and may underlie the cognitive and perceptual alterations in this disease. This study explored the characteristics of gamma oscillatory activity in an established mouse model of schizophrenia, the NRG1 transmembrane heterozygous mutant mouse. 18 (6 wild-type and 12 NRG1 mutant female) mice underwent surgery to be implanted with electrodes for EEG recordings. After recovery, EEG activity was recorded with each animal receiving ketamine (10 mg/kg s.c.) and saline (10 ml/kg s.c.) treatments (separate recordings). Mutant mice displayed significantly enhanced baseline cortical gamma power compared to controls (26.1% ± 0.2 vs 17.5% ± 0.01, $p<.0001$) as well as a reduced gamma response to the NMDA antagonist challenge (34.2% vs 64% relative increase in gamma power, $p<.0001$). This work establishes that animals with a genetic mutation relevant to schizophrenia and known behavioural deficits have a clear electrophysiological disturbance; alterations of gamma power in these animals further reinforces the relevance of gamma oscillation disturbances to the pathophysiology of schizophrenia.

ORAL-09-03

DISRUPTED-IN-SCHIZOPHRENIA 1 MODULATES GSK3β/β-CATENIN SIGNALING AND SURVIVAL OF HUMAN NEURAL STEM CELLS: A NEW FRAMEWORK FOR UNDERSTANDING HUMAN NEUROGENESIS AND PSYCHIATRIC DISORDERS

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Purpose: Disrupted-In-Schizophrenia 1 (*DISC1*) is a susceptibility gene for psychiatric disorders. In addition, *DISC1* has been implicated in key neurodevelopmental processes, including neural cell migration, dendrite formation and axon termination[1]. We have studied the role of *DISC1* in human neurogenesis using unique neural stem cell (NSC) models of *DISC1* loss-of-function (LOF). Methods: Transgenic NSC lines were generated from human hippocampus (HP; n=3) and subventricular zone (SVZ; n=3) *via* lentiviral delivery of short hairpin RNA targeting *DISC1*. The effects of *DISC1* LOF on NSC cycle, proliferation and survival were investigated using a panel of cellular and biochemical assays. Results: *DISC1* LOF caused a decreased NSC number ($p<0.05$) and concomitant reduction in the diameter of neurospheres ($p<0.001$). The effect of *DISC1* LOF appears to be mediated by activation of GSK3β with ensuing down-regulation of active β-catenin ($p<0.0001$). This is consistent with *DISC1* LOF increasing caspase-3/7 activity ($p<0.05$). Importantly, the GSK3β inhibitor lithium chloride, a mood-stabilizing drug often prescribed for bipolar disorder and schizophrenia, normalizes NSC number and neurosphere diameter caused by *DISC1* LOF. Conclusions: Our findings implicate *DISC1* in cell survival *via* GSK3β and canonical Wnt/β-catenin signaling. The results provide a novel framework for understanding how *DISC1* signaling may contribute to human neurogenesis and the etiology of psychiatric disorders. [1]Kobayashi NR et al. (2010) Mol Psychiatry 15:672-675.

ORAL-09-04

PROTEOGENOMIC ANALYSES OF LINGO-1 AND THE NOGO RECEPTOR IN SCHIZOPHRENIA

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Purpose: Schizophrenia is a severe neuropsychiatric disorder with an elusive aetiology, thought to result from abnormal brain development. Nogo is an oligodendrocyte bound molecule that signals by binding to its receptor NgR, located on axonal membranes that interacts with its co-receptor Lingo-1. Nogo signalling is responsible for CNS myelin regulation and neurite outgrowth during neurodevelopment, and plasticity in the mature brain. This study investigated polymorphisms within the *Lingo-1* and *NgR* genes in schizophrenia; and examined the Lingo-1 and NgR protein levels within the human dorsolateral prefrontal cortex (DLPFC) in schizophrenia. **Methods:** Human matched case control DNA samples (n=268/group) from the Australian Schizophrenia Research Bank were purified and then genotyped to assess polymorphisms within the *Lingo-1* and *NgR* genes using restriction fragment length polymorphism and Multiplex MassARRAY genotyping assays. Human DLPFC matched case control samples (n=37/group) from the NSW Tissue Resource Centre were used to assess Lingo-1 and NgR protein levels by immunoblotting. **Results:** One genetic marker, *NgR* rs701427, had a significant association with schizophrenia ($p=0.02$). NgR protein expression was significantly decreased by 16% ($p<0.001$) and Lingo-1 protein expression was significantly increased by 12% ($p=0.006$) in the DLPFC of schizophrenia subjects. There was a significant correlation between NgR and Lingo-1 protein levels ($r=-0.276$, $p=0.017$). **Conclusion:** This study shows strong evidence for the proteogenomic involvement of NgR/Lingo-1 in schizophrenia. The *NgR* and *Lingo-1* gene mutations found to be associated with schizophrenia in this study may result in the dysregulated NgR and Lingo-1 protein expression. Further studies are required in order to investigate the implications of these genetic and proteomic alterations to the aetiology and symptomatology of schizophrenia.

ORAL-09-05

VITAMIN D DEFICIENCY ALTERS DOPAMINE AND GLUTAMATE METABOLISM IN NEONATE BRAIN TISSUE

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Purpose: Epidemiological evidence suggests that prenatal vitamin D deficiency is a risk factor for schizophrenia. We have shown that this is biologically plausible in rodents, in which transient prenatal vitamin D deficiency is associated with changes in brain structure and adult behaviour, although the mechanism remains to be elucidated. Thus, the aim of the present study was to describe the neurochemical profile of vitamin D deficiency in discrete brain regions in newborn rats. Methods: Whole litters of vitamin D-deficient (n=6) and control Sprague-Dawley female rats (n=6) were collected on the day of birth and brain tissue was rapidly dissected into multiple brain regions, including hippocampus, basal ganglia, frontal cortex and cerebellum. HPLC was used to assay tissue amino acid content; including glutamate, glutamine and GABA, and catecholamines, such as dopamine and noradrenaline. Results: There was a significant ($p<0.05$) increase in the levels of dopamine in the basal ganglia of DVD-deficient rats, while in hippocampus there were higher levels of noradrenaline and lower levels of glutamate and GABA. However, the most consistent change was seen in levels of glutamine, which were significantly reduced ($p<0.05$) by 10-15% in all brain regions examined. Conclusions: DVD-deficient rats show changes in glutamate and dopamine signalling from birth. These data suggest that the glutamate-glutamine cycle is altered and that this may underlie the alterations in dopamine and glutamate signalling previously described in this model. Taken together, these data suggest that vitamin D deficiency during gestation alters neurotransmitter systems relevant to schizophrenia.

ORAL-09-06

MOLECULAR VALIDATION OF THE ACUTE PHENCYCLIDINE RAT MODEL FOR SCHIZOPHRENIA: TRANSLATION OF CHANGES IN ENERGY METABOLISM AND IN NEUROTRANSMISSION

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Purpose: Administration of the non-competitive NMDA receptor antagonist phencyclidine (PCP) to rodents is widely used as preclinical model for schizophrenia. Most studies on this model employ methods characterizing behavior or brain abnormalities. However, little is known about the corresponding peripheral effects. **Methods**: In this study we analyzed changes in behavior, serum proteins and brain molecules after acute PCP treatment of rats. **Results**: Acute PCP treatment induced hyperlocomotion and stereotypic behavior, which mimic some aspects of positive symptoms of schizophrenia. Multiplex immunoassay profiling of serum revealed abnormalities as seen in first onset, antipsychotic naive schizophrenia patients. Also, a state similar to insulin resistance was detected. Schizophrenia-relevant alterations in neurotransmitter-related molecules were found in the hippocampus using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. **Conclusions**: These studies identified behavioral and molecular alterations in the acute PCP rat model which can be translated to human schizophrenia. It is anticipated that these could be used as surrogate markers in this model to facilitate discovery and development of novel drugs for treatment of certain aspects of schizophrenia.

ORAL-09-07

THE EFFECT OF INTRACEREBRAL NEUREGULIN-1 APPLICATION ON PCP-INDUCED HYPERLOCOMOTION - POTENTIAL IMPLICATIONS FOR SCHIZOPHRENIA

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Phencyclidine (PCP) has been shown to mimic schizophrenia characteristics in both humans and animal models. Antagonism of PCP-induced hyperlocomotion has been previously used as an indicator for the antipsychotic potential of a compound. **Purpose:** In the present study, we explored the potential of intraventricular (icv) application of Neuregulin-1, mutations of which have been associated with the development of schizophrenia, to suppress PCP-induced hyperlocomotion. **Methods:** For this, C57Bl/6 mice (n=18) received intraperitoneal treatment with either 3 mg/kg or 5 mg/kg of PCP with or without simultaneous icv application of 20 ng recombinant Neuregulin-1 (NRG1β1 EGF Domain). **Results:** PCP application at both doses induced significant hyperlocomotion as frequently shown before. Analysing preliminary data, we found icv Neuregulin-1 application to act in a dose dependant manner by significantly suppressing hyperlocomotion induced by 3 mg/kg ($p<0.05$) but not 5 mg/kg PCP. **Conclusion:** These results suggest that Neuregulin-1 might have antipsychotic properties. In upcoming experiments we will address the effect of Neuregulin-1 on locomotion and schizophrenia-related neurotransmission to further explore its antipsychotic potential.

ORAL-09-08

NEUREGULIN-1 AND ERBB4 PROTEIN EXPRESSION IN SCHIZOPHRENIA AND THEIR RELATIONSHIP WITH THE NMDA RECEPTOR

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Purpose: Neuregulin-1 (NRG1), ErbB4 and NMDA receptors (NMDAR) are strongly implicated in schizophrenia pathology. However it is unclear how these proteins are altered and interact in the schizophrenia brain. The aim of this study was to determine if NRG1 and ErbB4 proteins are altered in the schizophrenia brain and if they are associated with alterations in NMDAR binding density. ***Methods:*** The cohort consisted of samples from the hippocampus, superior temporal gyrus (STG) and posterior cingulate cortex (PCC) from 20 schizophrenia and 20 matched control subjects. NRG1 and ErbB4 protein levels were assessed using immuoblotting. NMDAR binding density was examined using receptor autoradiography. ***Results:*** No statistically significant differences were found in NRG1 or ErbB4 protein levels between schizophrenia subjects and controls in the brain regions examined. NRG1 and ErbB4 protein levels strongly correlated in the STG ($r=0.606$, $p<0.001$) and PCC ($r=0.561$, $p<0.001$), but not the hippocampus ($r=0.123$, $p=0.463$). Compared to controls, schizophrenia subjects displayed a 7% decrease in NMDAR binding density in the STG ($p=0.011$) but not in the PCC. NMDAR binding density did not correlate to NRG1 or ErbB4 in any brain region. ***Conclusions:*** These results suggest that NMDAR binding density, NRG1 and ErbB4 protein levels are not robustly altered across the schizophrenia brain. However we cannot exclude the possibility of alterations in function or in specific subtypes of schizophrenia. Our findings highlight the brain region specific nature of interactions between NRG1 and ErbB4. Further research is required to complete our understanding of the role these signalling pathways play in the aetiology of schizophrenia.

ORAL-10-01

RESTORING THE BALANCE BY SEEING RED: A TREATMENT STRATEGY FOLLOWING PARTIAL INJURY TO A CNS TRACT

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Purpose: Partial CNS injury is followed by secondary degenerative events that result in further functional losses. Here we assess factors that may contribute to progression of secondary degeneration *in vivo* and outcomes of long term treatment of secondary degeneration with 670nm light delivered by LED array. **Methods**: We used a partial transection model of secondary degeneration in rat optic nerve (ON) and assessed Ca ions, cytochrome *c* oxidase activity, axon structure, neuroprotection and visual function. **Results**: Using NanoSIMS we demonstrated regions of elevated Ca ions in normal ON *in vivo* that were rapidly decreased following partial ON transection ($p \leq 0.05$). An increasingly punctate distribution at 5 minutes and 24 hours after injury indicated redistribution to the cytosol. Early increases in node and paranode length ($p \leq 0.05$) as well as chronic (3 month) increases in the node/paranode ratio were consistent with our published observations of chronic myelin abnormalities. In an effort to restore the balance by limiting oxidative stress throughout the injury process, we have used long term transcranial irradiation of the injury site with 670nm light to increase cytochrome *c* oxidase activity. The treatment restored normal visual function, as assessed using optokinetic nystagmus at 3 months ($p \leq 0.05$) and there was a trend towards protection of retinal ganglion cell numbers. **Conclusion**: The results indicate that Ca ion release from intracellular stores, associated with oxidative stress, are early events in secondary degeneration. 670nm light offers a potential non-invasive therapy to limit spread of secondary degeneration after CNS injury.

ORAL-10-02

HISTONE METHYLATION IN SPINAL CORD DORSAL HORN OF SPHK2$^{-/-}$ MICE AND C57/BL6 MICE IN RESPONSE TO NERVE DAMAGE

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Purpose & Methods: Differences in pain susceptibility and the development of pathological pain states might be connected by common underlying mechanisms such as variations in the epigenetic regulation of nociceptive neurons. We compared the patterns of histone H3 proteins that are triple methylated on lysine 27 and 4 (H3K27me3, H3K4me3) in spinal cord between controls and mice 6 days after peripheral nerve damage (sciatic nerve transection, ScNT) in C57/Bl6 wild-type and sphingosine kinase 2 deficient (Sphk2$^{-/-}$) mice. H3K27me3 is a marker of repressed chromatin, H3K4me3 facilitates transcriptional activation. GFAP and NeuN were used to label astrocytes and neurons, TOPRO-3 was used as a nuclear stain. Positive staining was analysed using Image J. **Results:** The dorsal horn contained a higher number of H3K27me3 & H3K4me3 positive nuclei relative to other parts of the spinal cord. Most of the positive nuclei belonged to NeuN-positive neurons in laminae I and II. In C57/Bl6 mice (n = 4), nerve damage induced a significant increase in H3K27me3 (plus 42±32 %) & H3K4me3 (plus 18±8 %) labelled nuclei as indicated by comparing treated (ipsi-) and non-treated (contralateral) sides. In Sphk2$^{-/-}$ mice (n = 4), the average number of H3K4me3 nuclei in untreated contralateral sides were slightly higher (350±28/150.000µm^2) than in C57/Bl6 (325±30 /150.000 µm^2) but there was no difference between sides in response to nerve damage. **Conclusion:** Histone H3 is trimethylated at K27 and K4 in neurons of the spinal cord dorsal horn and the pattern of methylation changes in response to nerve damage. This epigenetic response might be dependent on the synthesis of S1P through sphingosine kinase 2.

ORAL-10-03

TREATMENT WITH 670 NM (RED) PHOTOBIOMODULATION FOLLOWING SCIATIC NERVE INJURY PROMOTES FUNCTIONAL IMPROVEMENTS ASSOCIATED WITH REDUCED MACROPHAGE RECRUITMENT

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Purpose: Photobiomodulation (PBM) as a light therapy has been shown to have beneficial effects in several different neuronal injury models, however the mechanism for these improvements remain to be elucidated. This study aimed to examine the effect of 670 nm PBM treatment following sciatic nerve crush. **Methods:** The sciatic functional index (SFI) was used to examine functional recovery following a 2 mm sciatic nerve crush, in the absence or presence of PBM treatment (3 min exposure, 9 J/cm^2). The light-treated (PBM-T) group received PBM from a LED array delivered once daily for recovery periods of 3, 7 or 21 days, and were compared to non-treated (NT) sibling matched male Wistar rats. ED-1 immunoreactivity was assessed to quantify macrophage numbers at the conclusion of each recovery period. **Results:** At 3 d post injury, the PBM-T group showed significant reductions of cells labelled with the macrophage marker ED-1 at the injury site (PBM-T: 20 ± 8 cells/mm^2, n=4; NT: 55 ± 9 cells/mm^2, n=5, p=0.047). The reduced number of macrophages preceded the functional improvements of the PBM-T group at 5 d (PBM-T SFI: -72 ± 3, n=5; NT SFI: -90 ± 3, n=4, p=0.001), which remained significantly elevated up to 21 d (PBM-T SFI: -18 ± 5; NT SFI: -58 ± 3, $p < 0.0001$) post injury. **Conclusion:** These data demonstrate that 670 nm PBM has a potent inhibitory effect on the recruitment of macrophages in response to peripheral nerve crush, which may contribute to functional improvements after PBM treatment.

ORAL-10-04

STRETCH SENSITIVITY OF MUSCLE SPINDLE AFFERENTS IS NOT INCREASED IN HUMAN CHRONIC SPINAL CORD INJURY

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Purpose: Complete motor paralysis associated with chronic spinal cord injury is associated with spasticity, but it is not known whether this is due to an increased sensitivity of the muscle spindles to stretch, brought about by an increase in fusimotor drive to the relaxed muscles, or solely to disturbances in excitability of the spinal motoneurones. To test the hypothesis that resting fusimotor drive and stretch sensitivity are elevated in spinal cord injury, single-unit recordings were made from 19 muscle spindle afferents located in the pretibial flexors. **Methods:** Tungsten microelectrodes were inserted into motor fascicles of the common peroneal nerve in six individuals with complete paralysis of the lower limbs following spinal cord injury: 12 afferents were spontaneously active at rest and 7 were recruited during stretch of the receptor-bearing muscle. For comparison, unitary recordings were made from 17 spontaneously active and 9 stretch-recruited afferents in 12 neurologically intact subjects. **Results:** Spontaneous firing rates in the spinal subjects (9.8 ± 1.6 Hz) were not significantly higher than those recorded in the intact subjects (10.2 ± 1.3 Hz); the same was true for discharge variability at rest (8.1 ± 2.0 % vs 5.7 ± 0.9 %). Moreover, neither the peak firing rate (53.4 ± 13.6 % vs 63.5 ± 21.5 Hz) nor dynamic index (74.6 ± 6.6 % vs 70.2 ± 8.2 %) during muscle stretch were significantly higher in the spinal than in the intact subjects. **Conclusions:** It is concluded that the static and dynamic stretch sensitivity of human muscle spindles is not affected by chronic spinal cord injury, indicating that there is no difference in fusimotor drive following spinal cord injury. Furthermore, that mean firing rates and discharge variability at rest were not *lower* in the spinal than in the intact subjects supports the conclusion that there is negligible resting fusimotor drive to muscle spindles in relaxed muscles in intact subjects.

ORAL-10-05

DEFICIENCY IN THE COMPLEMENT ANAPHYLATOXIN RECEPTOR C3AR WORSENS THE OUTCOME FROM SPINAL CORD INJURY IN MICE

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Purpose: Cell death and axonal degeneration at sites of spinal cord injury (SCI) are inevitably linked to neuroinflammation. Resulting activation of the complement system is thought to be a major contributing factor to the development of secondary pathology after SCI. The precise contributions of the many individual components of the complement system remain, however, largely ambiguous. Here, we explored the role of the complement anaphylatoxin receptor C3aR in intraspinal inflammation and functional recovery following SCI in mice. **Methods:** Wild-type (WT) and C3aR$^{-/-}$ mice on a C57BL6/J background were subjected to moderate-severe contusive SCI. Open-field locomotor scoring was used to monitor functional recovery up to 35 days post-injury. Magnetic resonance imaging (MRI), together with standard histological and immunofluorescent staining procedures, was used to compare histopathology and lesion size between genotypes. Bone marrow (BM) chimeric mice were also employed to investigate the consequences of conditional C3aR deficiency on the outcome from SCI. **Results:** C3aR$^{-/-}$ mice showed significantly worsened recovery from SCI compared to their wild-type counterparts as evidenced by consistently poorer locomotor performance ($p<0.05$). These findings were paralleled by significantly greater lesion volumes as determined by MRI, reduced white matter sparing, and increased presence of Ly6B.2+ inflammatory infiltrate ($p<0.05$). The functional phenotype of C3aR-deficiency was rescued in [WT > C3aR$^{-/-}$] but not [C3aR$^{-/-}$ > WT] BM chimeric mice ($p<0.05$), indicating that the observed effect was largely mediated via altered leukocyte function. **Conclusion:** C3aR deficiency worsens the outcome from SCI at a functional and histopathological level. These findings demonstrate that signaling through this receptor serves a putative reparative role and that selective agonism may provide a novel treatment option to improve SCI outcomes.

ORAL-10-06

THE EFFECT OF AN NK1 RECEPTOR ANTAGONIST FOLLOWING SPINAL CORD INJURY

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Purpose: Following severe spinal cord injury (SCI), secondary injury processes contribute to tissue damage. Of particular importance is blood spinal cord barrier (BSCB) disruption, resulting in development of vasogenic oedema and increased intrathecal pressure. Previous studies have shown that the neuropeptide substance P (SP) causes barrier disruption following brain injury, and that inhibition of SP via its NK1 receptor attenuates vascular permeability and oedema formation. Such studies have not been performed in SCI. Moreover, it is important that experimental models be used that replicate a closed environment to ensure clinical relevance. Balloon compression SCI is such a model. Accordingly, this study characterised BSCB permeability and oedema after ballon compression SCI, and the effects of an NK1 receptor antagonist (N-acetyl- L-tryptophan) on both parameters. **Methods:** SCI was induced in anesthetised, adult male, New Zealand white rabbits and animals administered 2.5 mg/kg NAT or saline post-injury. Subgroups of animals were assessed for BSCB permeability (Evan's Blue, n=15), and oedema (wet weight/dry weight, n=21). **Results:** BSCB permeability was significantly increased at the injury epicentre in all injury groups compared to shams ($p<0.001$). There was no significant difference between vehicle and NAT treated animals. Spinal cord oedema at the injury epicentre was significantly increased post-injury at all time points ($p<0.001$). No significant difference was observed between NAT and vehicle treated animals. **Conclusion:** This study demonstrates that the balloon compression model of SCI produces significant BSCB disruption and oedema development, and thus is a strong candidate for future studies. Given that NAT does not attenuate BSCB permeability or oedema genesis, the role of SP following traumatic SCI requires further investigation.

ORAL-10-07

DEFICIENCY IN THE CHEMOKINE RECEPTOR CX_3CR1 DOES NOT ALTER THE OUTCOME FOLLOWING MODERATE-SEVERE SPINAL CORD INJURY IN MICE

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Purpose: Activated microglia and blood-borne macrophages are thought to play key roles in inflammation and associated secondary neuropathology following spinal cord injury (SCI). The chemokine receptor CX_3CR1 is thought to be a critical regulator of macrophage activation profiles. We therefore set out to examine the effect of CX_3CR1 deficiency on the outcome from SCI. **Methods:** Female C57BL6/J (n=9) and $Cx_3cr1^{gfp/gfp}$ mice (n=12) were subjected to a moderate-severe contusive SCI and let to recover for 35-42 days. Locomotor performance, sensory function and lesion volumes were contrasted between genotypes. **Results:** We did not find evidence for altered recovery of $Cx_3cr1^{gfp/gfp}$ mice after SCI, using both the Basso Mouse Scale (BMS) for locomotion as well as tapered ledged beam-walking. All experimental mice displayed hyposensitivity compared to uninjured controls ($p<0.001$), but again no differences were observed between genotypes. These behavioural findings were in line with high-resolution MRI analysis, which showed no difference in lesion volumes between genotypes. Finally, bone marrow (BM) chimeric mice were created to better discern the importance of CX_3CR1 expression on circulating monocytes and their progeny in response to SCI. [$Cx_3cr1^{gfp/+}$>WT] and [$Cx_3cr1^{gfp/gfp}$>WT] BM chimeras showed significantly worsened recovery compared to their [WT>WT] counterparts, starting at day 14 post-injury ($p<0.05$; n=10 per experimental condition). **Conclusion:** These findings suggest a critical role for CX_3CR1 expression on circulating monocytes and their progeny, blood-borne macrophages, in the outcome from SCI reduced whilst some compensatory mechanisms appeared to have developed in Cx_3cr1^{gfp} knock-in mice that conceal this effect.

ORAL-10-08

HOW DO THE BIOMECHANICS OF SPINAL LOADING AFFECT SPINAL CORD INJURY SEVERITY?

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Purpose: Spinal cord injury (SCI) is a rare occurrence but can have devastating consequences. Specifically, vertebral fracture-dislocation is one of the commonest mechanisms of SCI in adults, but the interactions between the spine and spinal cord and the effects of spinal loading conditions on spinal cord injury severity are not well understood. The aim of this study is to investigate the effects of different spinal loading conditions (speed and displacement) on the severity of SCI using a rodent model of vertebral dislocation. **Methods:** Thoracolumbar vertebral dislocation was induced in anaesthetised adult rats (~250g, N=60). The twelfth thoracic vertebra (T12) was secured while the first lumbar vertebra (L1) was dislocated laterally at a known speed and displacement. Speed of dislocation was varied between 100mm/s and 250mm/s and the displacement between 4mm and 10mm. These parameters were chosen to give a range of severities from no injury to high injury severity based on previous histological studies using our model. Animals survived for 5 hours under anaesthesia and were then euthanised. Spinal cord sections were stained to detect haemorrhage (haematoxylin and eosin) and axonal damage (β-amyloid precursor protein) as markers of acute SCI severity. Data were analysed using linear regression modeling with maximum speed and maximum vertebral displacement as predictor variables. **Results:** Data analysis shows that magnitude of vertebral dislocation is a significant predictor of haemorrhage volume ($p=0.008$), while speed of dislocation is not ($p=0.755$) and that neither speed nor magnitude of dislocation are significant predictors of axonal injury ($p=0.781$, $p=0.84$ respectively). **Conclusion:** Magnitude of vertebral dislocation is a significant predictor of acute SCI severity. This suggests that when developing strategies for SCI prevention, limiting spinal motion is an important factor to consider.

ORAL-11-01

DEVELOPMENT OF A STEM CELL-BASED STRATEGY FOR PARKINSON'S DISEASE

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Purpose: Stem cells are seen as a promising alternative to foetal tissue as a source of dopamine progenitors for use in transplantation procedures for Parkinson's disease patients. Although there has been early success in this field in the directed differentiation of therapeutic cell types, such as dopamine neurons, *in vitro*, the translation of this to an *in vivo* setting has been problematic. One serious issue has been the incidence of graft-derived tumours due to the incomplete nature of differentiation procedures. **Methods**: Flow cytometry represents a promising strategy for standardizing cell preparations generated from stem cells as well as eliminating unwanted, and potentially dangerous cell-types, prior to transplantation. We have recently completed a gene-array study that identified a number of novel genes encoding transmembrane proteins expressed selectively on dopamine neuron progenitors. In order to test the utility of these targets in cell-sorting procedures, we have used antibodies recognising extracellular domains of these proteins to isolate dopamine progenitors from foetal midbrain by fluorescence activated cell sorting for subsequent transplantation in an animal model of Parkinson's disease. **Results**: We have identified the cell-adhesion molecule *activated leucocyte cell adhesion molecule* (ALCAM) as a promising target for selective isolation of dopamine neuron progenitors from mixed cell populations. Grafts established from the ALCAM-expressing fraction of embryonic midbrain where enriched for dopamine neurons (relative to unsorted controls) and corrected amphetamine-induced turning behaviour. Grafts from the ALCAM-negative fraction did not contain dopamine neurons or correct turning behaviour. **Conclusion**: This study shows that immunological targeting of uniquely expressed transmembrane proteins is a promising strategy for standardising cell preparations used in neural transplantation procedures. ALCAM may be a useful target for isolating progenitors for dopamine neurons from mixed cell populations generated from partially differentiated stem cells. .

ORAL-11-02

A ROLE FOR GAP JUNCTIONS IN NEURODEGENERATION IN THE MONOAMINERGIC NUCLEI IN PARKINSON'S DISEASE

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Purpose: To establish the role of Connexin43 gap junctions in chronic inflammation in the monoaminergic nuclei in Parkinson's disease (PD). **Aim:** To investigate expression of Connexin43 in the Raphe, locus coeruleus and substantia nigra in control and PD brains. **Methods:** Immunohistochemistry was performed on adjacent, fixed frozen 50µm sections from five PD and five control brains. Antibodies against GFAP and Iba1 identified astrocytes and microglia and von Willebrand factor labelled endothelial cells. Sections were double labelled for Connexin43 and ImageJ software used to analyse confocal images. To investigate differences in protein levels in the three brain regions, a generalised linear mixed model was fitted to the means of the proportions within each region. **Results:** There was evidence for a difference in Iba1 expression overall between normal and PD brains ($p=0.03$) and specifically within the substantia nigra ($p=0.07$), but there was no difference in levels of GFAP across all three regions. There was evidence for increased Connexin43 expression in PD over normal ($p=0.08$), greatest in the locus coeruleus, but this did not correlate closely with changes in Iba1. Connexin43 co-localised with blood vessels, and significant loss of small vessels was seen in all three regions ($p<0.05$). **Conclusions:** Microglia in the substantia nigra may be important in chronic inflammation characteristic of PD but this is not necessarily Connexin43 related. However, it is known that Cx43 is upregulated in endothelial cells after CNS injury leading to blood-brain barrier permeability and endothelial cell loss. The results here show loss of microvascular in all monoaminergic nuclei in PD which may be mediated by increased Connexin43 expression.

ORAL-11-03

PARKINSON'S DISEASE ALTERS SPHINGOLIPID COMPOSITION IN THE ANTERIOR CINGULATE AND OCCIPITAL CORTICES

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Purpose: Only limited studies have undertaken lipidomic analysis of human Parkinson's disease (PD) brain regions. Changes in sphingolipid metabolism may be important in Lewy body disorders. The aim of the present study was to explore changes in brain lipid composition in PD. **Methods:** Lipids were extracted from frozen anterior cingulate (ACC; contains Lewy bodies) and occipital cortex (OCC; no Lewy bodies present) from PD patients (n=9) and age-matched controls (n=10) with approval by the Sydney Brain Bank and NSW Tissue Resource Centre. Lipid species (including ceramide and sphingomyelin) were analysed using electrospray ionisation mass spectrometry (ESI-MS) and quantified using appropriate internal standards. **Results:** A total of 24 sphingomyelin and 16 ceramide species were identified in both ACC and OCC. The amount of most sphingomyelin and ceramide species (nmol/g tissue) was significantly decreased in PD cases for ACC (19 and 14 species respectively), with similar trends in the OCC (2 and 1 species respectively were significant only). In the ACC only, fatty acyl chain length of ceramide and sphingomyelin species was also affected in PD, with a decrease in species with shorter chain length (≤20-carbon) and corresponding increase in species with longer chain length (>20-carbon) when compared to control subjects. **Conclusion:** Significant reductions in ceramide and sphingomyelin levels are evident in the ACC of PD brains, a region known to accumulate Lewy body-related pathologies, providing further evidence for the importance of sphingolipid metabolism in PD. Reductions in ceramide levels may be related to a loss of functional glucocerebrosidase, with glucocerebrosidase gene mutations considered the greatest genetic risk factor for PD.

ORAL-11-04

TYPE 1 INTERFERON SIGNALLING: DOES IT PLAY A ROLE IN PARKINSON'S DISEASE?

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Purpose: Parkinson's disease (PD) is the second most common neurodegenerative disorder in Australia, however current therapies are ineffective in slowing disease progression. Neuroinflammation has recently been proposed as contributing to the dopaminergic cell death observed in PD. Key players in the neuroinflammatory cascade are the type-I Interferons (IFNs), however the role these cytokines play in PD has not been explored. We propose that type I-IFNs contribute to the progression and exacerbation of neuronal cell death in PD. Methods: This study investigated the implications of reduced type-I IFN signalling in response to PD-associated environmental toxins. Human BE(2)M17 neuroblastoma (M17) cells stably expressing an Interferon Receptor-1 (IFNAR1) or scrambled negative control (NC) shRNA vector were generated. Parental M17, IFNAR1 or NC knockdown M17 cells were treated with environmental neurotoxins, rotenone or 6-Hydroxydopamine (6OHDA) and cells were harvested at time intervals (0, 2, 4, 8, 24 and/or 48 hours) for cell viability (MTT assay), western blot analysis or quantitative RT-PCR (QPCR). Results: MTT assay confirmed rotenone (10nM-1µM) and 6OHDA (10µM-150µM) induced cell death in M17 parental cells after 24 hours in a dose-dependent manner. This increased cell death was correlated with an upregulation in STAT-3 phosphorylation by western blot analysis and increased expression of IFNα and IFNβ by QPCR, confirming activation of the type-1 IFN signalling pathway. Significantly, IFNAR1 knockdown M17 cells treated with 6OHDA (50µM) displayed increased survival compared to NC M17 cells (89.77±2.77% versus 66.56± 4.21%) ($n=6$, $P<0.05$). A protective effect of reduced type-1 IFN signalling was also confirmed with rotenone (500nM) (83.73±4.20% versus 66.19±2.91% ($n=5$, $P<0.05$)). Conclusion: These results implicate type-I IFN signalling in the cellular response to rotenone and 6OHDA. Although the role of type-1 IFN signalling needs to be further investigated, therapeutics targeting the IFNAR1 subunit may be beneficial in reducing neuroinflammation and therefore neuronal cell death in PD.

ORAL-11-05

ASSOCIATIONS BETWEEN *GCH1* POLYMORPHISMS AND PRIMARY DYSTONIA AND PARKINSON'S DISEASE IN AN AUSTRALIAN CASE-CONTROL SERIES

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Purpose: Genes involved in familial dystonia syndromes are ideal candidates for investigating whether common genetic variants influence the susceptibility to sporadic primary dystonia. Mutations in the GCH1 gene are responsible for the inherited, Dopa-responsive dystonia, (DYT5 dystonia). *GCH1* encodes an enzyme that regulates tetrahydrobiopterin production, required for the synthesis of L-Dopa. As this essentially regulates dopamine production, variability in *GCH1* may also influence the PD susceptibility. We employed a haplotype-tagging strategy to assess if common variation in *GCH1* confers risk for primary dystonia and PD. **Methods:** Primary dystonia patients (n=230), PD patients (n=226) and healthy controls (n=228) were genotyped for 11 single nucleotide polymorphisms (SNPs) in *GCH1*. Genotype associations were calculated using logistic regression with adjustment for age and gender. **Results:** Modest associations between three SNPs and primary dystonia were observed – rs10483639, rs12147422 and rs3759664 ($P<0.01$). Associations between two of these SNPs and PD were also observed – rs10483639 and ($P<0.05$). **Conclusions:** Our study suggests that common polymorphisms in *GCH1* influence the susceptibility to primary dystonia and PD. Interestingly, rs10483639 and rs3759664 are highly correlated with a haplotype associated with reductions in *GCH1* expression and function. This potentially highlights a biological pathway perturbed in both primary dystonia and PD.

ORAL-11-06

GENERATION OF NOVEL PARKINSON'S DISEASE MODELS

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Purpose: Mutations in *PARK2* (*parkin*) can result in Parkinson's disease (PD). Parkin shares a small bidirectional promoter with a poorly characterised gene called *PArkin Co-Regulated Gene* (*PACRG*). We hypothesise that the function of PACRG is coupled to parkin by its co-regulation through the shared bidirectional promoter. **Methods:** We investigated the co-regulation of *Parkin* and *PACRG* by quantitative PCR analysis of human brain tissue. We also generated two novel knockout mice lines to investigate gene function. The first model is a knockout of both *parkin* and *Pacrg*, while the second model is a knockout of *Pacrg* alone. **Results:** 1. cDNA was generated from 22 discrete regions of the human brain including substantia nigra, and the expression of *parkin* and *PACRG* was determined by qRT-PCR. Co-incident expression of the two genes was observed in the majority of regions analysed, confirming for the first time the coexpression of the two genes in human brain. 2. Successful generation of two knockout strains was confirmed by molecular characterisation of gene and protein expression. At a gross level the mice did not display evidence of an overt molecular or behavioural phenotype. Neuropathological analysis suggested a decrease in the volume of the midbrain and an increase in tyrosine hydroxylase positive neuron number in the locus coeruleus. **Conclusion:** We have shown for the first time that *parkin* and *PACRG* are coexpressed in the human brain, including within nuclei that are important in the pathogenesis of PD. We have generated novel mouse lines with alterations in the structure and function of neuronal populations associated with the human disorder. Further characterisation of these models will delineate the functional significance of the co-regulation of *parkin* and *PACRG*.

ORAL-11-07

ADULT CORTICAL NEURONAL CULTURE TO MODEL AGE-DEPENDENT SUSCEPTIBILITY OF PARKINSON'S DISEASE

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The most robust determinant for Parkinson's disease is ageing. People, even carrying potent α-synuclein mutation, will not get the disease till after 40 years of age. **Purpose:** To understand the time-dependent pathogenesis at cellular level, we have established a primary neuronal culture of adult rats ranging from 7 to 18 month of age to examine the toxicities of α-synuclein and other Parkinson's disease relevant factors. **Methods:** In this study, we investigated the dose responses of adult neurons to various concentrations of α-synuclein fibrils. After 8 days in culture, varying concentrations (0.89μM, 3.78 μM and 7.69 μM) of α-synuclein fibrils were applied to the primary neurons for 24 h, monitored by propidium iodide and DAPI fluorescence staining. **Results:** We observed apoptotic profiles cultures treated with all three concentrations and their frequency corresponded to α-synuclein fibril concentrations used in dosage-dependent manner. **Conclusions:** Our result provided the evidence α-synuclein fibrils are toxic to primary adult neurons. **Keywords:** Parkinson's disease; α-synuclein; neuron; neurotoxicity; propidium iodide.

ORAL-11-08

THE NEURODEGENERATIVE LYSOSOMAL STORAGE DISORDER MPS IIIA (SANFILIPPO SYNDROME) IS A SYNUCLEINOPATHY

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Purpose: To characterise the spatiotemporal accumulation of α-synuclein-positive inclusions in a mouse with the neurodegenerative lysosomal storage disorder Mucopolysaccharidosis type IIIA (MPS IIIA; Sanfilippo syndrome). **Background:** MPS IIIA results from a mutation in the gene encoding the lysosomal enzyme sulphamidase. As a consequence, heparan sulphate accumulates inside lysosomes, accompanied by secondarily-accumulating glycolipids. Neuroinflammation, impaired macro-autophagy and ubiquitin-positive aggregates are observed. At present, the neurobiological basis for symptoms in MPS IIIA (cognitive regression, mild motor deficits) is unknown. Examination of human post-mortem tissues from children with Sanfilippo syndrome reveals phosphorylated α-synuclein-positive aggregates in the brain (Hamano et al. [2008] Acta Neuropathol. 115: 547-559), which sometimes co-localise with ubiquitin-positive structures. **Methods:** We have batch-stained tissues derived from 3- to 30 week-old MPS IIIA/unaffected mice (n=3-5/group), with antibodies to α-synuclein and ubiquitin. The distribution of immuno-positive lesions was mapped and co-localisation of the markers examined using confocal microscopy. **Results:** α-synuclein-positive lesions are present by 3-weeks of age (pre-symptomatically) in corpus callosum, the colliculi and brainstem of MPS IIIA mice. Increased numbers of aggregates are found in these structures with age. Other brain regions that exhibit α-synuclein-positive aggregates with time include the hippocampus, cerebral cortex, basal forebrain cholinergic structures, thalamus and striatum. Ubiquitin-positive aggregates were found in many, but not all of these regions over a similar, but not exact, timeframe. The two markers co-localised infrequently. **Conclusion:** The spatio-temporal distribution of α-synuclein-positive aggregates resembles the Braak staging for Parkinsons disease. The impact of the inclusions on cell function is unknown, however aberrant α-synuclein turnover may contribute to synaptic dysfunction, and may underlie the clinical changes observed.

ORAL-12-01

IDENTIFICATION AND CHARACTERISATION OF NOVEL GUIDANCE MOLECULES INVOLVED IN DEVELOPMENT OF THE NIGROSTRIATAL DOPAMINE PATHWAY

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Purpose: Target-directed connectivity of developing neurons is a fundamentally important feature of normal brain development. Unique combinations of surface molecules allow multitudes of distinct neuronal subtypes to navigate specific pathways to reach their target. While these molecules have been relatively well characterised in some systems, e.g. the cortico-thalamic and retino-tectal pathways, the key players in other pathways, including the dopamine pathway, remain poorly defined. **Methods:** In order to identify new genes involved in axonal growth and guidance in the dopamine nigro-striatal pathway, we used a combination of transgenic reporter mice and FACS to isolate progenitor dopamine neurons for microarray analysis (n=12). In addition to genes from many classic axonal guidance families, we identified a number of genes corresponding to cell-adhesion molecules. This lead us to explore the idea that developing midbrain dopamine neurons use cell-adhesion mediated mechanisms to reach their striatal target in the forebrain. **Results:** Immunohistochemical characterisation of developing and adult mouse brain revealed that the adhesion molecule activated leucocyte cell adhesion molecule (Alcam) is expressed initially in dopamine progenitors in the embryonic ventral mesencephalon, but is subsequently down-regulated and instead expressed in target striatal neurons. During the late stage of axon extension, ascending dopamine neuron axons are in close apposition to descending Alcam positive striatal axons, appearing to wind around the descending fiber bundles. This is consistent with a model where striatal neurons guide their dopaminergic synaptic-partners to a striatal target through a cell-adhesion mediated mechanism. **Conclusion:** Contact mediated axon guidance may act in addition to traditionally recognized soluble guidance cues to fine tune guidance during the final phase of axon extension in the development of the nigro-striatal dopamine pathway.

ORAL-12-02

QUANTIFICATION OF GROWTH CONE MORPHOLOGY USING PRINCIPAL COMPONENT ANALYSIS

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Purpose: Growth cone guidance by molecular gradients plays a crucial role in wiring up the developing nervous system. Growth cones have a complex morphology that is dependent on their local environment, and analysis of their morphology has the potential to reveal important information about the molecular mechanisms underlying axon guidance by molecular gradients. However, there has been little quantitative analysis of how this morphology depends on external gradient conditions. **Methods:** Here we first performed growth cone turning (pipette) assays using the response of rat P2 superior cervical ganglion axons to gradients of nerve growth factor as a model system. We systematically varied the concentration of NGF in the pipette to map the chemotactic sensitivity of both the attractive response, and of the repulsive response when intracellular Protein Kinase A was inhibited by KT5720. Time-lapse movies of these experiments (191 movies, 50-70 min duration, sampling rate = 1 frame/min) were analysed using a customised semi-automatic image segmentation program so as to determine the growth cone outlines from each movie frame. **Results:** From the population of growth cone outlines (11272 movie frames), we used Principal Component Analysis to extract the growth cone eigenshapes, i.e. the axes of shape space that reproduce the greatest variance of the growth cone population. This analysis reveals correlations between certain shape modes, particularly the growth-cone left/right asymmetry, and the experimentally observed chemotactic response.

ORAL-12-03

A MATHEMATICAL MODEL OF THE ROLE OF CALCIUM AND cAMP IN AXON GUIDANCE

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The ability of growing axons to locate appropriate targets is an essential part in the formation of correct circuits during neural development. This is facilitated in part by gradients of guidance cues, which can be either attractive or repulsive. However guidance cues for attraction can switch to promote repulsion and vice versa depending on levels of cAMP and calcium. Previously the mechanisms involved in modulating this switch have only been discussed qualitatively, and the interacting roles of cAMP and calcium have been unclear. Here we provide a mathematical model that is able to quantify the relationship between cAMP, calcium and axon turning, based on a transduction pathway previously used to model LTP and LTD. Besides reproducing a wide range of previous experimental data the model makes new predictions which we confirmed using the growth cone turning assay. One novel discovery is that, under certain calcium conditions, reducing cAMP promotes attraction. Overall the mathematical model provides a unifying explanation for many previous observations in axon guidance, and allows predictions regarding axon turning under a wide range of conditions.

ORAL-12-04

TEN_M3: ROLES IN THE DEVELOPMENT OF THE MOUSE THALAMOSTRIATAL SYSTEM

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Purpose: Ten_m3 is a transmembrane glycoprotein which regulates cell adhesion and axonal guidance. Within the thalamostriatal system, Ten_m3 is expressed in the parafascicular nucleus (PFN), and we show that it is expressed within a subregion of the striatal matrix compartment, a major target of PFN projections. We investigated a role for Ten_m3 in the guidance and targeting of these axons using tracer injections in adult mice. **Methods/Results:** Anterograde tracing using biotinylated-dextran amine into the PFN showed that thalamostriatal terminals filled a significantly larger striatal area in KOs than in WTs (KO: 54.26±5.28 (mean±SEM), n=7; WT: 25.24±6.23, n=9; p=0.026, t-test). Additionally, preliminary retrograde tracing experiments using two rostrocaudally isolated injections of fluorescently-tagged cholera-toxin subunit B into the striatum suggest a greater degree of overlap in the position of back-labeled PFN cells projecting to caudal and rostral striatum in KOs compared to WTs. This altered wiring pattern in adult KOs was further investigated in early development, when Ten_m3 expression is highest. Experiments using DiI crystals in fixed early postnatal brains suggest that thalamostriatal axons in KOs show less fasciculation, suggesting a mechanism for the wiring deficits observed in these animals. Further to this, immunohistochemistry for choline acetyltransferase (ChAT), labeling a postsynaptic target of these projections, the cholinergic interneurons, showed a redistribution of ChAT-positive interneurons in KOs such that the ratio of cells in the dorsomedial quadrant was significantly higher, compared to WTs (KO: 0.322±0.012 (mean±SEM), n=9; WT: 0.283±0.007, n=6; p=0.016, t-test). **Conclusion:** Together, these data suggest a complex role for Ten_m3 in the guidance and targeting of thalamostriatal afferents and distribution of a subset of striatal interneurons.

ORAL-12-05

FGF8 SIGNALLING REGULATES FORMATION OF THE CORPUS CALLOSUM AND HIPPOCAMPAL COMMISSURE

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The corpus callosum forms the principle connection between the cerebral hemispheres. Agenesis of the corpus callosum (AgCC) occurs in ≥50 congenital syndromes, including an autosomal dominant mutation in the transmembrane protein Fibroblast growth factor (FGF) receptor 2, known as Apert syndrome. **Purpose:** To elucidate the role of Fgf8 (an Fgfr2 ligand) in development of the corpus callosum and hippocampal commissure in a mouse model. **Methods:** Fgf8 and Fgfr expression were analysed with *in situ* hybridisation, immunohistochemistry and quantitative PCR. The guidance potential of Fgf8 was assessed with an *in vitro* guidance assay. Fgf8 conditional knockout mice were phenotyped with immunohistochemistry. **Results:** Fgf8 is ideally expressed *in vivo* to provide attractive axonal guidance for pioneering axons of the developing corpus callosum and hippocampal commissure (n ≥ 3). Concomitantly, pioneering axons of the corpus callosum and hippocampal commissure express Fgfr2, indicating competance to transduce Fgf8 signalling (n ≥ 3). We therefore tested whether Fgf8 can function as a chemoattractant for callosal and hippocampal axons. Recombinant Fgf8 induced significant attractive guidance ($p \leq 0.05$, Student's t-test) of both pioneering axons of the cingulate cortex (n = 51, control explants; n = 59, FGF8 explants) and hippocampus (n = 58, control explants; n = 55, FGF8 explants). Lastly, we have investigated the effects of Fgf8 knockdown *in vivo*. Co-ordinately, conditional knockdown of Fgf8 in *Fgf8$^{flox/flox}$;Nestin^{Cre}* mice disrupts pathfinding of the corpus callosum and hippocampal commissure (n ≥ 3). **Conclusion:** These findings suggest that Fgf8 regulates axonal guidance of the corpus callosum and hippocampal commissure, potentially through Fgfr2 signalling. Importantly, these findings are of clinical significance to the aetiology of AgCC in Apert syndrome.

ORAL-12-06

MYCBP2 CONTROLS GUIDANCE OF ROBO2 AXONS DURING DEVELOPMENT OF THE MURINE OLFACTORY SYSTEM

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ROBO and SLIT molecules are crucial axon guidance factors during the development and regeneration of the central nervous system. However, the molecular interactions that these molecules take part in to shape the axon tracts in the CNS remain largely unknown. MYCBP2 is a strongly conserved E3-ubiquitin ligase that regulates axon and synapse development through interactions with multiple signaling pathways. Here we describe how a subpopulation of ROBO2 expressing olfactory sensory neurons is severely misguided along the dorsoventral axis of the olfactory bulbs in *Mycbp2* loss of function mice, in a pattern strikingly reminiscent of that described for *Slit1* and *Robo2* mutant mice. We observed a significant loss of innervation in a large dorsal domain in the olfactory bulb. In addition, we showed that these dorsal ROBO2 expressing neurons do not die, but instead appear to stall in the ventral outer nerve layer, where they fail to refasciculate with homotypic dorsally targeting axons. In addition, we uncovered strong genetic interactions between *Mycbp2* and *Robo2* in double mutant mice. Alltogether, these data suggest that *Mycbp2* controls the guidance of ROBO2 expressing neurons during development of the olfactory system and provide important new insights into the genetic cascades that regulate axon guidance processes in the CNS controlled by classic guidance factors ROBO and SLIT.

ORAL-12-07

TEN-M3 INTERACTS WITH ZIC2 AND MEMBERS OF THE EPH FAMILY TO REGULATE VISUAL PATTERNING

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Purpose: Ten-m3 is a transmembrane glycoprotein which regulates guidance of ipsilateral retinal axons. The aim of this project is to determine the mechanisms by which Ten-m3 acts and to identify interacting molecules. **Methods:** The sequence encoding the intracellular domain of Ten-m3 (Ten-m3-IC) was fused to a glutathione-S-transferase (GST) moiety and a pull down assay was performed followed by Western blotting for candidate binding partners. Additionally, the expression levels of candidate genes in the retina, superior colliculus (SC), dorsal lateral geniculate nucleus (dLGN) and primary visual cortex (V1) of wildtype (WT) and Ten-m3 knockout (KO) mice were measured using quantitative real time polymerase chain reaction (qPCR) and in situ hybridisation. **Results:** The GST pull-down revealed the presence of a 60kDa Zic2 positive band in the GST-Ten-m3-IC lane which was absent from control lanes. In contrast, no evidence for a direct interaction was found between Ten-m3-IC and Zic4 or EphB1. Results of qPCR showed that Zic2 is significantly upregulated in SC (1.3 fold, $p=0.001$) and retina (2.3 fold, $p<0.001$) of Ten-m3 KOs. EphB1 also showed an approximately 2-fold upregulation in retina, SC, dLGN and V1 ($p<0.05$). Expression of EphA7 was significantly downregulated at all levels of the visual pathway ($p<0.05$). Changes in EphA7 expression were also seen by in situ hybridisation. **Conclusion:** These results identify the Zic2-EphB1 signaling pathway as a downstream target of Ten-m3. Further, Ten-m3 regulates Epha7 transcription. Together, these results indicate that Ten-m3 interacts with a number of key regulators of visual development to mediate the formation of functional binocular circuits.

ORAL-12-08

HETEROGENEOUS EXPRESSION OF GROWTH-ASSOCIATED PROTEINS IN REGENERATING ADULT RETINAL GANGLION CELLS

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Purpose: The rat visual system is frequently used to study the mechanisms associated with regenerative responses in adult CNS. Here we examined and compared the expression of a panel of key components of various cell signalling pathways in regenerating versus viable, non-regenerating retinal ganglion cells (RGCs). **Methods:** In anaesthetized (ketamine/xylazine) 8-10 week old Fischer (F344) rats, the left optic nerve (ON) was cut and a segment of autologous peripheral nerve (PN) grafted onto the cut end. Four and 11 days after surgery, the grafted eye was injected with saline (control, n=7) or ciliary neurotrophic factor (CNTF) and a cell permeant cAMP analogue chlorphenylthio-cAMP (CPT-cAMP) (n=18). Surviving RGCs were visualized using anti-βIII-tubulin antibody, and regenerating RGCs identified by injecting fluorogold (FG, n=14) or FG and fast blue (n=11) into PN grafts 4 weeks after surgery. Retinal sections were also immunostained with antibodies to phospho-Akt, phospho-CREB, arginase-1, phosphorylated ribosomal protein S6 (p-S6), the neurotrophin receptor TrkB, growth associated protein GAP-43, the immediate early gene c-Jun, Bax, Brn3a or the anti-apoptotic protein Bcl-2. **Results:** There was increased RGC survival and axonal regeneration in eyes injected with CNTF and CPT-cAMP, associated with increased expression of c-jun and GAP-43. The majority of regenerating RGCs expressed these markers, however there was variability in expression. Some regenerating RGCs were not immunoreactive for c-jun, others negative for GAP-43, while some surviving but not regenerating RGCs expressed high levels of these proteins. Similarly, there was increased expression of p-S6 although only 30-40% of regenerating RGCs were immunoreactive, often the larger cells. Expression of other growth-associated proteins was seen in both surviving and regrowing RGCs, more frequently in the latter, but again expression was heterogeneous. **Conclusion:** The results suggest that individual adult RGCs may harness more than one potential growth promoting signalling pathway when initiating a regenerative response.

ORAL-13-01

BDNF AND TRKB MRNAS ARE MODULATED BY ANDROGENS IN THE ADOLESCENT MALE RAT MIDBRAIN

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Positive symptoms of schizophrenia (hallucinations, delusions) result from increased subcortical dopamine but mechanisms underlying increased dopamine neurotransmission in schizophrenia are uncertain. Males have greater symptom severity, earlier (typically adolescent) onset and a higher rate of schizophrenia than females. We found that testosterone (T) increases dopamine synthesis in the adolescent male brain and we hypothesize that increased T in adolescent males increases susceptiblity to schizophrenia by altering growth and/or function of midbrain dopamine neurons. Brain-derived neurotrophic factor (BDNF), regulates dopamine neuron differentiation and survival. BDNF and its receptor, TrkB, can be upregulated by estrogen (E). **Purpose:** To investigate if sex steroids regulate the responsiveness of dopamine neurons to BDNF. **Methods:** Pre-adolescent (45 day old; n=13-15/group) male rats underwent sham gonadectomy or were gonadectomised and given replacement T, dihydrotestosterone (DHT), E or no steroid (Gdx), via silastic implants, until 60 days old and dopamine neurotransmission was investigated in the midbrain. **Results:** T, not DHT or E, increased tyrosine hydroxylase (TH) protein levels compared with sham-treated controls, suggesting T can increase dopamine synthesis capacity. Some BDNF mRNA transcripts were lower after Gdx and I, IIA and IV were increased by T and I and III by DHT compared to Gdx. TrkB.FL was increased by T, DHT not E and TrkB.T1 was increased by T, DHT and E compared to Gdx but TrkB.T2 was increased by Gdx and this was prevented by replacement T, DHT not E. **Conclusion:** T upregulates TH protein and BDNF pathways in the midbrain, suggesting a role for T in stimulation of dopaminergic neurotransmission.

ORAL-13-02

RAPID DOWN-REGULATION OF MICRORNA FOLLOWING INDUCTION OF LONG-TERM POTENTIATION IN VIVO

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Rapid up-regulation of gene networks is associated with persistence of the long-term potentiation (LTP) model of memory, but regulation of these networks is poorly understood. MicroRNA are recently identified negative regulators of gene expression, which act through RNA degradation or translation inhibition. We hypothesized that rapid, LTP-induced up-regulation of gene networks is mediated in part by down-regulation of microRNA expression. **Purpose:** To identify microRNA regulated rapidly following LTP induction *in vivo*. **Methods:** We used Affymetrix GeneChip and Agilent microRNA arrays to identify microRNA regulated in dentate gyrus 20 min following LTP induction at perforant path synapses of awake, adult male Sprague-Dawley rats (n=4). **Results:** The Affymetrix and Agilent screens predicted differential expression of 69 and 7 microRNA respectively, when compared to non-tetanised control hemispheres (fold change ± 0.15; P<0.05). Five microRNA (miR-132, miR-181c, miR-214, miR-24-1, and miR-34a) were chosen for further investigation. TaqMan reverse transcription quantitative PCR confirmed down-regulation of miR-132 (0.30 ± 0.09; average ± SEM), miR-181c (0.44 ± 0.12), and miR-34a (0.38 ± 0.14) following normalization to miR-16, Y1, and U6 (P<0.05). Interestingly, microRNA target prediction algorithms identified known LTP-related molecules ARC, FOS and NR4A3 as likely targets of LTP-regulated microRNA. Up-regulation of these genes was confirmed within our dataset (ARC: 22.40 ± 8.13; FOS: 4.02 ± 1.32 and NR4A3: 1.48 ± 0.20). **Conclusion:** These data show that LTP induced *in vivo* is associated with rapid down-regulation of specific microRNA and concomitant up-regulation of potential target mRNA. Thus, microRNA appear to play an important role in regulating LTP-related gene expression and may thereby contribute to LTP stabilisation.

ORAL-13-03

INDUCTION OF LONG-TERM POTENTIATION *IN VIVO* ACTIVATES LATENT PRECURSOR CELLS IN THE MOUSE DENTATE GYRUS

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Purpose: The recent discovery of a large latent population of hippocampal precursor cells in the dentate gyrus of adult mice led us to investigate whether activation of this population is regulated by synaptic activity. **Methods:** Mice were unilaterally implanted for acute *in vivo* electrophysiology, in which perforant path-evoked synaptic potentials were recorded from the dentate gyrus region of the hippocampus. High-frequency stimulation (HFS) was delivered, after which the mice recovered for 48 hours (n=13) or 96 hours (n=3) before they were processed for the neurosphere assay. **Results:** We found that only the induction of long-term potentiation (LTP) activated the latent precursor pool, resulting in a nearly 2-fold increase in the number of neurospheres cultured from the stimulated hippocampus, compared to the non-stimulated hemisphere (n=6, p<0.05). No increase in proliferation was observed when the stimulation protocol induced early-LTP (n=3) or failed to induce any potentiation (n=4), or when the mice recovered for 96 hours post-HFS (n=3). Furthermore, there was strong correlation between the magnitude of potentiation 60 min post-HFS and the extent to which precursor proliferation was activated (r^2=0.8165, p<0.0001). **Conclusion:** These results show that synaptic activity sufficient for inducing LTP also activates latent neural precursor cells in the dentate gyrus. These findings may help explain how environmental signals can affect neurogenesis, through the activation of proliferation by mechanisms underlying synaptic plasticity.

ORAL-13-04

DEVELOPMENTAL PROFILE OF NRG1/ERBB4 PROTEIN IN THE MOUSE BRAIN: EFFECTS OF PERINATAL NR2B ANTAGONISM

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Purpose: The NRG1-ErbB4 signalling system is implicated in schizophrenia and altered NRG1/ ErbB4 signalling has been found to affect NMDA receptor levels and function, particularly phosphorylation of the NR2B subunit. NMDA receptor hypofunction in early brain development is also linked to the pathophysiology of schizophrenia and NR2B subunit expression is the most prominent at this time. This study aimed to determine the influence of perinatal NR2B antagonism on NRG1/ ErbB4 protein levels in the mouse brain throughout development. **Methods:** Male and female mice were treated with either the NR2B antagonist Ro 63-1908 (30mg/kg) or saline from postnatal day (PN) 7 to 14. Brain tissue was collected eight hours after the last treatment (PN14), at adolescence (PN35) and adulthood (PN70) (n=5 per group). Western blot was used to determine NRG1/ErbB4 protein levels in the prefrontal cortex and hippocampus. **Results:** NRG1 protein was low at PN14 and increased to PN35 (+330%; p<0.001) in both brain regions, with a further increase to PN70 in the prefrontal cortex (+34%; p=0.012) but not the hippocampus (p=0.745). There was no difference in ErbB4 protein levels throughout development and no effect of NR2B antagonist treatment on NRG1/ ErbB4 protein levels in either brain region. **Conclusion:** NRG1 protein appears to increase from the juvenile to adult period in the mouse brain, in contrast to the NRG1 mRNA developmental profile in rat and primate brain. Perinatal NR2B antagonist treatment did not alter this developmental profile, or that of ErbB4, suggesting that it does not alter NRG1-ErbB4 signalling in the prefrontal cortex and hippocampus of the mouse brain.

ORAL-13-05

USING ZEBRAFISH TO STUDY ADULT NEUROGENESIS AND BRAIN REGENERATION

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The mammalian brain has a limited regenerative capacity. In contrast, zebrafish display widespread life-long neurogenesis and can regenerate parts of its CNS. Yet, the cellular and molecular mechanisms that allow brain regeneration in zebrafish are poorly understood. We have studied the zebrafish regenerative capacity by using telencephalic and cerebellar lesions as models. We show that most cell types are restored following a traumatic lesion. However, the regenerative capacity is linked to adult neurogenesis i.e. only cell types that are homeostatically produced in the adult brain regenerates well. Interestingly, it seems like that the zebrafish brain provides a permissive environment for regeneration because we do not detect scarring or a persisting inflammation. Furthermore, we have identified genes directly involved in regulating the regenerative response in zebrafish by micro-array analysis. In summary, zebrafish provides exciting potential as a new powerful model for studying brain regeneration and neuronal stem cell diversity in the vertebrate brain.

ORAL-13-06

CEREBELLAR CONTRIBUTIONS TO MOTOR LEARNING IN ZEBRAFISH

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Purpose: Our group in interested in how the cerebellum drives motor learning and coordination, and we address these issues using optogenetics in the zebrafish model system. There are two parts to this approach: the development of novel behavioural preparations that allow motor learning to be quantified, and the establishment of optogenetic lines for observing and manipulating neural activity in the cerebellum. **Methods and results:** To develop an assay for studying motor learning, we have shifted zebrafish larvae to water of increased viscosity, and found that while they initially struggle to swim, they make adjustments to their motor programs in order to meet their normal swimming goals. Within five minutes the shift, the larvae (n=30) increase the magnitude and number of tail beats, but do not adjust the velocity of their tail movements. These adjustments increase their swim performance in high viscosity water, and by 15 minutes, they have restored normal swimming. We have also developed a swim simulator, in which the tail movements of a head-immobilized larva dictate the visual stimuli presented to it (using a high-speed camera and a closed-loop feedback program). This allows us to adjust the gain of their swimming performance, effectively presenting them with the same challenges that they face during viscosity shifts. **Conclusions:** We have developed two assays for motor learning in zebrafish larvae. For both of these behaviours, we are investigating the neural mechanisms underlying this motor learning. Using optogenetic tools, we are observing, driving, or blocking neural activity in the cerebellum, and looking for the ways in which this impacts the acquisition, storage, and recall of motor learning.

ORAL-13-07

ANAESTHETIC HYPERSENSITIVITY AND RESISTANCE BY MANIPULATING ONE MOLECULE IN THE FLY BRAIN

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Purpose: A clear understanding of the molecular mechanism of general anaesthesia has yet to emerge. Research in the nematode worm and human cell lines suggest that a target site for general anaesthetics might involve the presynaptic vesicle release machinery, in particular the H3 domain of the protein syntaxin1A. We are investigating this hypothesis using *Drosophila melanogaster* as a model, by testing various molecular constructs that express altered syntaxin1A proteins in *Drosophila*. **Methods:** We have developed two new behavioural assays to study general anaesthesia phenotypes in *Drosophila*. Using these clinically-relevant assays, we quantified isoflurane sensitivity for a number of syntaxin1A genetic lesions. We utilised the Gal4/UAS expression system to localise these molecular lesions to specific circuits in the fly brain. **Results:** Flies with a deletion to the C-terminal region of the H3 domain of syntaxin1A showed resistance to isoflurane (n=8, 10 flies per genotype, $p<0.05$). In contrast, flies with a deletion to the N-terminal region of the H3 domain of syntaxin1A are hypersensitive to isoflurane (n=8, 10 flies per genotype, $p<0.05$). Syntaxin1A truncations, expressed in the central fly brain, also showed altered isoflurane sensitivity (n=10, 10 flies per genotype, $p<0.05$.) **Conclusion:** Our results demonstrate that different regions of the H3 domain of syntaxin1A can confer an 8-fold difference in isoflurane sensitivity, suggesting that this molecule is a likely target of volatile anaesthetic drugs.

ORAL-13-08

LINEAGE ORIGINS AND GENETIC CONTROL OF NEURONAL SUBTYPE FATE DETERMINATION IN THE DEVELOPING ZEBRAFISH RETINA

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During neurogenesis, multipotent progenitors generate diverse neuronal subtypes using highly co-ordinated gene expression. **Purpose:** Using molecular manipulations in developing zebrafish, we study mechanisms and genes involved in subtype specification of retinal neurons. **Methods:** Comparable expression of different genes within progenitors and their resulting fate were studied using time-lapse imaging of transgenic lines. The role of genes was assessed using functional studies (n > 20 embryos / condition). **Results:** We previously identified multiple lineages generating distinct excitatory or inhibitory neurons. The pancreas transcription factor 1a (Ptf1a) acts as a switch towards the inhibitory fate, though the final inhibitory neuron subtype fate depends on the lineage of origin. We show that Ptf1a within the *ath5* lineage is necessary for the expression of *barh-like 2*. Functional manipulations reveal that Barhl2 acts as a switch to drive the subtype fates of particular inhibitory neurons such as GABAergic amacrine cells within this lineage. Barhl2 is cell-autonomously necessary and sufficient in specifying these subtype fates. Loss of Barhl2 causes a switch towards alternate inhibitory neuron subtypes, whereas mis-expression of Barhl2 drives the generation of only the inhibitory subtypes that usually express Barhl2. **Conclusion:** We are characterising the gene networks that generate neuronal subtype diversity. We reveal a sequence of gene expression within individual progenitors that influences the final subtype fates. Understanding developmental subtype diversification can contribute to efforts of regenerating specific neuronal subtypes.

ORAL-14-01

OVEREXPRESSION OF RCAN1 CAUSES DOWN SYNDROME-LIKE HIPPOCAMPAL STRUCTURAL, CELLULAR AND FUNCTIONAL DEFICITS

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Purpose: To investigate the overexpression of RCAN1 in the hippocampus and its ability to replicate the morphological and functional alterations characteristic of Down syndrome (DS). **Methods:** The hippocampus of RCAN1 transgenic (RCAN1-TG) mice underwent morphological analysis to assess size and neuronal content. Behavioural tests (novel object recognition, Y-maze and Morris water maze) to measure cognitive ability were performed in conjunction with electrophysiological studies on hippocampal slices to measure Long Term Potentiation (LTP), a physiological measure of learning and memory. Finally, a series of biochemical assays to examine key molecular pathways involved in LTP were performed. **Results:** RCAN1-TG mice had a reduced hippocampal volume (n=4, P=0.0038) that was due to a reduction in the number of neurons within the dentate gyrus (n=4, P=0.0065). Hippocampal-dependent behavioural studies revealed that RCAN1-TG animals had reduced visual recognition memory (n=11, P=0.0008) and defects in both short and long-term visuo-spatial learning and memory (n=15, P=0.0089 and P=0.0399, respectively). Upon stimulation, LTP was reduced in RCAN1-TG mice (n=6, P=0.0012), presumably due to the reduction in post-synaptic calcium influx (n=120) that affects the activation of a number of down-stream calcium-sensitive signalling pathways such as CaMKII (n=9, P=0.0108) and ERK (n=6, P<0.05). **Conclusion:** Overexpression of RCAN1 replicates the hippocampal defects characteristic of DS. This mouse model will allow us to further dissect the molecular pathways involved in the learning and memory alterations associated with DS.

ORAL-14-02

RISPERIDONE RESCUES A NOVEL AGGRESSION PHENOTYPE IN THE NEUROLIGIN 3$^{(R451C)}$ MOUSE MODEL OF AUTISM

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Aggression is common in patients with Autism spectrum disorders (ASD) along with the core symptoms of impairments in social interaction, communication and repetitive behaviour. Risperidone, an atypical antipsychotic, is widely used to treat aggression in ASD. We have discovered that mice expressing a gene mutation associated with ASD (Neuroligin 3^{R451C}, NL3) show heightened aggression. We aimed to further characterise the aggressive phenotype in NL3 mutant mice and to ascertain if this model is responsive to risperidone treatment. After 1 week in social isolation housing, NL3 mice were tested for aggressive behaviour using the resident-intruder test following acute injection of saline or risperidone (0.05mg/kg). The latency of the resident mouse to attack an intruder mouse was recorded 15 mins post treatment. Corticosterone blood serum levels were quantified immediately following aggression testing and an independent cohort were tested for their ability to locate buried food as an indicator of olfactory function. NL3 mutant mice displayed heightened aggression towards intruder mice, including reduced latency to attack and increased duration of fighting compared to wild type (WT) littermates. Treatment with 0.05mg/kg risperidone reduced aggression in NL3 mutant mice to a level comparable to WT mice. No change in corticosterone serum levels following aggressive behaviour was observed in NL3 mutant mice indicating that this phenotype is not due to an altered stress response. Intriguingly, NL3 mutant mice also displayed a longer latency to locate food in the buried food test indicating that they have impairments in olfaction. In conclusion, NL3 mutant mice show heightened aggression, a phenotype that is reversed with risperidone. This is the first demonstration of predictive validity in a genetic mouse model of autism displaying an aggression phenotype. Further work is required to investigate the effects of the NL3 mutation on brain areas implicated in aggression and olfaction.

ORAL-14-03

P75 REDUCTION ATTENUATES COGNITIVE DEFICITS IN A MODEL OF ALZHEIMER'S DISEASE

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Purpose: A hallmark of Alzheimer's disease (AD) is degeneration of cholinergic neurons in the basal forebrain and subsequent loss of hippocampal innervation. Ablation of the p75 neurotrophin receptor (p75) increases cholinergic innervation of the hippocampus, enhances synaptic plasticity and improves spatial memory performance in normal mice. We have thus investigated if p75 reduction can improve spatial memory in AD. We compared cognitive deficits in transgenic AD mice expressing the human amyloid precursor protein (APP) gene with familial AD Swedish mutation (Tg2576) to AD mice with decreased p75 gene expression (Tg2576 p75$^{+/-}$). **Methods:** Cohorts of Tg2576 and Tg2576 p75$^{+/-}$ mice aged 5 to 7 months (n=13 and n=11 respectively) and 13 months (n=15 per genotype) underwent Barnes maze testing to assess spatial memory. **Results:** At 13 months of age there was a striking difference between genotypes, with Tg2576 p75$^{+/-}$ mice performing better on the Barnes maze than Tg2576 mice. A smaller but statistically significant difference was seen at 5-7 months of age, again indicating improved spatial learning and memory performance in Tg2576 p75$^{+/-}$ mice. **Conclusions:** Cognitive decline is substantially attenuated in aged AD mice with reduced p75 compare to control AD mice. Comparatively better learning and memory is also observed in younger Tg2576 p75$^{+/-}$ compared to Tg2576 mice, before the pathological features of AD are prominent. We are currently investigating whether p75 reduction alters pathological features of AD, including brain Aβ levels and plaque burden, or whether the improved spatial memory seen in Tg2576 p75$^{+/-}$ mice occurs independently of the disease process.

ORAL-14-04

CANNABIDIOL AND CLOZAPINE REVERSE MK-801-INDUCED SOCIAL INTERACTION DEFICITS IN RATS IN A NOVEL TWO BOX PARADIGM

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Purpose: Recently a novel paradigm has been designed to assess social investigative behaviour in pairs of rats which involves physical separation whilst ensuring they are able to maintain contact through other social cues. We have modified this paradigm in order to assess social behaviour and locomotor activity alterations induced by the NMDA receptor antagonist MK-801. The ability of the clinically used antipsychotic clozapine and a phytocannabinoid with purported antipsychotic activity called cannabidiol were administered to determine whether the MK-801 induced behavioural effects could be reversed. **Methods:** Following acclimatisation to a holding room for 5 days, rats were habituated to the novel test setup on day 6 with unfamiliar weight-matched conspecifics for 30 min. On day 7, rats were administered cannabidiol, clozapine or vehicle followed 20 min later by vehicle or MK-801 (n = 10-12 per group). 20 min after the last injection unfamiliar weight-matched pairs that had received the same treatment were tested in the novel paradigm for 30 min. **Results:** MK-801 (0.3 mg/kg) treated rats displayed reduced social investigative behaviour, hyperactivity as well as reduced attention span. Pretreatment with cannabidiol (3 mg/kg) not only normalised social investigative behaviour but increased it beyond control levels. Pretreatment with clozapine (1 and 3 mg/kg) also normalised social investigative behaviour. Both cannabidiol and clozapine inhibited MK-801-induced hyperactivity. However, there were no effects of pretreatment on impairments to attention span. **Conclusion:** Our findings reinforce several aspects of the validity of the MK-801 model of social withdrawal and hyperactivity and also support the use of this novel paradigm for further investigations to assess antipsychotic potential of drugs.

ORAL-14-05

OVER ACTIVATION OF HIPPOCAMPAL SERINE/ THREONINE PROTEIN PHOSPHATASES PP1 AND PP2A AS A NOVEL MECHANISM OF LEAD-INDUCED DEFICITS IN LEARNING AND MEMORY IN YOUNG RATS

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Purpose: Serine/threonine protein phosphatases PP1 and PP2A regulate several key events in the brain, including learning and memory. We investigated whether these phosphatases are implicated in lead (Pb)-induced deficits in learning and memory. **Methods:** Wistar rat pups (n = 10) were exposed to 0.2% Pb-acetate via their dams' drinking water from postnatal day (PND) 1 to 21 and directly in drinking water until PND30. Control rats (n = 10) received regular water. Pb levels in blood, brain and hippocampus were measured and expression of PP1, PP2A, PP2B and PP5 in hippocampus was analyzed. Total phosphatase activity, and PP1 and PP2A activities were determined. Tau phosphorylation at various epitopes was determined by Western blot. Spatial learning and memory was determined by Morris water maze test. **Results:** Pb exposure significantly ($p < 0.05$) increased levels of Pb in blood, brain and hippocampus, reduced the number of synapses in hippocampus and impaired learning and long-term memory (LTM). Short-term memory (STM) was only affected in rats at PND21. Pb exposure increased the expression and activity of PP1 and decreased phosphorylation of tau at T231 in hippocampus at both PND21 and PND30. Pb-induced phosphorylation of tau at S199/202 (AT8) paralleled with PP2A activity; at PND21 PP2A activity increased and AT8 phosphorylation decreased; at PND30 PP2A activity decreased and AT8 phosphorylation increased. Increased PP1 activity in hippocampus by Pb is associated with learning and LTM impairment, whereas, increased PP2A activity is associated with STM impairment. **Conclusion:** These findings suggest the over-activation of PP1 and PP2A, together with changes in tau phosphorylation, as a novel mechanism of lead-induced deficits in learning and memory.

ORAL-14-06

DISCRIMINATION OF GENETIC DETERMINANTS CONTRIBUTING TO FEAR RESPONSIVENESS AND FEAR MEMORY

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PURPOSE: Part of the human variation in response to fear and memory of fearful experiences is genetically determined. However, few genes have been identified which underlie this variation. Two mouse strains, C57Bl/6J (B6) and DBA/2J (D2), show variation in fear responsiveness and fear memory. We, and others, have linked this variation to loci on chromosomes (Chr) 1 and 12. What is unclear is whether the variation in fear responsiveness is responsible for variation in fear memory, or whether fear and fear memory can be separated. These studies were done to address this issue. **METHODS:** We studied congenic mouse strains harbouring D2-derived DNA from Chr1 or Chr12 on a B6 genetic background. Cohorts of D2, B6 and congenic mice were tested for fear responsiveness in an open field arena and for fear memory following Pavlovian context fear conditioning. **RESULTS:** The Chr1 congenic mice (n=33) showed clear deficits in fear memory compared to B6 mice (n=37), which established the presence of a QTL on Chr1 directly influencing fear memory. The Chr12 congenic mice (n=38) also showed alterations in fear conditioning, but this was more associated with alterations in fear responsiveness. **CONCLUSION:** These findings provide evidence for the localisation of independent genetic determinants for fear memory and fear responsiveness. We are currently in the process of identifying the genes involved in this variation; with a focus on the Chr12 D2-derived region.

ORAL-14-07

MOUSE MODELS OF ADVANCED PATERNAL AGE: RELEVANCE TO NEUROPSYCHIATRIC DISORDERS

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Purpose: Advanced paternal age (APA) is associated with an increased risk of adverse health outcomes in offspring, including autism and schizophrenia. With respect to biological mechanisms for this association, there are many more germline cell divisions in the life history of a sperm relative to that of an oocyte. This leads to more opportunities for copy error mutations in germ cells from older fathers. Rodent models provide an experimental platform to examine the association between APA and brain development. Three APA mouse models have been published, however existing mouse models vary in key features creating a lack of consistency within the literature. Methods: Our published model showed increased anxiety-related and exploratory behaviours. Another study demonstrated reduced social behaviour, while the third showed reductions in avoidance learning and locomotion. The present study focused on refining the APA mouse model and examining factors that could contribute to this variability. We have carefully considered several potential modifiers of APA effects in the mouse and investigated the effects of breeding programme and APA sire age range on behavioural phenotypes in offspring. Results: We have demonstrated that breeding programme does not contribute to offspring outcomes. However, increasing the APA sire age to 24 months reliably recapitulates previously reported increases in anxiety-related behaviour on the elevated plus-maze (p=0.005), hole board (p=0.03) and light/dark (p=0.03) tests. Furthermore, a preliminary exploration of genomic correlates of these challenging phenotypes revealed that particular copy number variation (CNV) expansions, only found in APA mice, were correlated with changes in specific behavioural domains. Conclusions: These experiments demonstrate that variation in APA sire age and CNV load could explain the phenotypic variability in APA mouse models. Investigating factors that contribute to behavioural phenotypes in APA mouse models and modifying protocols accordingly will allow further exploration of mechanisms that may underpin these changes. Modelling APA in mice may provide clues to upstream mechanisms of action in ASD and schizophrenia.

ORAL-14-08

PROFILING TRAIT ANXIETY: TRANSCRIPTOME ANALYSIS REVEALS CATHEPSIN B (CTSB) AS A NOVEL CANDIDATE GENE FOR EMOTIONALITY IN MICE

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Behavioral endophenotypes are determined by a multitude of counteracting but precisely balanced molecular and physiological mechanisms. In this study, we aim to identify potential novel molecular targets that contribute to the multigenic trait "anxiety". We used microarrays to investigate the gene expression profiles of different brain regions within the limbic system of mice which were selectively bred for either high (HAB) or low (LAB) anxiety-related behavior, and also show signs of comorbid depression-like behavior. We identified and confirmed sex-independent differences in the basal expression of 13 candidate genes, using tissue from the entire brain, including coronin 7 (*Coro7*), cathepsin B (*Ctsb*), muscleblind-like 1 (*Mbnl1*), metallothionein 1 (*Mt1*), solute carrier family 25 member 17 (*Slc25a17*), tribbles homolog 2 (*Trib2*), zinc finger protein 672 (*Zfp672*), syntaxin 3 (*Stx3*), ATP-binding cassette, sub-family A member 2 (*Abca2*), ectonucleotide pyrophosphatase/phosphodiesterase 5 (*Enpp5*), high mobility group nucleosomal binding domain 3 (*Hmgn3*) and pyruvate dehydrogenase beta (*Pdhb*). Additionally, we confirmed brain region-specific differences in the expression of synaptotagmin 4 (*Syt4*). Our identification of about 90 polymorphisms in *Ctsb* suggested that this gene might play a critical role in shaping our mouse model's behavioral endophenotypes. Indeed, the assessment of anxiety-related and depression-like behaviors of *Ctsb* knock-out mice revealed an increase in depression-like behavior in females. Altogether, our results suggest that *Ctsb* has significant effects on emotionality, irrespective of the tested mouse strain, making it a promising target for future pharmacotherapy.

ORAL-15-01

TYPE-1 INTERFERON SIGNALLING IS INVOLVED IN NEURO-INFLAMMATION FOLLOWING TRAUMATIC BRAIN INJURY

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Introduction: Traumatic brain injury (TBI) is the major cause of death in young individuals in industrialised countries. TBI triggers acute neuro-inflammation, which exacerbates primary brain damage. We propose that type-1 Interferons (IFNs), which signal through the interferon α receptor-1 (IFNAR1), are a major driver of neuro-inflammation in TBI. **Methods:** TBI was induced using a controlled cortical injury model in 8-week-old male C57BL/6J wild type (WT) and IFNAR1$^{-/-}$ mice. WT mice were intra-venously administered, in a blinded fashion, either a monoclonal antibody isotype control (0.5mg) or an IFNAR1-blocking monoclonal antibody (MAR1, 0.5mg) 30 minutes after TBI. Brains were excised at 2, 4 and 24 hours after TBI for infarct volume measurement and qPCR. Type-1 IFN levels were analysed by qPCR in human post-mortem brain samples obtained from the National Trauma Research Institute. **Results:** IFNα mRNA levels were increased up to 5-fold in WT mice compared to IFNAR1$^{-/-}$ mice ($p<0.05$, n=3). IFNβ levels were increased up to 10-fold in post-mortem brains ($p<0.05$, n=8). mRNA levels for the pro-inflammatory genes IL-1β and TNF-α were elevated in WT mice up to 13- and 50-fold, respectively, compared to IFNAR1$^{-/-}$ mice ($p<0.05$, n=3). MAR1-treated mice had decreased infarct volumes compared to control-treated mice (3.41mm3 compared to 4.84mm3, respectively, $p<0.05$, n=5). **Conclusion:** This study confirms involvement of type-1 IFN signalling in neuro-inflammation after TBI. The therapeutic potential of MAR1 has been validated in a mouse model of TBI conferring protection 30 minutes post-injury. This suggests reduced type-1 IFN signalling may be a novel therapeutic target in reducing damage in humans following TBI.

ORAL-15-02

MONOCLONAL ANTI-LYSOPHOSPHATIDIC ACID ANTIBODIES ARE NEUROPROTECTIVE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Purpose. Traumatic brain injury (TBI) is the major cause of adolescent death and disability worldwide. Neural cell death following TBI is a result of direct (immediate mechanical disruption of brain tissue) and indirect (secondary or delayed) damages. The indirect damages involve the initiation of an acute inflammatory response, including breakdown of the blood-brain barrier (BBB), edema formation and swelling, infiltration of peripheral blood cells and activation of resident immunocompetent cells. Lysophosphatidic acid (LPA) is a bioactive lysophospholipid released by activated platelets. LPA targets all cells in the central nervous system through the binding of its five specific G-protein-coupled receptors. LPA accumulates in the nervous system following the impairment of the blood brain barrier and is suggested to play a detrimental role in brain injury responses. Methods. Male mice (8-10 weeks) of C57BL/6 background (n=6) were given two treatments in a blinded fashion: either 0.5mg of monoclonal antibody targeted to the bioactive Lysophosphatidic acid (B3) or 0.5mg isotype control pre or post-CCI (controlled cortical impact mouse model). Magnetic Resonance Imaging (MRI) was used to analyze mouse brain infarct volumes following CCI on day 1 and day 7. Results. We found that B3 potently inhibited LPA's effect in vivo by binding to LPA thereby preventing it from interacting with its complement of receptors. When administered 60 min before or 30 min after injury in our CCI mice model of TBI, B3 significantly and substantially reduced infarct volumes measured by MRI (both on day 1 and day 7) and standard histological staining. For the post-CCI treatment the B3 reduced about 15-25% of the infarct volume on day1 and 50% on day7 (statistic significant). Conclusion. This study provides a promising therapeutic approach for the treatment of acute brain injury. The protective effect of the B3 monoclonal antibody, prior to or after TBI, raises the prospect of translating monoclonal antibodies targeting LPA into clinical trials.

ORAL-15-03

FGF MEDIATES GLIA BRIDGE FORMATION AFTER SPINAL CORD INJURY IN ZEBRAFISH

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Spinal cord injury (SCI) usually results in a very limited regeneration of lesioned axons, followed by permanent impairment of function. A major barrier to axonal regeneration is glial scar formation as a result of the astrocytic response to injury. As opposed to mammalian spinal cord, adult Zebrafish show a remarkable capacity to regenerate their spinal column after injury. **Purpose:** By using different transgenic lines, we studied mechanisms involved in gliogenesis that occurred during the regeneration process. **Methods:** Glia proliferation and morphology was examined at the lesion site in zebrafish expressing GFP under the GFAP or nestin promoters. In addition, the role of FGF in gliogenesis was examined in transgenic lines in which there is either a loss or gain of Fgf function. **Results:** By 3 weeks after SCI, zebrafish glia form an elongated morphology that bridges the resected region. These elongated glia act as a bridge over which regenerating axons actively regrow through the lesion site, as demonstrated by tracer labelling. Zebrafish with loss of Fgf function display inhibition of glial cell proliferation and bridging across the two transected sides and therefore inhibit axon regeneration through the lesion site. In contrast, zebrafish with over-activation of Fgf signalling show an accelerated glia proliferation and bridging that supports axonal regeneration in vivo. In addition, mammalian astrocytes can be activated by Fgf signalling to produce a similar bipolar morphology and behaviour. **Conclusion:** Our study suggests that differential Fgf regulation could well underlie the distinct response of mammalian and zebrafish spinal column to injury and could be manipulated to provide therapeutic benefit.

ORAL-15-04

VEGF AND PDGF REDUCE SECONDARY DEGENERATION AND IMPROVE LOCOMOTOR BEHAVIOUR AFTER CONTUSION SPINAL CORD INJURY IN RATS

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Inflammation in most tissues is initially protective, facilitating removal of damaged tissue. Later the response transitions to an anti-inflammatory state, initiating wound healing. After traumatic spinal cord injury (SCI) the inflammatory response is prolonged and the anti-inflammatory response delayed, leading to secondary degeneration, greatly increasing the lesion size. Vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) increase angiogenesis and vascular permeability and lymphocyte extravasion. Here, we investigated whether VEGF and PDGF alter the outcome after SCI. Adult Wistar-Kyoto rats received 150 kDyne weight-drop contusion injuries at T10 spinal level. They were randomly assigned to treatments (n=6 per group) delivered over 7d via implanted catheter and osmotic mini-pump loaded with VEGF+PDGF (Low dose; 5µg each) (High dose; 15µg each) or saline (Control). One month later the animals were killed. The cords were cut horizontally and the lesion cavity was defined by immunoreactivity to NF200 to identify axons. VEGF+PDGF treatment reduced the lesion cavity to 5% of the Controls: High dose, 0.001+0.001 mm^2; Low dose, 0.10± 0.03 mm^2; Control 0.53±+0.12 mm^2. High and Low dose treated cords, but not Controls had high levels of axon filaments within the lesion extending into the lesion cavity beyond the region of astrogliosis (GFAP immunoreactivity). High and Low dose treatments altered the distributions and morphologies of macrophages/microglia, defined by Iba1 immunoreactivity, and revealed macrophages that were immuno-positive for Iba1 and NG2, a chondroitin sulphate proteoglycan indicative of a regenerative phenotype of inflammatory cells. VEGF+PDGF treatment significantly improved the BBB locomotor score (treatment 19.4±+1.2, Vehicle control 16.0±+1.6 Lesion Control 16.5±1.1) and prevented the increase in gait parameters associated with SCI (increased stepping frequency (Prelesion 35.8±6.2 Control 30.0±7.5 Treatment 37.7±12.9 frames) and stance width (Prelesion 2.9±0.02 Control 3.06±0.4 Treatment 2.85±0.06 mm)). We conclude that VEGF+PDGF may alter the dynamics of macrophages/microglia activation in the inflammatory sequelae following injury, preventing secondary degeneration of the spinal cord tissue and reducing the loss of locomotor functions.

ORAL-15-05

INVESTIGATING THE MECHANISMS OF AXONAL DEGENERATION AFTER LASER-MEDIATED MICRO LESION IN THE ADULT BRAIN USING IN VIVO 2-PHOTON MICROSCOPY

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Purpose: Axonal damage is a hallmark of many neurological disorders. Axonal degeneration is an active process triggered by a range of insults to the central nervous system and is a secondary result in several neurological disorders, for example following neuroinflammation. The factors that trigger axonal degeneration are not fully understood. **Methods:** We studied the axonal degeneration response caused by a focal lesion in the cerebral cortex using in vivo two-photon imaging through a cranial window. Excitatory axons were visualised using transgenic mice expressing GFP in a subset of neurons and were cut using a pulsed high-energy femtosecond laser (800nm). Damaged axons were tracked over time to assess the fragmentation kinetics of the disconnected part. **Results:** The detached axon rapidly underwent beading within the first few minutes of the lesion (n=28axons, 17mice), commenced fragmentation and took a variable length of time to be removed (1.5-70 hours). Interestingly, axon subtypes responded differently to lesions, with some cortical arbors undergoing fragmentation within minutes of insult, and others surviving for longer than 2 days. Once disconnected from the cell body, axonal segments >800µm took longer to start fragmenting (29.6±4.7hours, n=7) compared to shorter axons (1.6±0.4 hours, n=21; $p<0.01$), however they fragmented at a faster rate than shorter axons (>800µm: 1.6±0.3 µm/min; <800µm: 0.7±0.2µm/min; $p<0.01$). **Conclusion:** Elucidating the underlying mechanisms of cortical axonal degeneration may lead to new potential therapies to delay or prevent it in a variety of neurological conditions that are characterised by axonal injury.

ORAL-15-06

INVOLVEMENT OF THE KYNURENINE PATHWAY IN MULTIPLE SCLEROSIS PROGRESSION: THE LINK BETWEEN NEUROINFLAMMATION AND NEURODEGENERATION

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PURPOSE: The kynurenine pathway (KP) has increasingly drawn awareness in multiple sclerosis (MS), for which abnormal levels of KP metabolites have been found. The KP may be involve in neurological deficit from two aspect: firstly, it has been shown that the first rate limiting enzyme of the KP, Indoleamine, 2-3 dioxygenase (IDO) is involve in immune regulation of inflammatory process in the brain; and/or secondly, metabolites from the KP are associated with neurodegeneration. We hypothesize that the progression of MS is likely to be associated with the dysregulation of the kynurenine pathway metabolism. METHODS: Our studies involve quantifying levels of several KP metabolites in the serum and cerebrospinal fluid of MS patients (combine n=90) with early and late stages diagnosis using HPLC and GC/MS. These patients had not received any medications known to interfere with the KP at the time of sample collection. RESULTS: We found that IDO is upregulated at all the stages of MS compare to healthy controls. We also observed an increased production of excitotoxin, QUIN in MS patients compared to controls implying abnormal alteration to the KP metabolism. Furthermore, QUIN was also present in active lesion of human MS postmortem brain tissue suggesting their involvement with neurodegeneration in MS progression. CONCLUSION: All the above data suggests the significant involvement of KP metabolism in MS progression.

ORAL-15-07

IDENTIFICATION OF ANTI-INFLAMMATORY COMPOUNDS FROM CINNAMON

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Purpose: In traditional oriental therapies, cinnamon has a long history of medicinal use, including for the treatment of diabetes and infections. In addition, cinnamon has been demonstrated to have anti-oxidant properties. This study was performed to characterize its anti-inflammatory properties and to identify the responsible compounds. Methods: Cinnamon powder was extracted by sequential extraction using solvents with increasing polarity or by two single extraction procedures using 10% ethanol or water, respectively. The extracts were analysed using GC-MS, and the compounds identified using a compound library. Anti-inflammatory activity was assessed in LPS + Interferon-gamma activated RAW 264 macrophages (n=3, in duplicates) by inhibition of nitric oxide (NO) and tumour necrosis factor (TNF) production. Results: In the sequential extraction, the EtOAc and EtOH fractions were the most potent, with IC50 values of 0.02 and 0.072 mg/ml (NO) and 0.047 and 0.034 mg/ml (TNF), respectively. Among the single extracts, the ethanolic extract was most potent with IC(50) values of 0.026 mg/ml (NO) and 0.088 mg/ml (TNF). In total, 13 individual compounds were identified, including cinnamyl alcohol, p-cymene, benzyl benzoate, trans-cinnamaldehyde, o-methoxycinnamaldehyde, eugenol and β-caryophyllene. Among those, the most potent anti-inflammatory compounds were β-caryophyllene and trans-cinnamaldehyde, both exhibiting IC(50) values for both inhibition of NO and TNF production below 0.05 mg/ml. Discussion: These findings indicate that the components of C. zeylanicum, provided they reach therapeutic concentration in the brain, may be used as an anti-inflammatory treatment for chronic neuroinflammatory diseases including Alzheimer's disease.

ORAL-15-08

ACTIVATED SCHWANN CELL COMBINED BONE MESENCHYMAL STEM CELL: THE BEST CELL STRATEGY FOR REPAIR AFTER RAT'S SPINAL CORD INJURY

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Aim: To explore the repair effect of cell combined therapy with activated Schwann cells and bone mesenchymal stem cells in rat's traumatic spinal cord injury. Materials and Methods: In the present research, activated Schwann cells (ASCs) and bone mesenchymal stem cells (BMSCs) were applied for combined transplantation to treat rat's acute spinal cord injury and both of these two types of cell can be obtained from SCI patients. ASCs were obtained by prior ligation of saphenous nerve and BMSCs by flush of marrow cavity with DMEM solution. Our experiment in vitro confirmed that ASCs promoted BMSCs to differentiate into mature neural cells. This further indicated BMSCs hold the potential to repair CNS injury. ASCs and BMSCs were co-transplanted into the injured epicenter of spinal cord made by the NYU impactor machine with the drop weight of 10g-50mm. Complete ASCs, BMSCs and DMEM were also transplanted in rats with spinal cord injury respectively as control. Recovery of rat's hindlimb function was serially evaluated by Basso, Beattie, Bresnahan (BBB) locomotor rating scale and footprint analysis. Changes of neurological potential were recorded by nerve electrophysiologic test. Improvement in the microenvironment of the injured spinal cord was evaluated by HE staining, glial fibrillary acidic protein (GFAP) staining, biotinylated dextran amine (BDA) anterograde tracing and electron microscopy. Results: From the results of BDA anterograde tracing, we demonstrated that more regenerative axons of corticospinal tract (CST) surrounding and passing through the injured cavity to the caudal cord in ASC-BMSC co-graft group than those in the other three groups, and we also confirmed it further by quantitative analysis. Immunostaining for GFAP exhibited the smallest population of astrocytes in the injury epicenter in ASC-BMSC group than those in other three groups. Relatively complete myelin sheaths and organelles were found in ASC-BMSC group than those in other three groups under electron microscopy. Conclusion: ASCs and BMSCs co-transplantation effectively promotes rat's hindlimb functional recovery and reduces the formation of glial scar as well as remyelinates the injured axons as compared with other three groups. This conclusion was also in accordance with the observation of immunohistochemistry staining and electron microscopy, suggesting the possible clinical application for the treatment of spinal injury.

ORAL-16-01

LOOK HERE. DO NOT PAY ATTENTION TO DETAIL: ROLE OF LOCATION AND FEATURE CUES IN GUIDING ATTENTION

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Purpose: One of the most debated questions in neuroscience is how the brain's attentional system selects what to process from the rich environments we live in. Top-down information about either locations or features in a visual scene can effectively bias selective attention. However, there is no clear consensus as to which of these mechanisms plays the more dominant role. This study assesses the degree to which location, colour and direction cues are each capable of biasing attention in a global motion perception task. Methods: Five observers were tested using a stochastic motion stimulus. In the first motion-direction discrimination task, attention was cued to one of four locations of moving dots that had coherently moving stimuli, while the rest had randomly moving distractors. The second task was similar, but employed a mixed display of red and green moving dots, with the colour of the coherently moving dots cued instead of location. The third task involved directing attention to one of four directions of monochromatic coherent movement and detecting the location of coherent motion. Coherence thresholds in the presence and absence of cues in each task were compared. Results: Observers demonstrated lower motion coherence thresholds when location was cued than when direction ($P<0.05$) or colour was cued ($P<0.05$) (RM-ANOVA). Colour and direction cueing showed similar effects ($P>0.05$). Conclusions: In order for top-down processes to bias selection of a particular object in a cluttered visual scene, it appears that spatial information is more effective in engaging attention than non-spatial information. Hence, in attention demanding tasks, providing location-based cues would have greater benefits than feature based cueing.

ORAL-16-02

ODOURS FACILITATE DETECTION OF MATCHING PICTURES IN THE ATTENTIONAL BLINK

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Purpose: The sense of smell is our most ancient and basic sense and plays a role in fundamental processes of everyday life. While it is well known that vision influences human olfaction, few studies have provided conclusive evidence that odours can impact on the visual sense. We investigated whether odours have an effect on a well-established cognitive paradigm, the *attentional blink* (AB). This phenomenon is displayed in rapid serial visual presentation (RSVP) when people fail to see the second of two targets if it appears within 500ms of the first target. The AB deficit is believed to be due to a bottleneck in the temporal allocation of selective attention. A salient T2, however, has been found to attenuate this effect. By pairing an odour with T2 in this study, we could determine whether a matching odour would enhance the salience of T2 and increase its detection during the AB. **Methods:** Participants (n=17) monitored a rapid stream of colour photographs at fixation to discriminate which visual object with a characteristic smell (T2; lemon, orange, rose or mint) appeared after an initial target. During the task, they were exposed to an odour that was congruent, incongruent or neutral with respect to T2. **Results:** As expected, neutral, incongruent and irrelevant odours resulted in a clear AB deficit. In contrast, a congruent odour increased T2 detection and attenuated the AB. **Conclusion:** The results indicate that an odour can enhance the salience of a matching visual object and make it easier to detect in a limited attention situation. Therefore, the well-learnt associations between odours and objects can lead to interactions where olfaction influences visual perception.

ORAL-16-03

STIMULUS AND RESPONSE CONFLICT PROCESSING THROUGHOUT THE ADULT LIFESPAN: A COMBINED ERP AND EMG INVESTIGATION

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Purpose This research examines the neural activity that underlies limitations in conflict processing at two critical transitions in the adult lifespan; the end of adolescence and the beginning of old age. **Method** To develop a better understanding of the neural changes in conflict processing throughout adult life, the electrical activity of the brain was examined as adolescents (16-17), young adults (20-30) and older adults (45-65) preformed a Stroop task. Electromyography (EMG) was also examined in response hands. This task was designed to isolate stimulus and/or response level processing when task irrelevant distracting information (i.e. word reading as opposed to colour naming) induces conflict in the brain. **Results** Adolescents, young adults and older adults performed similarly in terms of reaction time and accuracy however, the underlying brain activity was significantly different. Specifically, the P3a component was significantly larger and delayed in older adults indicating additional processing was required for reorienting attention ($p=0.0001$). Additionally the onset of the P1 and N2 was significantly delayed in the adolescent group however the onset of the P3b was earliest in this group ($p=0.0001$). This indicates that adolescents' visual attention had stronger processing demands and this aided subsequent stimulus categorization. Also between 420-480ms after stimulus presentation there was a negative depression (N450) which is related to response conflict. This was significantly enhanced in older adults and showed an increased left activation in adolescents indicating additional processing requirements for response conflict resolution. **Conclusion** Overall even though adult level performance was exhibited across the three groups the underlying neural activity indicates that the brain employs different cognitive strategies to compensate for limitations at both the stimulus and response levels of processing.

ORAL-16-04

EEG-BASED FUNCTIONAL NETWORKS IN HEALTH AND DISEASE

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Purpose: Techniques from graph theory are increasingly being applied to model the functional and/or structural networks of the brain. Large-scale brain networks, comprising anatomically or functionally distinct regions and inter-regional pathways, exhibit specific non-random patterns with the small-world and/or scale-free properties. Studying dynamical networks along with traditional methods complements our knowledge of the brain. In particular, graph theoretical analysis of anatomical and functional networks of the brain has revealed its economical small-world structure. Here we aim at studying the network properties of functional brain networks in Alzheimer's disease (AD) and schiphrenia. Methods: We considered two datasets; one consisting 17 patients suffering from AD and 17 matched controls, and another consisting 14 schizophrenic patients and 14 matched controls. The EEGs were obtained in closed-eyes resting states. Brain functional networks were extracted from subjects' EEG time-series through unpartial and partial cross-correlation analysis. The extracted networks were then examined for various graph theory metrics such as small-worldness, efficiency, modularity, assortativity, and synchronizability. The statistical significance of the changes was tested using Wilcoxon's ranksum test. Results: Our graph theory analysis of brain networks showed widespread abnormalities in both AD and schizophrenia. Both groups showed decreased synchronizability as compared to healthy control subjects, which was consistent in all frequency bands. Schizophrenia group showed frequency-specific anomalies in the small-worldness, while this metric did not show significant changes in AD group. The assortativity and modularity of the brain networks were also changed in patients suffering from these disorders. Conclusion: Analyzing EEG-based brain functional networks is an alternative approach for studying binding capabilities of the brain. Our graph theory analysis of brain networks showed widespread abnormalities in schizophrenia and AD that might shed light on how these disorders influences the brain functional networks.

ORAL-16-05

MINIMAL EFFECTS OF AGE ON NON-FOVEAL VISUAL LOCALISATION AND POINTING PRECISION

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Purpose: Pointing precision to non-fixated targets is similar in magnitude to visual localisation precision, implying a similar performance limit on both tasks. Although foveal hyperacuity declines with age, non-foveal visual localisation is less studied, and it is unknown whether visuo-motor interactions for such stimuli are altered. Our experiments aimed to determine the effects of ageing on these tasks. **Methods:** Twenty five younger (20 to 31 years) and 25 older (60 to 72 years) adults participated. Visual localisation and pointing precision were measured radially at eccentricities of 5, 10 and 15° visual angle, with targets (0.5°, circular white dots of 174 cd/m^2) presented randomly at 45°, 135°, 225° and 315° on a black background. The visual task was performed in both the presence and absence of visual references, in order to modulate the degree of difficulty of the task. **Results:** Age groups were not significantly different for either visual or pointing precision ($p>0.05$), with similar eccentricity effects (interaction: eccentricity x group: $p>0.05$). There was no group x task interaction for visual localisation and pointing precision ($p>0.05$). Older adults showed a greater relative decline for the unreferenced compared to the referenced visual localisation task (significant interaction: task x group: $F_{1,1000.5} = 6.8$, $p=0.009$). **Conclusions:** Normal ageing has minimal effect on non-foveal visual localisation and pointing precision for highly visible stimuli. However, performance for older adults did decline more than younger adults when the visual task was made more difficult by removing local visual reference cues. This finding is consistent with previous reports of age-related performance reduction on sensory tasks that are revealed when task demands increase.

ORAL-16-06

THE CONTRIBUTION OF MUSCLE RECEPTORS TO BODY REPRESENTATION IN THE ABSENCE OF VISION

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Purpose: Muscle receptors contribute to the sense of body ownership (Walsh *et al.* (2011), *J Physiol* **589** 3009), but it is not known if vision is required. **Methods:** To assess this 20 subjects placed their right hand on a lower table and their left hand on an upper table with 12 cm of vertical distance between the index fingers. The right index was connected, via a shaft co-linear with the proximal interphalageal joints, to a rubber finger on the upper table. When the shaft was coupled, movement between the rubber finger and the subject's finger was congruent. When decoupled, incongruent movement was possible. With the subject's arms hidden, the experimenter used the subject's left index and thumb to hold and move the rubber finger, at the same time moving the subject's right index finger, congruently or incongruently with the rubber finger. **Results:** Simply passively holding the rubber finger was enough for the perceived vertical spacing between the subject's index fingers to reduce from 10 cm [5.5,12] to 4.0 cm [2.5,7.5] (median [IQR] ($p<0.0005$), although the difference between the perceived absolute height of the left and right index fingers was not significantly different (6.0 cm [2.5,9.0] to 5.5 cm [3.5,7.0]). There was a further reduction (2 cm) in perceived vertical spacing between the index fingers after congruent movement ($p<0.05$). Removing skin and joint receptors by blocking the digital nerves of the right index finger with local anesthetic did not significantly change the effect of congruent movement. **Conclusion:** There are three novel findings. First, muscle receptors contribute to the sense of body ownership in the absence of vision. Second, simply holding a rubber finger with vision excluded is enough to alter perceived hand position. Finally, perceived finger position depends upon whether we consider each finger separately or together.

ORAL-16-07

AGE IS NO BARRIER TO LEARNING TO LINK GLOBAL CONTOURS

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Purpose: Older adults have decreased ability to extract contours from noise. This study examined whether training improves contour integration in older adults. **Methods:** 15 younger (20- 34 years) and 17 older (62- 78 years) adults attended 6 sessions. The first 5 were conducted over several weeks (3-7 days between visits). The final session was 3 months after the 5th. Contour processing was measured using closed contours (circles or ellipses) constructed of Gabor elements embedded in noise (identical Gabors of random orientation). Measures were: 1) the threshold aspect ratio for discriminating between a 15 element elliptical and circular contour; 2) the threshold number of elements to discriminate contour shape with aspect ratio fixed (3 x individual threshold). At visits 1, 5 and 6, Glass pattern coherence thresholds were measured to determine whether learning transferred to a different task requiring shape extraction from noise. **Results:** Thresholds for the older group were elevated relative to younger adults for all measures ($p<0.05$). Groups showed similar magnitudes of learning on the contour tasks (main effect of session: $p<0.05$; no session x group interaction: $p>0.05$), which was retained after 3 months. Glass pattern coherence thresholds did not vary between sessions for either group ($p>0.05$), hence improvements were specific to the trained tasks. **Conclusions:** Our results indicate plasticity of the aging human visual system for spatial visual tasks. Evidence from primates suggests that improvements to contour integration from training arise from top-down modulation of neural circuitry in V1 (Li, Pietch & Gilbert, Neuron, 2008). If similar processes are engaged in humans, our results suggest that the capacity for top-down influences to modify neuronal systems in V1 is not compromised by aging.

ORAL-16-08

CHANGES IN MOVEMENT COMPLEXITY ARE ASSOCIATED WITH INCREASED POSTURAL INSTABILITY IN PARKINSONS DISEASE

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Introduction: Decline in physiological function with ageing and neurological disease may result in a loss of system complexity resulting in reduced movement coordination and loss of motor control. Impaired postural control is particularly critical for older people and people with Parkinsons disease (PD) because it can lead to increased risk and incidence of falling. **Methods:** Bilateral resting hand tremor of 63 people with PD (disease duration= 5.6±4.3 yrs; Hoehn and Yahr =1.7±0.6) and 43 age-matched controls was assessed (Coulbourn accelerometers) while they stood quietly on a force plate (Bertec, 100 Hz). Postural sway measures (RMS and Approximate Entropy) in the anterior-posterior (AP) and mediolateral (ML) directions were calculated from force plate centre of pressure recordings. Falls were recorded in calendars over 12 months. **Results:** Resting tremor (4-7 Hz) was of greater magnitude and more regular (lower entropy) for PD fallers compared to PD non-fallers. Older people's tremor was more broadband in appearance and there were no differences between fallers and non-fallers. PD patients had greater AP but less ML postural sway than older people. PD Fallers and Older Fallers had greater ML sway than non-fallers. AP and ML sway were less complex (more regular) for PD participants than older controls. ML sway was more regular for PD Fallers. AP sway was more irregular for Older Fallers. **Conclusions:** Larger and more regular tremor is a characteristic of PD fallers but not older people. PD fallers have more regular (less complex) postural sway whereas older fallers have more irregular postural sway. The pathophysiology underlying postural instability and falls is different for people with Parkinsons and older people.

ORAL-17-01

THE SODIUM CALCIUM EXCHANGER (NCX) AND PLASMA MEMBRANE CALCIUM ATPASE (PMCA2) TOGETHER INFLUENCE PRE-SYNAPTIC RESIDUAL CALCIUM DYNAMICS AT THE CEREBELLAR PARALLEL FIBRE TO PURKINJE NEURON SYNAPSE

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At the facilitating cerebellar synapse between granule cells and Purkinje neurons (the parallel fibre (PF) to Purkinje neuron (PN) synapse) the control of pre-synaptic calcium dynamics is critical for the timing of neurotransmitter release. The plasma membrane Na^+/Ca^{2+} exchanger (NCX) is a key regulator of the distribution of intracellular [Ca^{2+}] and [Na^+] in a variety of excitable cells and is expressed within the cerebellar molecular layer and cerebellar granule cells. NCX is regarded as a low affinity, high capacity calcium clearance mechanism; in contrast PMCA2 is regarded as a higher affinity lower capacity mechanism. Although both mechanisms are present at synapses their cooperation during fast excitatory synaptic transmission and facilitation is unknown. We used wild type and $PMCA2^{-/-}$ transgenic mouse (age 22-32 days) cerebellar slices to record fast fluorescent pre-synaptic calcium signals in response to PF stimulation and whole-cell patch clamp electrophysiology from the post-synaptic PN whilst manipulating NCX activity. Low $[Na^+]_e$ to reduce NCX activity did not significantly influence either the early or later phase of the recovery, or the amplitude of single stimulus-evoked PF calcium transients ($p>0.1$, paired t-test, n=9). In contrast, when the calcium load was increased by paired PF stimulation at 100Hz, and / or by removing the plasma membrane calcium ATPase, PMCA2, low $[Na^+]_e$ prolonged both the early and late phases of PF calcium recovery ($p<0.01$, paired t-test, n=6) without effect upon amplitude ($p=0.2$, paired t-test, n=6). In separate experiments, only in the absence of PMCA2 did inhibition of NCX by low $[Na^+]_e$ and bepridil prolong the recovery of facilitation of PN excitatory post-synaptic currents. Our results indicate that NCX influences calcium recovery from PFs only when the calcium load is sufficiently high (high frequency stimulation) and that other mechanisms such as PMCA2 effectively control calcium during low frequency PF activity.

ORAL-17-02

ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTORS (α7NACHR) CONTRIBUTE TO CEREBELLAR EXCITATORY SYNAPTIC TRANSMISSION

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The cerebellum performs a well-known motor control function but accumulating evidence supports its role in cognitive functions. The nicotinic cholinergic system alters cognition through its actions elsewhere in the brain but its role in cerebellar processing is not understood. However, expression of a subtype of nicotinic acetylcholine receptors is increased in the cerebellum of human autism patients. Here, we explore the expression, localisation and functional contribution of these receptors at cerebellar synapses. Longitudinal cerebellar sections (30 µm) were prepared from C57BL/6 and Swiss Webster male mice (28-48 days old). Positive immunoreactivity for α7nAChR (Abcam) and established excitatory synaptic proteins, Vesicular Glutamate Transporter 1 (VGLUT1; Synaptic Systems) and Post Synaptic Density-95 (PSD-95; Abcam) was visualised using secondary fluorescent antibodies Alexa488 and Alexa594 (Invitrogen) and confocal microscopy. Sagittal cerebellar slices (250µm thick) (C57BL/6 male mice, 21-31 days old) were prepared in artificial cerebrospinal fluid (aCSF) for whole-cell patch clamp recordings from Purkinje neurons (PNs). We recorded excitatory post-synaptic currents (EPSCs) following parallel-fibre (PF) stimulation in aCSF (containing 50µM picrotoxin to block GABA-A receptors) before, during and after 15 minutes application of 10nM methyllycaconitine (MLA; Tocris) a potent α7nAChR antagonist. Series and input resistances varied by <10% throughout. α7nAChRs were abundantly expressed throughout the cerebellar cortex where they overlapped with the PF excitatory pre-synaptic marker protein VGLUT1 (10±1%) and more strongly with the excitatory post-synaptic marker protein PSD-95 (54±3%) ($p<0.0001$, n=3 animals, Mann-Whitney U-test). Overlapping expression of α7nAchRs was not evident at inhibitory synapses. Electrophysiological recordings revealed that 10nM MLA reduced EPSC amplitude, compared with controls, by 30±10% ($p<0.05$, n=5, ANOVA). This study provides the first line of evidence to show that α7nAChR expression in the cerebellum contributes to excitatory synaptic transmission at the important PF-PN synapse. Our findings have wider implications for how nicotinic cholinergic inputs influence cerebellar processing.

ORAL-17-03

ACTIVATION AND DESENSITIZATION INDUCE DISTINCT CONFORMATIONAL CHANGES AT THE GLYCINE RECEPTOR EXTRACELLULAR-TRANSMEMBRANE DOMAIN INTERFACE

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Most ligand-gated channels exhibit desensitization, which is the progressive fading of ionic current in the prolonged presence of agonist. This process involves conformational changes that close the channel despite continued agonist binding. Despite the physiological and pathological importance of desensitization, little is known about the conformational changes that underlie this process in any Cys-loop ion channel receptor. Here we employed voltage clamp fluorometry to identify conformational changes that occur with a similar time course as the current desensitization rate in both slow- and fast-desensitizing α1 glycine receptor (GlyR) chloride channels. Wild-type and cysteine-mutant GlyRs were expressed in Xenopus oocytes, and introduced cysteines were labeled by a sulfhydryl-reactive rhodamine. Current and fluorescence responses were recorded by voltage-clamp and photomultiplier, respectively. Voltage clamp fluorometry provides a direct indication of conformational changes that occur in the immediate vicinity of residues labeled with environmentally-sensitive fluorophores. We compared the rates of current desensitization and fluorescence changes at nine labeled extracellular sites in both wild type slow-desensitizing and mutated (A248L) fast-desensitizing GlyRs. We recorded from >5 oocytes expressing each mutant GlyR. As labels attached to three sites at the interface between the ligand-binding domain and transmembrane domain reported fluorescence responses that changed in parallel with the current desensitization rate, we concluded that they experienced local conformational changes associated with desensitization. These sites included A52C in loop 2, Q219C in the pre-M1 and M227C in the M1. Activation and desensitization were accompanied by physically distinct conformational changes at each labeled site. Since activation is mediated by a specific reorganization of molecular interactions at the extracellular-transmembrane domain interface, we propose that desensitization is mediated by a distinct set of conformational changes that prevents this reorganization from occurring, thereby favouring channel closure.

ORAL-17-04

THE AXON INITIAL SEGMENT IS NOT A SITE FOR HOMEOSTATIC PLASTICITY IN A MOUSE MODEL OF CHILDHOOD ABSENCE EPILEPSY

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Purpose: A novel form of neuronal homeostatic plasticity, occurring at the axon initial segment (AIS), has recently been described. Lengthening of the AIS and movement away from the soma are consequences of changes in neuronal input and result in alterations in neuronal excitability. We hypothesized that such plasticity may play a role in epilepsy, where chronic changes in neuronal activity are a hallmark of seizures. For homeostatic plasticity we expected to see changes in AIS properties which would reduce the excitability of neurons exposed to seizure activity, including movement away from the soma or reduction in length. **Methods:** AIS length and position were analysed using immunohistochemistry in a mouse model of childhood absence epilepsy (CAE). These mice suffer from chronic ictal activity with an average of 30 seizures per hour. We analysed pyramidal neurons in deep layer 5 of the somatosensory cortex from 4 wild-type mice and 5 mice with genetic epilepsy. **Results:** We found no difference in the AIS length or position in these neurons between wild-type and epileptic mice. A power analysis performed on the results indicated that a difference of the magnitude detected in previous studies would have been identified. **Conclusion:** Whilst we did not identify homeostatic plasticity at the AIS, we cannot rule out changes in the distribution of AIS channels and receptors. The absence of detectable AIS plasticity is significant when considering physiological conditions under which plasticity of this type might occur. Examination of graded alterations in neuronal excitation would indicate pathophysiological states in which homeostatic AIS plasticity might occur. Ongoing studies are investigating AIS plasticity in more severe models of epilepsy.

ORAL-17-05

DISTINCT DENDRITIC LOCATION OF CONVERGING INPUTS IN THE MEDIAL AMYGDALA

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PURPOSE: The medial nucleus of the amygdala (MeA) plays a key role in generating defensive and reproductive responses to chemosensory information. It receives direct vomeronasal inputs from the accessory olfactory bulb (AO) as well as processed information from the associational cortical amygdala (CoA). We have previously shown that inputs from both the AO and CoA converge onto neurons in the posteroventral nucleus of the MeA (MePV), moreover, the kinetics of AO evoked responses were significantly slower than those of CoA inputs. Here we examined whether this difference reflects a differential dendritic distribution of AO and CoA synapses. **METHODS:** Whole cell recordings were obtained from MePV neurons (GFP-) in GAD67-eGFP mice in acute brain slices. Synaptic responses were evoked by independent electrical stimulation of the CoA and AO afferents. Cells were filled with fluorescent indicators (Oregon Green – Fluo and Alexa 594) to measure calcium and visualize neuronal morphology. **RESULTS:** To test whether the AO synapses are formed on the distal dendritic branches in this layer, we blocked the synaptic transmission at these branches by focal pressure application of TTX (1μM). TTX blocked the AO synaptic input, while leaving the CoA evoked responses intact (80±0.6 % vs. 6.2±1.6% block; n=6, p<0.001). Stimulation of AO afferents evoked calcium rises that were restricted to the distal dendritic branches in the molecular layer whereas action potentials evoked calcium rises in all compartments **CONCLUSION:** These results indicate that AO and CoA synapses are spatially segregated on MePV neurons. AO inputs are restricted to the distal dendritic tree whereas CoA inputs are more proximally located. These results suggest that vomeronasal and associational inputs in the MeA are differentially processed.

ORAL-17-06

GAMMA HYDROXYBUTYRATE ACTIVATES EXTRASYNAPTIC GABA(A) RECEPTOR SUBTYPES

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Gamma hydroxybutyrate (GHB) is a small molecule with complex pharmacology. At low concentrations, it acts as a neuromodulator. When taken exogenously, it is used to treat narcolepsy and to ameliorate the withdrawal effects of alcohol. It is used as a recreational drug at higher concentrations, sometimes used as a date-rape drug. Despite this, the pharmacology of GHB is unclear. It has been demonstrated that GHB activates the $GABA_B$ receptor at high concentrations, but the receptor that mediates other actions of GHB has yet to be identified. Through biochemical and electrophysiological studies, we have identified that GHB activates a subset of $GABA_A$Rs. The $GABA_A$R is a ligand-gated ion channel that forms a pentameric complex surrounding a central chloride-conducting pore. It is formed by a combination of α(1-6), β(1-3),δ, π, and ε subunits according to a predetermined set of rules. We expressed a variety of known subtypes (>10, n>3 each) in Xenopus oocytes and determined that GHB elicited a chloride current from oocytes expressing α4, β1-3 and δ subtypes. GHB activated oocytes injected with α4, β1 and δ subunits with an EC_{50} of 140nM (n=5). GHB demonstrated selectivity for the β-subunit with rank potency order of β1> β3> β2 when co-expressed with the δ-subunit. This activation was blocked by gabazine, a selective inhibitor of $GABA_A$Rs (n=4). Binding studies of brains from knockout α4 and δ-$GABA_A$R mice demonstrated reduced binding in α4 but not δ-knockout mice. Taken together, these data demonstrate GHB activates α4βδ $GABA_A$Rs with different potencies, but requiring the δ-subunit for the maximum elicitation of a chloride current.

ORAL-17-07

LONG-TERM MODULATION OF N-TYPE CALCIUM CHANNELS BY ORL1 RECEPTORS IN RAT SENSORY NEURONS

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The opioid-related receptor, ORL1, is activated by the neuropeptide nociceptin/orphanin FQ (N/OFQ) and inhibits high-voltage activated calcium channels (VGCCs) via a G-protein coupled mechanism. Endocytosis of ORL1 during N/OFQ exposure was proposed to cause N-type VGCC internalization via physical interaction between ORL1 and the N-channel (Altier et al. Nat Neurosci 9:10, 2006). However, there is no electrophysiological evidence for this mechanism in dorsal root ganglion (DRG) neurons. **Purpose:** To identify a population of N/OFQ-responsive DRG neurons and determine changes in the N-current following prolonged exposure to N/OFQ. **Methods:** We performed whole-cell patch clamp recordings of VGCC currents in DRG neurons and primary afferent eEPSCs in spinal cord slices from male SD rats (2-7 weeks). DRG neurons were classified on the basis of diameter, isolectin-B4 binding and responses to capsaicin-, N/OFQ- and a μ-opioid, DAMGO. **Results:** IB4-negative neurons sized < 20 μm were highly responsive to N/OFQ and DAMGO. ORL1 desensitization in these neurons was not followed by a decrease in proportion of N-current, determined using the N-channel selective blocker, ω-conotoxin CVID (300 nM). There was also no decrease in the proportion of N-current when neurons were incubated at 37°C with 300 nM N/OFQ for 30 min. In spinal cord slices, N/OFQ (1 μM) consistently inhibited primary afferent eEPSCs onto dorsal horn neurons. As observed in DRG neurons, incubation of slices in 10 μM N/OFQ for 30 min produced no decrease in the proportion of eEPSCs inhibited by CVID. **Conclusion:** No internalization of the N-type VGCC occurs in either the soma or nerve terminals of DRG neurons following prolonged exposure to high, desensitizing concentrations of N/OFQ.

ORAL-17-08

EXPRESSION OF CHLORIDE TRANSPORTERS IN THE DEVELOPING MOUSE OLFACTORY SYSTEM

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Purpose: Olfactory sensory neurons (OSN) are uniquely different from other adult CNS neurons as they maintain high intracellular Cl^- concentration [Cl^-] of 50-80 mM to drive Cl^- efflux, which results in a high-gain, low noise amplification of the odorant receptor current. It is thought that the high [Cl^-] in OSN is likely due to the expression of the inward transporter NKCC1 and the lack of expression of the outward transporter KCC2. However previous studies have suggested that there must be other transporters that also accumulate chloride within the OSN. In this study we examined the expression of the Cl^- transporters; KCC2, NKCC1 and NCC, which, like NKCC1 pumps Cl^- into cells using the Na^+ gradient but has previously thought to only be expressed in the kidney. Methods: BALBC mice aged P1 to P60 were euthanised with an overdose of sodium pentobarbital and were perfusion-fixed with 4% paraformaldehyde. Olfactory epithelium and olfactory bulb (OB) were examined using immunohistochemistry to determine the expression and localization of transporters NKCC1, KCC2 and NCC. Results: At P1 (n=4), OSN expressed NCC, NKCC1 and KCC2, while only NKCC1 and KCC2 were expressed in OSN axons that project to the OB and form part of the glomeruli. By P7 (n=4), KCC2 expression had switched to the adult profile and was not detected in the OSN, but was expressed in the mitral cells of the OB. NCC expression was detected in the OSN axons and glomeruli of the OB from p14 onwards. Conclusion: We show that NCC is expressed in the OSN throughout development and may be the missing transporter responsible for the high intracellular [Cl^-] in OSN.

ORAL-18-01

EXPOSURE TO COMPLEX ENVIRONMENTS RESULTS IN MORE SPARSE REPRESENTATIONS OF SPACE IN THE HIPPOCAMPUS

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It is well understood that the neural circuitry mediating sensory and motor representations is adaptively tuned by an animal's environment. **Purpose:** Here we sought to investigate brain mechanisms mediating changes in higher order representations such as spatial memory following exposure to a complex environment (CE). **Methods:** Young male Sprague-Dawley rats were exposed for at least 3 months to CE or social control (SC) conditions, and then tested for either CA1 "place cell" properties or Arc expression 90 min following a 5 min exposure to a novel open field. **Results:** Compared to social controls (SC; 113 cells, 13 animals), prolonged CE exposure was associated with a reduction in the number of active hippocampal place cells that fired in any one location (104 cells, 13 animals). Similarly, fewer CA1 and DG neurons in the CE group (n=4 animals) expressed high levels of Arc protein, a marker of recent activation, following exposure to a novel environment. The reduced Arc expression was not attributable to overall changes in cell density or number. **Conclusion:** These data indicate that CE exposure increases the sparsity of spatial coding within hippocampal networks. High sparsity is potentially a more efficient coding system allowing for rapid learning. Consistent with this hypothesis, CE-treated animals (n=10) habituated more rapidly to a novel environment.

ORAL-18-02

SINGLE-UNIT ELECTROPHYSIOLOGICAL RECORDINGS IN THE NUCLEUS ACCUMBENS AND THE AMYGDALA BEFORE AND AFTER PAVLOVIAN FEAR CONDITIONING IN FREELY-BEHAVING RATS

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Purpose: Substantial data indicate that nucleus accumbens (NAc) neurons encode a variety of appetitive events, including Pavlovian conditioned stimuli (CSs) that have been paired with food. Recent data indicate that these CS-elicited responses in NAc are driven, in part, by projections from the basolateral amygdala (BLA). Given the prominent role of the BLA in aversive conditioning, we explored whether NAc neurons also encode aversive CSs. **Methods:** We used multiple individually-driven tetrodes to simultaneously record from neurons in the NAc and BLA. Following a baseline tone habituation recording session, rats were presented with five tone (2kHz, 10sec, 80dB)-footshock (1sec, 0.6mA) trials. One hour later they received a test session consisting of 20 CS-alone trials. **Results:** Rats showed robust CS-elicited fear at test as measured by freezing. A total of 42 and 62 neurons from LA and NAc were recorded, respectively. In the LA, 35.7% of neurons were tone-responsive (TR; Z-score >3 within 100ms of CS) during habituation. Additionally, 16.7% of LA neurons became TR following conditioning. In contrast, only 1.6% of NAc neurons exhibited TR during habituation, and 6.5% of NAc neurons became tone-responsive following conditioning. Lastly, significantly lower proportion of NAc neurons was TR at any given phase compared to the LA ($Z = 4.83$, $p < 0.01$). **Conclusion:** It appears that the processing of aversive CSs by NAc neurons is fundamentally different than that in the LA. An aversive CS with considerable motivational valence drives LA neuronal firing, whereas the same cue has little effect on NAc firing. Therefore, it is likely that dissociated amygdalo-striatal interactions underlie appetitive and aversive learning.

ORAL-18-03

ADAPTATION IMPROVES NEURAL CODING EFFICIENCY DESPITE INCREASING CORRELATIONS IN VARIABILITY

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Purpose: We quantified the functional effect of adaptation in cortical neuronal responses in the rat whisker-barrel system. We chose this system because of its functional efficiency and its well-studied anatomical organisation. **Methods:** A multi-shank array of electrodes was used to record extracellularly the activity of barrel cortex neurons (n = 73 single units and 86 multi-unit clusters) in anaesthetised rats. We characterised the response of neurons to sinusoidal whisker vibrations of varying amplitude in 3 states of adaptation. Test stimuli comprised vibrations ranging 0-33μm while adaptors had amplitudes of 0, 6μm or 12μm. Mutual Information analysis was employed to quantify the effect of adaptation on neuronal responses and trial-by-trial coding efficiency. **Results:** Neuronal responses, as a function of test stimulus amplitude, were well-fit by a sigmoid curve. Across the population of neurons, the adaptors produced a systematic rightward shift in the response function. Shift magnitude was proportional to adaptor amplitude with no aggregate gain modulation. Mutual information revealed that peak discrimination performance was not aligned to the adaptor but to test amplitudes 3-9μm higher. Simultaneous recordings from multiple neurons (6-25 units) allowed us to quantify trial-by-trial signal- and noise- correlations across neurons. Noise correlation tended to be in the same direction as the signal and decreased as stimulus intensity increased. **Conclusion:** The results reveal that sensory adaptation in the whisker-barrel system enhances discrimination performance most prominently at amplitudes greater than that of the adaptor. Across all population sizes, adaptation improved the total information despite increasing the noise correlation between neurons.

ORAL-18-04

RESPONSE PROPERTIES OF NEURONS IN AREA PROSTRIATA OF THE MARMOSET MONKEY

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Purpose: Area prostriata is located between the rostral tip of the primary visual cortex (V1) and the parahippocampal/retrosplenial cortex. Although prostriata has been hypothesized to be visual based on its proximity to V1, there has been no systematic study of its physiological properties. Here, we report the response properties of prostriata neurons to various types of visual stimuli. **Method**: Single-unit recordings were obtained in marmosets anaesthetised with sufentanil (6 μg.kg-1.h-1) and N2O. The visuotopic organization was mapped densely in one hemisphere (201 receptive fields). Quantification of tuning properties was based on another 98 cells. **Results**: Cells in prostriata have very robust, short-latency visual responses. Despite the large receptive fields (median 36° in diameter), prostriata is visuotopically organized, forming a simple, first-order representation of the visual space. It forms a congruent border with the V1 map, which represents the far peripheral visual field. Whereas prostriata neurons respond strongly to moving bars, they are weakly tuned to orientation, direction of motion and length. They are selective to very high speeds (median optimal speed 115.8°/s). Many prostriata neurons can be driven by stimuli consisting of a few (2-3) large moving dots in their receptive fields, but most do not respond well to large kinetic dot patterns, or flow fields. Only half of the sample exhibited any tuning to the spatial and temporal frequency of gratings. **Conclusion**: These results, together with anatomical evidence of wide-ranging connectivity to non-visual areas, are consistent with the hypothesis that prostriata forms a pathway that rapidly distributes potentially threatening signals throughout the cerebral cortex.

ORAL-18-05

CORRELATION BETWEEN SPIKING AND LOCAL FIELD POTENTIALS IN BEE AND FLY BRAINS

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Purpose: To examine how visual information is processed in the insect brain, we recorded from insect brains at the level of single spiking neurons and local field potentials (LFPs) from neuronal populations while presenting single or competing visual stimuli. Methods: Honeybees (*Apis mellifera*) and fruit flies (*Drosophila melanogaster*) were immobilized and multi-electrodes (Neuronexus Technologies) were inserted into the brain at different visual processing stages from the eye to the central complex. Visual stimulation was performed with light emitting diodes (LEDs). Electrodes were coated with dye to map the recording location. Results: Mapping the anatomical location of the recording electrodes in both bees (n=50) and flies (n=30), revealed that central brain structures exhibited significantly higher LFP variance compared to the primary visual processing centres at rest ($p<0.05$ for all comparisons). Stimulation with light cues showed characteristic LFP responses similar to event-related potentials found in human electro-encephalograms (EEG). The event related potentials varied with location of the electrodes in both flies and bees, with a positive going peak in the eyes, a negative going peak closer to central brain areas, as well as complex waveforms in the central brain which varied in amplitude depending on the location of the light stimulus. Examination of the relationships between a total of 133 spiking cells in bees and event-related potentials revealed that correlated activity between the spiking cells and LFP depended on electrode brain location. Conclusions: We found LFP activity varies similarly across brain structures in two different insect species. Relating LFPs to spiking activity in the bee will enable us to investigate convergent neural mechanisms of processing complex visual scenes, whereas genetic analysis in *Drosophila* will allow us to functionally test those mechanisms.

ORAL-18-06

RELATION OF LOW FREQUENCY ELECTROENCEPHALOGRAM SIGNALS TO KONIOCELLULAR PATHWAY ACTIVITY IN ANAESTHETISED MARMOSETS

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Purpose: Koniocellular (KC) cells in the lateral geniculate nucleus (LGN) exhibit slow fluctuations in maintained discharge rate in the absence of patterned visual stimuli. The discharge rate is inversely proportional to low frequency (<15 Hz) electroencephalogram (EEG) power [1]. Here we examined this phenomenon more closely by relating KC cell activity to different subdivisions of the <15 Hz EEG frequency range. **Methods:** Extracellular spike activity of KC cells (n=19) in LGN and local field potential over primary visual cortex (V1) were recorded in sufentanil-anaesthetised marmosets (*Callithrix jacchus*, n=5). Visual stimulus was a uniform grey field ~15 degrees square at close to 50 Cd / m^2. Linear regression analysis was performed on LGN cell maintained discharge rate and V1 local field potential strength across four different frequency bands (delta [0.3 – 5 Hz], theta [5 – 8 Hz], alpha [8 – 12 Hz] and beta [12 – 30 Hz]). The KC cells that showed spike rate variation > 10 impulses per second (7/19, 37%) over the recording epoch were analysed. **Results:** KC cells show a negative correlation between maintained discharge rate and the delta frequency band (-0.23±0.10, mean±SD, n=7), and the theta frequency band (-0.17±0.12). The other frequency bands showed no correlation with maintained discharge rate. **Conclusion:** These results indicate that delta and theta frequency oscillations can influence sensory information processing in the KC layers of the LGN. **Reference:** [1] Cheong S.K. et. al., (2011) PNAS 35, 14659-14663.

ORAL-18-07

SUPRACHOROIDAL VISUAL PROSTHESIS: ASSESSING THE EFFICACY OF VARYING STIMULUS PARAMETERS BY CHARACTERIZING CORTICAL POPULATION RESPONSE

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Purpose: To characterize responses in the primary visual cortex to different stimulus parameters using suprachoroidal visual prostheses. **Methods:** A 7x12 platinum disc electrode array was acutely implanted in the suprachoroidal space in normal sighted feline eyes (n=10). Constant biphasic current pulses were presented through a single suprachoroidal electrode (420 µm diameter) with a remote return (n=15). We varied the pulse polarity, pulse width and inter phase gap of the constant current pulses. Cortical responses (n>350) to electrical stimulation were recorded using multichannel electrodes implanted in the (V1) primary visual cortex. **Results:** Cortical response thresholds to anodic and cathodic pulses were not significantly different (Paired t-test, $p>1.000$). Monophasic pulses evoked lower thresholds (4 dB) than biphasic pulses with the same polarity and duration leading phase. Increasing the temporal separation of the two phases of a biphasic pulse resulted in up to a 3 dB reduction in threshold. There was also a systematic reduction in current threshold with increasing pulse width, which asymptoted at pulse widths greater than 1000 µs.**Conclusion:** These results show that the threshold for suprachoroidal stimulation is time dependent at shorter pulse widths < 800 us and charge-dependent at the longer pulse widths >800 us. Furthermore, these results suggest that pulse widths between 400-800 us should be used for suprachoroidal visual prostheses.

ORAL-18-08

SIMULTANEOUS MULTI-SITE NONLINEAR PHOTOSTIMULATION IN 3D

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Purpose: We report the first demonstration of simultaneous 3D multisite nonlinear photostimulation on dendrites. Scanning techniques offer pseudo-simultaneous photostimulation but are restricted to a single optical plane. Using holographic projection of multiple foci in 3D, we describe a means to extend synaptic integration studies to the entire dendritic tree. **Methods:** Our setup includes a two-photon fluorescence microscope for rendering the 3D structure from which the stimulation sites are chosen. A spatial light modulator encoded with the appropriate phase-hologram is used to project the stimulation foci. At each focus, highly localized two-photon uncaging of neurotransmitters is achieved. To verify this technique, experiments were done in 300µm thick slices of rat somatosensory cortex (15-19d). Whole-cell recordings of layer II pyramidal cells were done with an AxoClamp-2B. For imaging, 10mM fluorescein was added to the pipette solute. 10mM MNI-glutamate was released from a blunt glass electrode close to the cell at a constant pressure (0.5kPa). Uncaging was done with a Ti:S laser (Mira-900s pumped with 5W Verdi). **Results:** Glutamate-induced currents (25-60pA) were found to vary quadratically with power, characteristic of 2P-absorption. Sample lateral and axial resolutions of uncaging based on laser power setting and pulse-widths (5-10ms) were 3.9 and 4.1µm (half-widths), respectively. We performed simultaneous uncaging at multiple sites on different dendrites extending into different planes and observed sublinear summation at synaptic inputs onto shafts. **Conclusion:** This technique allows simultaneous uncaging at multiple 3D-locations on dendritic trees. The holographic generation of any desired spatial light pattern offers unprecedented flexibility in designing input patterns for synaptic integration studies.

ORAL-19-01

HIGH FAT FEEDING AND COGNITIVE IMPAIRMENT

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Recent evidence demonstrates an association between obesity and cognitive decline. The present study aimed to determine whether very high fat (HF) or western diet (WD) can affect working or spatial memory in rats; and whether diet-induced cognitive impairment is linked to the brain acetylcholine system. Three groups of male Long Evans rats were fed either normal chow (Con), WD (21% fat, 0.15% cholesterol) or HF diet (60% fat) for 12 weeks (n=12/group). Body weight, food intake and blood pressure were measured weekly. Behavioural testing (Y-maze and object recognition) was carried out at week 12. At the end of the study brain choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) levels were estimated. Consumption of a WD significantly increased body weight ($p<0.001$) and blood pressure ($p<0.05$), while a HF diet produced no significant differences compared to Con. WD ($p<0.05$) and HF ($p=0.05$) diets had a significant negative impact on performance of rats in the Y-maze; but not on discrimination scores in the object recognition task. AChE activity increased significantly in the striatum of the rats after a HF diet ($p<0.05$), whereas levels of ChAT did not change with a HF or WD diet. These results demonstrate that consuming a HF or WD for 12 weeks impairs spatial memory but not working memory in rats. This effect is independent of changes in weight or blood pressure and does not appear to be associated with changes in the brain acetylcholine system.

ORAL-19-02

NATURAL VS SYNTHETIC ESTROGENS: EFFECTS ON PREPULSE INHIBITION AND HIGHER-ORDER COGNITIVE FUNCTION

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PURPOSE: The oral contraceptive pill is the most common form of contraception amongst young Australian women. Given its prevalent use, this study aimed to investigate its effects on cognitive function and compare this group to women at two different stages of the menstrual cycle and to men. **METHODS:** This study tested age- and IQ-matched healthy women at different stages of the menstrual cycle (early-follicular "Low E/P" n=15, mid-luteal "High E/P" n=14), women taking the combined oral contraceptive pill ("Pill" n=16) and men ("Male" n=15). Assessment of prepulse inhibition (PPI) included 21 pulse-alone trials (115 dB) and 42 prepulse-pulse trials (7 of each prepulse: 74, 78, 86 dB). The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used to assess five cognitive domains: immediate memory/learning, delayed memory, visuospatial ability, language and attention. **RESULTS:** Overall, there were no significant differences in PPI between the four groups. However, when comparing only the High E/P and Low E/P groups, there was a significant difference at the 60 ms stimulus onset asynchrony, whereby the Low E/P group had greater PPI. The Pill and High E/P groups significantly outperformed the Male group on the Total RBANS score, a measure of overall cognitive function. Further, for the immediate memory/learning and attention domains, the Pill group outperformed the Male group. For the delayed memory domain, the Pill and High E/P groups outperformed Males. **CONCLUSION:** Different hormonal states in women, whether natural variations across the menstrual cycle or induced with the contraceptive pill, had little effect on baseline prepulse inhibition. In contrast, high levels of circulating estrogen and progesterone had a positive effect on higher-order cognition.

ORAL-19-03

EARLY TRANSIENT IMPAIRMENTS IN DOPAMINE SYNTHESIS PRODUCE PERSISTENT BEHAVIORAL ALTERATIONS IN THE ADULT

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Introduction: Schizophrenia is both a disorder of neurodevelopment and dopamine (DA) signaling. Our group is pursuing the idea that gestational events, which are known epidemiological risk factors for schizophrenia i.e. infection, hypoxia and low vitamin D, produce early alterations in DA neuron development which contribute to disease phenotype in the adult. **Methods:** To obtain a better understanding how early alterations in DA signaling could induce long-term effects on DA related behavior we used morpholino oligonucleotides (MO) in zebrafish embryos to transiently suppress Tyrosine hydroxylase (TH) (the rate-limiting enzyme for DA synthesis) gene function, during early development. TH content was assessed by qPCR and western blot analysis, the distribution of TH-positive cells by immunofluorescence and DA levels by HPLC. Subsequently, various behavioral assays such as novel open field, novel diving test and acoustic startle response (ASR) were performed in adult fish. **Results:** Depending on the MO used, we could achieve either a partial or complete loss of TH positive neurons in the larval brain with corresponding levels of DA produced. Consistent with the transient nature of MO interventions, TH expression and DA content had returned to normal levels in adult brains. Our data show that although locomotor behaviour in the adult fish appeared normal, the morphants show anxiolytic behaviour and increased latency in their startle response at mild startling frequencies. **Conclusions:** Here we demonstrate that early alterations in DA signaling induce anxiogenic and impaired escape behavior in the adult fish even though TH and DA content have returned to normal. Prepulse inhibition and behavioral pharmacology studies are ongoing. TH loss-of-function zebrafish may represent a useful model for examining how early abnormalities in DA ontogeny produce persistent abnormalities in DA signaling in adults.

ORAL-19-04

THE RESPONSE OF HONEYBEES TO MULTIMODAL STIMULI IN A VIRTUAL REALITY FLIGHT ARENA

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Purpose: In the natural world, honeybees use sensory information to facilitate a range of complex behaviours. However, the majority of these behaviours have been studied in free flight, where it can be difficult to precisely control the environment the insect perceives. To address this, we have developed an assay where a tethered honeybee can be exposed to precisely controlled visual and wind stimuli whilst in flight. **Methods:** Tethered honeybees fly inside a virtual reality flight arena, and are exposed to open loop stimuli consisting of combinations of optic flow (100 - 600 deg/s) and airflow (0 - 3 m/s). The honeybee's abdomen angle, or streamlining response, is measured as a behavioural indicator. **Results:** Optic flow appears to be required for reliable flight in tethered honeybees. Without wind, optic flow at 260 deg/s ensures that all tested bees complete two minute flights. Without optic flow, wind at 3 m/s elicits sustained flight in only 70% of honeybees (four airflow conditions, n = 8 per condition). When exposed to increasing optic flow (with or without airflow), honeybees actively raise their abdomens into a streamlined position. Exposing honeybees to increasing airflow generally results in increasingly streamlined flight positions, peaking at 2.5 m/s (seven airflow conditions, $n \geq 7$), but with a second, unexpected peak at 0.5 m/s. **Conclusion:** In tethered honeybees, optic flow rather than airflow is required to elicit reliable sustained flight. However, both of these stimuli interact to affect the honeybee's active streamlining of its abdomen. This tethered flight assay provides a novel approach to investigating the honeybee's response to multimodal stimuli.

ORAL-19-05

SLEEP STAGES IN *DROSOPHILA*

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Purpose: All animals sleep. Thus, sleep must perform a crucial function that justifies these periods of increased vulnerability. Nevertheless, its function is still debated. Sleep is characterized by immobility, increased arousal thresholds and increased rest after prolonged wake. During sleep, different stages (REM and nREM, or dreaming and deep sleep) can be distinguished electrophysiologically in vertebrates. However, sleep in *Drosophila* is still quantified as a binary process, where flies are either immobile and asleep or mobile and awake. We investigated whether different sleep stages exist in *Drosophila*. **Methods:** Adult *Drosophila* (male and female, n = 51 per group) were collected, individually housed in glass 65 mm tubes and periodically subjected to mechanical startle stimuli over several consecutive days. Fly activity was measured from filmed experiments, and increased locomotor activity was used to quantify arousal thresholds. Sleep states were inferred from arousal thresholds, where increased arousal thresholds correlate with deeper sleep. **Results:** *Drosophila* arousal thresholds showed circadian variation (flies were more responsive to stimuli by day and less responsive at night ($p<0.001$)), and night-time sleep was deeper than daytime sleep ($p<0.001$). Interestingly, during prolonged periods of inactivity, arousal levels were not uniform but cycled through periods of high and low arousability. Periods of lowest responsiveness occur after ~15 and ~45 minutes of inactivity. 24 hours of sleep deprivation decreased daytime responsiveness by 45-55% and night-time responsiveness by 40-60%, suggesting that this decreased responsiveness plays an important role in sleep homeostasis. **Conclusion:** Like other animals, sleep in *Drosophila* consists of alternating stages of light and deep sleep, even though both stages are characterized by extended immobility. During daytime sleep, flies are more arousable than during night-time sleep, suggesting that day and night-time sleep fulfill different functions. We are currently investigating whether sleep patterns are altered in a *Drosophila* model of schizophrenia.

ORAL-19-06

SENSORY INTEGRATION IN THE RAT WHISKER SYSTEM: SPEED VERSUS ACCURACY IN A PERCEPTUAL DECISION PARADIGM

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Purpose: Perceptual decision making is often explained in terms of an integration process that accumulates sensory information over time towards a decision criterion. Here we investigate sensory integration in the whisker system; a highly efficient sensory apparatus that is actively used by rodents to navigate the environment. **Method:** We designed a behavioural paradigm for vibro-tactile detection/discrimination. Rats initiated a trial by nose-poking into an aperture where their whiskers came into contact with two meshes. A continuous nose-poke for a random duration (0.5 to 0.6sec) triggered stimulus presentation. Rats indicated the target stimulus by choosing between two reward spouts. In Exp.1 stimuli consisted of a sequence of discrete Gaussian deflections of the mesh that increased linearly in amplitude over time. Time to maximum amplitude varied from 0.5 to 8 seconds. Exp.2 used similar stimuli but amplitude remained constant within trial. In both experiments the target was the stimulus with the higher intensity. Two rats completed 1425 trials per condition in Exp.1 and 598 trials per condition in Exp.2. **Result:** For both experiments, regardless of condition, rats' performance improved as a function of stimulus sampling duration, until approximately 250msec of sampling and plateaued thereafter. Rats' performance dropped with increasing task difficulty. For both rats and all conditions the median reaction time was longer for correct trials compared to incorrect trials. At any given sampling duration, the performance was used to quantify signal integration. Integration times increased systematically across 5 levels of task difficulty. **Conclusion:** In a vibro-tactile decision task, rats show evidence of sensory integration and speed accuracy trade off. During training rats learned to adjust their sampling time to optimise the reward collection.

ORAL-19-07

NEUROPHARMACOLOGICAL INVESTIGATION OF N40 SENSORY GATING IN THE RAT BRAIN: RELEVANCE TO SCHIZOPHRENIA

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Purpose: Schizophrenia patients have disruptions in sensory gating, a normal brain filtering mechanism which occurs in response to repeated auditory stimuli. Sensory gating can be measured by presenting two clicks of sound and measuring the response with electroencephalography (EEG). In healthy subjects the response to the second click is diminished, however in schizophrenia patients the response is similar for both sounds. In rodent studies this filtering mechanism is referred to as N40 sensory gating, however little is known about the brain pathways that underlie this mechanism. We therefore investigated the acute effects of several psychoactive compounds that interact with dopaminergic, glutamatergic and/or 5-HT receptors. **Methods:** Male Sprague-Dawley rats (n=9-13 per group) were implanted with cortical surface electrodes and allowed one week to recover. Dose-response test sessions were conducted 2-3 days apart and comprised of 150 presentations of two 85 dB bursts of white noise, 500 ms apart (S1 and S2). **Results:** Treatment with the dopamine receptor agonist, apomorphine, the dopamine releaser, amphetamine, the NMDA receptor antagonist, phencyclidine, the serotonin-2A receptor agonist, DOI, and the serotonin releaser, MDMA (Ecstacy), all caused a dose dependent increase in N40 sensory gating ratios (S2/S1) in the rat brain, indicating a disruption of normal sensory gating. We are currently undertaking component analysis to reveal if selected aspects of the neurophysiological responses (P15 vs. N40) show a differential pattern of change depending on the drug treatment used. **Conclusion:** These results help us to understand neural processes that underlie N40 sensory gating. Pharmacologically-induced sensory gating deficits in rats were reminiscent of those observed in schizophrenia patients.

ORAL-19-08

THE CONTINUOUS PERFORMANCE TASK: ASSESSING COGNITIVE DEFICITS IN RATS

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Purpose: Schizophrenia is characterised by hallucinations and delusions however cognitive symptoms have a strong influence on both daily functioning and patient outcomes. In particular, attentional deficits have been persistently reported in patients and first-degree relatives using the continuous performance task (CPT). The aim of this study was to assess behaviour on two attentional tasks using a rodent model of schizophrenia, Developmental Vitamin D (DVD) deficiency, and relate these data to the human CPT task. **Methods:** Adult DVD-deficient (n=16) and control rats (n=12) were assessed on the 5-choice serial reaction time task (5CSRTT) and the 5-choice continuous performance task (5C-CPT). Measures such as accuracy, impulsivity and response inhibition were recorded. The effects of antipsychotics on 5C-CPT were then assessed. **Results:** Performance was not altered on the 5CSRTT. However on the 5C-CPT DVD-deficient rats demonstrated reduced response inhibition on no-go trials ($p<0.05$). Under these conditions, they also showed increased impulsivity ($p<0.05$). Clozapine, but not haloperidol, reversed the inhibition deficits in DVD-deficient rats ($p<0.05$). The rodent tasks measure attention and impulsivity, however in contrast to the human CPT they do not utilise working memory or context cues. **Conclusion:** While DVD-deficient rats are able to successfully perform the basic task, under a more demanding paradigm deficits were observed. Clozapine was able to reverse this deficit. It was noted that some key aspects of the human CPT task are not implemented in either the 5CSRTT or 5C-CPT. Application of these rodent tasks in the translational study of cognitive symptoms relevant to schizophrenia is discussed.

ORAL-20-01

INSIGHTS INTO THE MECHANISM REGULATING THE APPROACH AND DOCKING OF SECRETORY VESICLES TO THE PLASMA MEMBRANE IN CHROMAFFIN CELLS

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Neurotransmitter release is a highly regulated process allowing neurons and neurosecretory cells to communicate with their target cells. This process relies on the timely interaction between proteins and lipids driving the process of neuro-exocytosis leading neurotransmitter-containing vesicles to fuse with the plasma membrane thereby releasing their content. Phosphoinositides are a group of cytosol-facing phospholipids that are involved in a multiple of physiological processes including exocytosis, endocytosis, cell death etc. Their level of phosphorylation depends on a number of PI-kinases and phosphatases whose activity and localization underpins the landscapes of phosphoinositides in the cells. In neurosecretory cells, vesicles undergo docking and priming before Ca^{2+}-dependent fusion with the plasma membrane. The synthesis of the phosphoinositide phosphatidylinositol(4,5)-bisphosphate (PtdIns(4,5)P_2) is required for both priming and the latest stage of exocytosis. **Purpose:** Although the role of PtdIns(4,5)P_2 in triggering fusion is relatively well-characterised its precise contribution to priming is still unclear. **Method:** We use IC87114, an inhibitor of type I PI3-kinase δ isoform, to promote a transient increase in PtdIns(4,5)P_2, leading to a potentiation of exocytosis in chromaffin cells. We exploit this pathway to examine the effect of a transient rise in PtdIns(4,5)P_2 on neurosecretory vesicles behavior, outside the context of a secretagogue stimulation. **Results:** Our data demonstrate that such PtdIns(4,5)P_2 transient rise is sufficient to trigger the mobilization and recruitment of secretory vesicles to the plasma membrane via Cdc42-mediated actin reorganization. **Conclusion:** PtdIns(4,5)P_2 therefore controls the conveyance of secretory vesicles to the plasma membrane through cdc42-mediated actin polymerization.

ORAL-20-02

VITAMIN D SIGNALLING IN DEVELOPING BRAIN

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Purpose: Schizophrenia is a neurodevelopment disorder. We have shown that Developmental vitamin D (DVD) deficiency is a risk-modifying factor for schizophrenia. Vitamin D is a nuclear steroid with well-described anti-proliferative actions in brain tissue. We have recently described the ontogeny of the receptor for vitamin D (VDR) in the embryonic rat mesencephalon. As part of that study we discovered what appeared to be an interesting post-translational modification of this receptor. The aim of this study was to understand the nature of this post-translational change. **Method:** Forebrains were obtained from Sprague-Dawley rats at both embryonic day E18 and as adults. Animals were euthanized with sodium pentobarbital and both adults and embryos were perfused-fixed for immunofluorescence. A separate cohort of forebrain and kidney samples again at both ages were obtained, nuclear and cytosolic fractions prepared, and VDR expression examined by Western blots. **Results**: In the embryonic brain VDR immunoreactivity in brain was strictly nuclear. In the adult brain again VDR presence was strongly nuclear but also appeared to be have a faint somal distribution. However western blots unambiguously confirmed that VDR was strictly nuclear at both ages. Closer examination of these blots revealed the VDR in the embryonic brain was a slower migrating form. This general pattern of expression was confirmed in kidney but there appeared to be far less of the slower migrating band in the embryonic kidney compared to embryonic brain. The embryonic VDR band was abolished when pre-treated with alkaline phosphatases suggesting the slower migrating band may represent a phosphorylated form of the receptor. **Conclusion**: We have previously shown that the timing of VDR appearance in developing brain coincides the cessation of proliferation. Our latest data lead us to speculate that the VDR in proliferating tissue is phosphorylated and this may be more important in developing brain. We are now attempting to determine the sites of phosphorylation within the embryonic VDR by mass spectrometry. Depending on the exact phosphorylation site these findings may help to explain the anti-proliferative actions of vitamin D in developing brain.

ORAL-20-03

STEM CELL THERAPY PLUS VOLUNTARY RUNNING RESCUES MEMORY DYSFUNCTION IN A RODENT MODEL OF BRAIN AGEING

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Background: Age-related memory loss is characterized by degeneration and dysfunction of distributed synaptic networks. Both voluntary running (VR) and stem cell transplantation are possible therapeutic strategies, but to date the advantage of combining these approaches has not been investigated. **Methods:** 18-month older female Fischer rats (N=31) were randomly allocated into one of 4 possible factorial conditions: 1) 6 weeks VR plus sham transplant (VR+SHAM), 2) VR plus skin-derived neuroprecursor transplantation (VR+NPC), 3) standard housing plus NPC transplantation (SH+NPC), and 4) standard housing plus sham surgery (SH+SHAM). Pre- and post- testing included Place- and Object- recognition memory tests (PRM and ORM). After sacrifice, hippocampal synaptic density, neurogenesis, NPC migration and survival were measured based on single and combined histological markers. **Results:** Older rats were impaired on PRM but not ORM compared to 3-month old animals, which correlated with loss of synaptic density in several hippocampal subfields. VR by itself led to the restoration of synaptic density and synaptic network topology, which together predicted improvement in PRM performance (Effect size relative to SH+SHAM *d=1.89*). VR stimulated neurogenesis, but this was not linked to PRM or ORM. Intrahippocampal NPC transplantation also led to significant PRM improvement (*d=1.07*), however, NPC+VR led to better PRM outcomes *(d=1.74).* Initial mapping of NPC migration and survival indicates that VR increases the likelihood of cells engrafting into the dentate gyrus and CA1. **Conclusions:** In a naturalistic animal model of memory dysfunction, we found that VR plus stem cell transplantation led to better memory outcomes than cell therapy alone. Physical exercise may enhance donor cell targeting of hippocampal memory circuits.

ORAL-20-04

PHYSICAL EXERCISE INCREASES PRECURSOR CELL NUMBERS WITHIN THE NEUROGENIC REGIONS OF THE VERY AGED MURINE BRAIN

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Purpose: Voluntary physical exercise has been demonstrated to have positive effects in relation to hippocampal neurogenesis in both young and aged animals. More recently we described, for the first time, that physical exercise activates precursor cells within the subventricular zone (SVZ) of aging mice. Interestingly, following an acute running paradigm of 21 days, animals of very advanced age (24-months) demonstrated a decrease in neurosphere number, rather than the expected increase seen in younger animals. We therefore investigated whether the precursor cell population could be activated with a longer exercise regime. **Methods**: Female C57Bl/6 animals 24 months of age were housed either with or without access to running wheels. At weekly intervals, animals were sacrificed and precursor cell activation was assessed in the SVZ and the hippocampus, using the neurosphere assay. **Results**: Up to three weeks of exercise resulted in a decrease in SVZ neurosphere number (472±23 to 221±25, $p<0.01$) and no change within the hippocampus (12.6±2.6 vs. 13±2.5). However, the 35-day paradigm resulted in significant increases in SVZ (567±36 vs. 872±60, $p<0.01$) and hippocampal (6.7±2 vs. 27.3±4.1, $p=0.01$) neurosphere numbers. **Significance**: As activation was observed in both of the major neurogenic regions, populations of endogenous neural precursor cells exist in very aged animals. However, the activation process appears to require a more prolonged stimulation than in younger animals. Determining the optimal stimulation period will have important implications for people affected with cognitive decline.

ORAL-20-05

INVESTIGATION INTO THE ANTIOXIDANT AND CYTOPROTECTIVE PROPERTIES OF "ANTI-AGEING" HERBAL MEDICINES

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Purpose: Age is the leading risk factor for neurodegenerative diseases. Herbal medicines have been used for centuries to ameliorate deleterious effects of ageing-related diseases and increase longevity. Oxidative stress plays a role in normal ageing as well as in neurodegenerative processes. Since many of the constituents of herbal extracts are known antioxidants, it is believed that restoring oxidative balance may be one of the underlying mechanisms by which medicinal herbs can protect against ageing and cognitive decline. **Methods:** Thirteen herbal extracts purported by traditional Chinese medicine to possess anti-ageing properties were tested for their ability to protect U373 human astrocytes from hydrogen peroxide toxicity using the resazurin reduction assay. To determine the contribution of antioxidant activity to the cytoprotective ability of extracts, total phenol content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity and oxygen radical absorbance capacity were determined for each extract. **Results:** *Polygonum multiflorum*, amongst others, was identified as possessing potent antioxidant and cytoprotective properties (IC50=≤0.2µg/ml). We also identified that total phenol content of extracts was strongly correlated with antioxidant capacity (r=0.808). However, when total phenol content and radical scavenging capacities of extracts were compared to the cytoprotective properties of extracts, only moderate correlations were observed (r=≤-0.699). *Conclusion:* Results showed that anti-ageing herbal medicines are the source of many potent antioxidant and cytoprotective agents. Furthermore, total phenol content and antioxidant activity of extracts are only moderately correlated with their cytoprotective ability, suggesting the involvement of multiple protective mechanisms in the beneficial effects of medicinal herbs. Herbal medicines might therefore provide potential therapeutic options for complex, multi-factorial diseases, such as Alzheimer's disease and other neurodegenerative and oxidative stress related disorders.

ORAL-20-06

SEZ-6 PROMOTES EXCITATORY SYNAPSE DEVELOPMENT THROUGH α2δ

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Development of appropriate neuronal circuitry in the mammalian central nervous system is essential for learning, memory and cognition. Seizure-related gene 6 (Sez-6) is required for dendritic arborization and excitatory synapse development of cortical pyramidal neurons. As Sez-6 proteins contain CUB and SCR protein interaction domains, we hypothesized that Sez-6 would signal through a protein complex at the cell surface. **Purpose:** To investigate Sez-6 signaling pathways promoting excitatory synapse development. **Methods:** Precipitation of Sez-6 from embryonic brain (E17 wild-type and Sez-6 null controls) using a Sez-6 polyclonal antibody was followed by identification of co-immunoprecipitated proteins by mass spectrometry. Candidate proteins were verified using specific antibodies, exogenous expression in Cos-7 cells and lipid raft sub-cellular fractionation. Synaptogenesis was measured in Sez-6 knockout mouse cortical neuron cultures and in rat retinal ganglion cell cultures treated with secreted Sez-6. **Results:** The voltage-sensitive calcium channel (VSCC) subunit α2δ was precipitated with Sez-6 from brain extracts and from Cos-7 cells after exogenous expression. α2δ was enriched, and Sez-6 was also present, in lipid raft fractions from E17 brain although most Sez-6 co-localized with transferrin receptor in the recycling endosome. Excitatory synapses were initially more abundant on Sez-6 knockout neurons (relative to controls) although this situation was reversed by the third week in culture. Secreted Sez-6 induced excitatory synapses in retinal ganglion cells and blockage of this effect by gabapentin indicated that it occurred through α2δ. **Conclusion:** Sez-6 enhances excitatory synapse development through an interaction with α2δ.

ORAL-20-07

MOLECULAR MECHANISMS OF NDFIP1 DURING BRAIN DEVELOPMENT

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The modification of proteins by ubiquitination is important in brain development. Ndfip1 (Nedd4 family-interacting protein 1) is an important player in the ubiquitination of target proteins through the Nedd4 ligase pathway. Purpose: In the present study, we investigate the expression pattern and molecular function of Ndfip1 in cortical development. Methods: Immunohistochemistry and western blot analysis were performed to investigate the level of Ndfip1 and Spry2 during cortical development. Co-immunoprecipitation assay was performed to confirm the interaction of Ndfip1 and Spry2. Result: During cortical development, Ndfip1 is differentially expressed over time in different regions of the cortical wall. In the early stages (E11), Ndfip1 is highly expressed in the proliferative cells of the germinal zones where neurons are born. As development progresses (E15 onwards), Ndfip1 expression shifts to the mature neurons in the cortical plate, with concomitant reduction in the ventricular zone. This dynamic shift from proliferative to non-proliferative regions of the cortex suggests important roles for Ndfip1 in the cell proliferation. To explore this, we have investigated the relationship between Ndfip1 and Sprouty2 (Spry2), an inhibitor of cell proliferation via the MAPK-ERK pathway. Our work shows that Ndfip1 binds to Spry2, in endogenous and over-expression systems. In a neuronal cell line (SY5Y), artificial over-expression of Ndfip1 results in reduction of Spry2, suggesting that Ndfip1 can regulate Spry2 levels. Conversely, in *Ndfip1-/-* fibroblasts, levels of Spry2 are upregulated. This upregulation of Spry2 is associated with attenuated epidermal growth factor-elicited ERK1/2 signalling. Conclusion: The association of Ndfip1 with Spry2 might be important for the regulation of cell proliferation via the ERK-signalling pathway.

ORAL-20-08

DEFECTS IN NFIB KNOCKOUT MICE REVEAL NOVEL MECHANISMS OF PREPLATE SPLITTING

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Formation of the cortical preplate and its subsequent splitting into the marginal zone and subplate are crucial steps in the lamination of the cortex. Nuclear factor I (Nfi) genes are transcription factors known to regulate brain development and axon pathfinding. Purpose: To investigate the phenotype of Nfib knockout mice which display an increase in the number of cells within the marginal zone but a decrease in the number of cells that will eventually form the subplate. Methods: BrdU birthdating, immunohistochemistry and in utero electroporation were used to examine the phenotype of Nfib and Nfib;Golli-tauGFP mice. Results: NFIB was co-localised with reelin in the marginal zone but was not expressed in GABAergic interneurons. At E18, there was a 30% increase in the number of calretinin-positive cells and reelin-positive Cajal-Retzius cells in the marginal zone of Nfib knockout mice, but not an increase in calbindin positive cells (n=3). Thus, the increase in cell number in the marginal zone is not due to an increase in the number of GABAergic interneurons migrating from the ventral forebrain. Rather, the number of preplate cells that differentiate into marginal zone neurons is increased in Nfib knockout mice. No differences in proliferation or cell death nor other gross defects in the lamination of the cortex accounted for this. Nfib; Golli-tauGFP reporter mice corroborated these findings. Conclusion: Nfib regulates the development of the preplate, marginal zone and subplate, but that this disruption in preplate splitting does not result in other lamination defects within the cerebral cortex.

ORAL-21-01

THE GABAA γ2R43Q MUTATION CAUSES TWO DISTINCT SEIZURE PHENOTYPES THROUGH DIFFERENT MOLECULAR MECHANISMS

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Patients with a *GABRG2* mutation resulting in a R43Q substitution in the encoded γ2 subunit of the GABAA receptor (GABAA γ2R43Q) have childhood absence epilepsy and febrile seizures (FS). A syndrome-specific mouse model based on the mutation recapitulates both these seizure phenotypes. Haploinsufficiency (the loss of function of one allele) and a dominant impact, where the disease allele influences the expression of other GABAA receptors have both been proposed as molecular mechanisms underlying seizure genesis. **Purpose:** We compare the seizure phenotypes of the GABRG2 knockout (GABAA γ2KO) haploinsufficient mouse with the knockin GABAA γ2R43Q model to determine the molecular mechanism underlying pathogenesis. **Methods:** Video-EEG recording were made from P40 heterozygous mice for a total of 12 hours to assess absence seizure phenotype. The FS phenotype was ascertained by exposing mice to a constant 42°C and measuring the latency to first clonic-tonic seizure. **Results:** EEG analysis revealed that the GABAA γ2KO had clear spike and wave discharges on EEG associated with behavioural arrest that were sensitive to the first line antiepileptic drug ethosuximide. Seizure severity in the GABAA γ2KO was similar to the GABAA γ2R43Q model suggesting the haploinsufficiency was sufficient explain absence seizures in both models. In contrast heightened susceptibility to thermally triggered seizures was only seen in the GABAA γ2R43Q model. **Conclusion:** GABRG2 haploinsufficiency is the underlying molecular basis of the absence seizure phenotype while the molecular basis of FS depends on a dominant effect of the GABAA γ2R43Q mutated channel.

ORAL-21-02

ENVIRONMENTAL ENRICHMENT AND LIMBIC EPILEPSY: ANXIETY, LEARNING AND SYNAPTIC REORGANISATION

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Purpose: Temporal lobe epilepsy is commonly accompanied by neuropsychiatric and neurocognitive comorbidities. Recent animal studies have highlighted enhanced seizure susceptibility, mood disturbances and neurocognitive impairments following stress. We therefore hypothesized that positive experiences, in the form of environmental enrichment (EE), may have neuroprotective, anti-epileptic and psychoprotective effects. **Methods:** At weaning, male Wistar rats were randomly allocated into either EE (large plastic cages containing running wheels and toys) or Impoverished Housing (IH; standard laboratory cages). At P63, a bipolar electrode was implanted into the left amygdala, followed by rapid amygdala kindling until rats experienced five class V seizures (fully-kindled). The Elevated Plus Maze (EPM) and Morris Water Maze (MWM) behavioural tests were conducted to assess anxiety and spatial learning. Animals were then transcardially perfused, brains removed and processed for histological analysis of hippocampal mossy fibre sprouting. **Results:** EE delayed the time-course of seizure progression, with enriched rats (n=18) requiring a significantly greater number of kindling stimulations to reach a fully-kindled state compared to IH rats (n=18; $p<0.05$). EE also reduced anxiety-like behaviour in the EPM (EE: n=43, IH: n=39; $p<0.05$) and facilitated superior performance in the MWM (EE: n=27, IH: n=31; $p<0.05$). Timm's staining analysis revealed significant reductions in aberrant hippocampal mossy fibre sprouting following EE (EE: n=13, IH: n=19; $p<0.05$). **Conclusion:** Our data demonstrates a beneficial effect of prolonged EE on vulnerability to limbic epilepsy, co-morbid anxiety and neurocognitive function, possibly underpinned by a neuroprotective effect of EE at the structural level.

ORAL-21-03

A SYNDROME SPECIFIC MODEL OF AN EARLY ONSET EPILEPTIC ENCEPHALOPATHY REVEALS A CELLULAR DEFECT AND SUGGESTS A TARGETED THERAPEUTIC INTERVENTION

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Purpose: Early onset epileptic encephalopathies (EOEE) are a severe form of epilepsy with developmental regression often with a genetic basis. Dravet syndrome is a common EOEE typically caused by SCN1A mutations although SCN1B homozygous mutations have also been implicated. To elucidate the underlying pathology of SCN1B EOEEs we investigated a Scn1b(C121W) homozygous mouse model and examined behavioural and neuronal phenotypes. **Methods:** Thermal seizure susceptibility, gait analysis and survival analyses were undertaken. Whole-cell patch clamp was used to compare electrophysiological properties in homozygous and control subicular neurons. **Results:** Homozygous mice exhibited the heightened susceptibility to thermally triggered seizures, possessed an abnormal gait, shared pharmacosensitivity with EOEE patients, and died prematurely at around P21. Neurons from homozygous mice had left-shifted action potential (AP) input-output curves (n=10, $p<0.05$) suggesting increased excitability. While the voltage threshold for AP firing was unchanged, neurons from homozygous mice had a significantly higher input resistance (Rm), which could readily account for this hyperexcitability. We tested a novel antiepileptic drug, retigabine, a potassium channel activator that lowered Rm, in neurons from mutant mice and showed a significant decrease in AP firing (n=4, $p<0.05$). When retigabine was given in whole mice there was a profound reduction in thermogenic seizure susceptibility (n=9, $p<0.05$). **Conclusion:** These data suggest that a primary neuronal deficit caused by the homozygous mutation is increased input resistance, as a consequence of either altered ion channel distribution or dendritic arborisation, that can be rescued by drugs that specifically decrease input resistance.

ORAL-21-04

PERSISTENT FIRING IN NEUROGLIAFORM CELLS: AN ENDOGENOUS MECHANISM FOR SUPPRESSING SEIZURES?

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It has recently been reported that GABAergic interneurons in the hippocampus and neocortex can exhibit an unusual kind of slow integration of synaptic inputs (Sheffield *et al.*, *Nature Neurosci*, 2011). In these interneurons, hundreds of action potentials, evoked over tens of seconds, eventually initiate spontaneous persistent firing (PF) that sometimes lasts for minutes. **Aims:** To identify the classes of interneurons exhibiting PF and to examine possible roles for PF. **Methods:** Whole-cell recordings were made in 300 µm-thick slices from 18-25 d-old GAD67-GFP mice, in which GABAergic neurons express GFP. **Results:** PF was triggered using either depolarizing current steps or excitatory synaptic stimulation to elicit trains of evoked action potentials at 20-50 Hz. PF, when present, typically emerged after 5-20 s of stimulation. PF was very common in neurogliaform cells in the piriform cortex, hippocampus and neocortex (86% of cells; n = 107). In the piriform cortex, PF was present but much less common in fast-spiking multipolar cells (23%; n = 22), and was never seen in any other class of interneuron (n = 33) or in layer II principal cells (n = 20). Pair recordings from a synaptically-connected neurogliaform cell and a pyramidal cell showed that PF in the neurogliaform cell could generate persistent synaptic inhibition in the pyramidal cell. **Conclusions:** Neurogliaform cells are ubiquitous in the cortex, where they can inhibit many surrounding neurons by spillover transmission. If excess synaptic excitation triggers PF in neurogliaform cells, then the resultant persistent release of GABA may help to suppress the excitability of nearby neurons. Thus, PF in neurogliaform cells may function as an endogenous anticonvulsant.

ORAL-21-05

THE IMPACT OF DIET MANIPULATION IN A MOUSE MODEL OF ABSENCE EPILEPSY

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Absence seizures are common and frequently pharmacoresistant form of epilepsy. Dietary manipulation and metabolic agents have become increasingly important in the treatment of epilepsy. However, clinical studies are confounded by a variety of problems that include compliance and efficacy issues. Syndrome-specific animal models can be studied to investigate new and more compliant diets as well as provide a basis to select those patients that are most likely to benefit. **Purpose:** We tested efficacy of the ketogenic diet and anaplerotic (triheptanoin) diets on the GABAA γ2R43Q absence mouse. **Methods:** Mice were fed on the two diets for 21 days with standard mouse chow serving as control. Video-EEG recordings were made from P40 mice for a total of 14 hours to assess absence seizure occurrence and duration. **Results:** Mice gained weight in all groups except those fed the ketogenic diet. Absence seizures were significantly reduced in mice fed the triheptanoin diet (0.9±0.2%, n=12) as compared to control (2.1±0.3%, n=9, $p<0.05$). In contrast the ketogenic diet had no significant impact on absence seizure severity (1.2±0.3%, n=9) as compared to control (1.1±0.3%, n=6, p=0.884). Insulin-mediated acute hypoglycemia is a known trigger for absence seizures. Interestingly both diets reduced the impact of acute hypoglycemia suggesting a protective role. **Conclusion:** While both diets are protective against triggered absence seizures only the triheptanoin diet was effective in reducing spontaneous seizures in genetic absence epilepsy. The triheptanoin diet had the added advantage of maintaining mouse body weight suggesting that it may be a better long term alternative to the ketogenic diet.

ORAL-21-06

THE ENDURING EFFECTS OF EARLY-LIFE STRESS ON LIMBIC EPILEPTOGENESIS ARE MEDIATED BY HPA AXIS HYPER-REACTIVITY

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Purpose: Enduring effects of early-life stress on the brain promotes vulnerability to the later development of limbic epilepsy. This process is possibly mediated by hyper-reactivity of the hypothalamic-pituitary-adrenal (HPA) axis with increased corticosterone release. Using the maternal separation (MS) model of early-life stress in rats, this study assessed whether inhibiting corticosterone synthesis could ameliorate the adverse effects of MS stress on kindling epileptogenesis and kindling-induced neurogenesis. **Methods**: From postnatal days 2-14, female Wistar rats were subjected to MS stress for 3h/day (MS, n=17) or early-handled for 15min/day (EH, n=21). At 8w, rats were assessed for seizure threshold via a bipolar electrode implanted in the left amygdala, and subsequently subjected to electrical amygdala kindling. Throughout the kindling period, rats were administered either metyrapone (corticosterone synthesis inhibitor: 50mg/kg) or vehicle 60min before each stimulation. Kindling-induced neurogenesis was assessed by administration of 5-bromo-2-deoxyuridine (BrdU, 100mg/kg) and subsequent post-mortem histology. **Results:** Vehicle-treated MS rats displayed reduced seizure threshold compared to EH rats (p=0.03), a vulnerability attenuated by metyrapone treatment. Vehicle-treated MS rats also exhibited longer seizure duration compared to EH rats (p=0.02), an effect also reversed by metyrapone treatment. Further, metyrapone treatment in MS rats retarded the progression of kindling (p=0.03). Kindling induced neurogenesis in all rats compared to sham-kindled rats (p=0.0001). Further, MS rats displayed a pattern of reduced neurogenesis, which was reversed in metyrapone-treated rats. **Conclusion:** Inhibition of corticosterone synthesis with metyrapone alleviated the enduring effects of MS stress on seizure susceptibility and severity, and removed inhibition on kindling-induced neurogenesis. This suggests that HPA axis hyper-reactivity is critical to the effect of MS stress in increasing vulnerability to limbic epileptogenesis, an effect that may involve suppression of kindling-induced neurogenesis.

ORAL-21-07

FLUOXETINE ACCELERATES KINDLING EPILEPTOGENESIS IN RATS

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Purpose: Due to the high comorbidity of epilepsy and depression, antidepressant treatment is commonly indicated for patients with epilepsy. Studies in humans and animal models suggest that serotonin specific reuptake inhibitors (SSRIs) may reduce seizure frequency and severity, and these drugs are therefore considered safe for use in epilepsy. However no studies have investigated the effects of SSRIs on epileptogenesis: the neurobiological processes leading to the development of the epileptic state, continuing even after seizures emerge. **Methods:** 9-11 week old male Wistar rats received surgical implantation of a bipolar electrode into the left amygdala for electrical kindling and an osmotic mini-pump filled with fluoxetine (10mg/kg/day, n=19) or vehicle (n=22). All rats received 30 kindling stimulations and seizure threshold and kindling rates were compared between treatments. Anxiety-like behaviour (elevated plus maze) and endocrine response (corticosterone response to swim stress) were also tested. **Results:** Fluoxetine-treated rats showed an accelerated rate of kindling epileptogenesis compared to vehicle-treated rats (18.3±1.8 versus 23.6±1.4 stimulations to class V, $p<0.05$). There was no difference in seizure threshold before or after kindling ($p>0.05$). Overall, kindling increased anxiety-like behaviour ($p<0.05$), with no difference between fluoxetine and vehicle treatment. Fluoxetine-treated rats had lower stress response than vehicle-treated rats ($p<0.05$). **Conclusion:** In this study, fluoxetine was found to accelerate the rate of epileptogenesis without affecting seizure threshold. This has implications for patients with epilepsy taking SSRIs: although seizure frequency and severity may not be altered, the underlying neurobiological epileptogenic changes may be accelerated with SSRI treatment. However, fluoxetine itself has several other molecular targets and cellular effects, and future studies with other drugs should therefore investigate whether this is a specific effect of fluoxetine, or a more general effect of SSRI treatment.

ORAL-21-08

ALTERATIONS IN EXPRESSION OF NEUROPEPTIDE Y AND ITS RECEPTORS IN A GENETIC MODEL OF ABSENCE EPILEPSY

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Gill KP.[1], Powell KL.[1], Morris MJ.[2], O'Brien TJ.[1] [1]Department of Medicine, Melbourne Brain Centre, The University of Melbourne, Victoria, Australia. [2]School of Medical Sciences, the University of New South Wales, Sydney, Australia. **Purpose:** Neuropeptide Y (NPY) is a 36 amino acid peptide that is abundantly expressed in the mammalian CNS. There is accumulating evidence that NPY is an endogenous modulator of seizure activity. Administration of NPY to Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a genetic model of generalised epilepsy, has been shown to suppress seizures. Multiple receptors have been implicated in mediating NPY's seizure suppression action. Here we investigated if endogenous expression of NPY and its receptors (Y1R, Y2R and Y5R) are altered in GAERS, compared with Non-Epileptic Control (NEC) rats at different epileptogenic time points. **Methods:** RNA was extracted from the motor cortex (MCx), somatosensory cortex (SCx) and thalamus of 7 day (pre-epileptic) and >13 week (epileptic) old GAERS and NEC rats. Quantitative PCR was performed for NPY, Y1-, Y2-, and Y5-receptor mRNA. **Results:** Epileptic GAERS (n=7-9) showed a significant increase in NPY, Y1R and Y5R mRNA expression in the SCx ($p<0.05$, $p<0001$, $p<0.001$ respectively) and MCx ($p<0.001$, $p<0.05$, $p<0.001$ respectively), whereas in the thalamus only Y1R and Y5R mRNA expression ($p<0.001$, $p<0.01$ respectively) was increased compared to NEC rats (n=7-10). At the pre-epileptic time point only Y2R mRNA expression was increased in the thalamus of GAERS (n=8) compared to NEC (n=10, $p<0.05$). **Conclusions:** Following the onset of spontaneous seizures, NPY expression is significantly increased in GAERS compared to NEC rats accompanied by a modification in receptor subtype expression. This data is consistent with the hypothesis that NPY expression is up-regulated in an attempt to suppress the seizure activity.

ORAL-22-01

PROFILES OF $GABA_A$ RECEPTORS IN HUMAN BRAIN TO UNDERPIN IMAGING STUDIES

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Purpose: PET is increasingly used to investigate human neurochemistry *in vivo*. Studies on $GABA_A$ receptors use [^{11}C]flumazenil and [^{11}C]Ro15-4513. Flumazenil binds to $GABA_A$ receptors containing any α subunit to portray overall receptor density, Ro15-4513 is selective for α5-containing receptors. Alcoholics show reduced [^{11}C]Ro15-4513 binding in hippocampus and nucleus accumbens; the former may relate to memory impairment, the latter to addiction and reinforcement. Zolpidem, a non-benzodiazepine sedative, binds to type I and II $GABA_A$ sites differentially depending on the α-isoform present: to α1 complexes at high affinity, to α2, α3 and α5 sites at low affinity. The binding parameters of the tracers are usually derived from animal studies. **Methods:** We determined the pharmacological and quantitative characteristics of key radioligands, and the effects of receptor modulators, in membrane samples prepared from controls and alcoholic cases (both n=6) matched for age and post-mortem interval. Five representative regions were taken for regional comparisons. Tissue was obtained from the Queensland Brain Bank under informed written consent of the next of kin and human ethical clearance from University of Queensland. **Results:** While [^{14}C]flunitrazepam affinity was relatively invariant, [^{14}C]flumazenil affinity showed significant regional differences and [^{14}C]Ro15-4513 affinity varied both regionally and between case-groups. In contrast, the benzodiazepine ligands delineated clear up-regulation of binding capacity in alcoholics but [^{14}C] Ro15-4513 was muted in this regard. Zolpidem modulation of both [^{14}C] Ro15-4513 and binding showed strong regional differences in all cases, with much lower affinity in hippocampus and caudate. Differences in Hill slope were indicative of regional variations in α-subunit composition, notably in occipital cortex. **Conclusion:** These variations may inform the interpretation of imaging studies in alcohol misuse.

ORAL-22-02

UNDERSTANDING THE ROLE FOR THE ZINC FINGER PROTEIN RP58 IN THE ETIOLOGY OF TERMINAL 1Q DELETION SYNDROME

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Terminal 1q deletion syndrome is a congenital neurological disorder that is characterised by microcephaly, agenesis of the corpus callosum and severe intellectual disability. Recent innovations in genome sequencing technology have led to the identification of the relevant genes which are deleted in patients suffering this chromosomal disorder. One such gene encodes the zinc finger protein known as Rp58 (also known as Znf238 or Zfp238). Our descriptive studies demonstrate the Rp58 is expressed during mouse brain development, and our functional studies show that loss of Rp58 expression impairs neuron production and their maturation, including an impairment of their migration within the developing cerebral cortex. Together, these studies not only implicate important roles for Rp58 in normal brain development, but also provide a basis through which we can investigate the potential effects of the other candidate genes which are deleted in patients which suffer Terminal 1q deletion syndrome.

ORAL-22-03

NEW INSIGHTS ON DOWN SYNDROME THROUGH STUDIES OF THE TC1 MOUSE MODEL

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Mouse models are an effective tool for understanding the underlying molecular mechanisms which underlie the clinical features of Down Syndrome (DS; Trisomy 21). Current partial trisomy models are valuable, but do not fully replicate the genetics of this disorder. The novel Tc1 (transchromosomic) mouse line carries a virtually complete copy of human Chromosome 21 so as to model this disorder in an almost genetically identical way to human DS (O'Doherty et al 2005). Tc1 mice exhibit neurological endophenotypes reminiscent of human DS (including poor working memory and motor function). Since previous studies have suggested a neurodevelopmental origin for the cognitive deficits in human DS (including defects in cortical lamination, neuronal arborisation and connectivity), we have now conducted in vivo labelling studies to examine the cerebral cortices of Tc1 mice for similar changes. Our data reveals that embryonic Tc1 neurons do not show aberrant neuronal migration during brain development, and their gross neuronal morphology (such as the degree of dendritic branching) did not differ from wildtype litter mates. However, Tc1 neurons did show specific changes to their dendritic spine morphologies, with more spines adopting a short and stubby morphology, as opposed to wildtype neurons, which had significantly more thin and large mushroom shaped spines. Taken together, these studies provide a cellular mechanism to explain changes to the brain behaviour of this novel mouse model of DS.

ORAL-22-04

IMPACT OF ADVANCED PATERNAL AGE ON COPY NUMBER VARIATION AND DNA METHYLATION IN C57BL/6J MICE

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Purpose: Advanced paternal age (APA) is associated with autism and schizophrenia. *De novo* (*dn*) copy number variants (CNVs) and alterations to the epigenome in the male germ line may be transmitted to the next generation. Here we aimed to investigate the impact of APA on CNV load and DNA methylation in offspring using an inbred mouse model. **Methods:** 4 month-old and 12-16 months-old sires were used to create control and APA offspring. Using array technology (Agilent), offspring (6 control, 6 APA) were screened genome-wide for CNVs. CNV were validated and their prevalence established in additional 77 offspring by Sequenom. Genome-wide DNA methylation was examined in brain (8 control, 8 APA offspring) applying MeDIPseq. **Results:** Seven distinct CNVs were detected. Considering the initial and extended set together (n=89), 6 *dn*CNV events were evident in APA offspring, none in control offspring. Therefore, APA offspring were 16 times more likely to have a *dn*CNV (odds ratio, mid-P-exact=0.005). 538 genomic loci were differentially methylated. **Conclusions:** Our results support the hypothesis that an increased CNV load may underlie the association of APA with autism and schizophrenia and provide preliminary evidence of altered brain DNA methylation in offspring of aged sires.

ORAL-22-05

HOW IS COPPER REGULATED IN THE HUMAN BRAIN?

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Purpose: Disturbances in brain copper levels result in serious neurological disorders and may play a role in the pathogenesis and progression of multiple neurodegenerative diseases. Despite this, the mechanism by which copper levels are regulated in the human brain remains poorly understood. **Methods:** We quantified regional copper concentrations and the distribution and cellular localisation of copper transport proteins in post-mortem human brains without neurological or neuropathological abnormalities (n=10, obtained with approval from the NSW Tissue Resource Centre), using inductively coupled plasma-mass spectrometry, western blot and immunohistochemistry. **Results:** We demonstrated the presence of the copper transport proteins, Ctr1, Atox1, ATP7A, and ATP7B in multiple regions of the human brain. Interestingly, the cellular localisation of these proteins varied across brain regions and differed to the cellular localisation reported in other tissues, suggesting that copper may be regulated differently, or used for different purposes, in human brain tissue. Further, the substantia nigra contained twice as much copper compared with other brain regions ($p<0.001$), suggesting a more important role for copper in this region. There was a significant relationship between brain copper concentration and levels of Ctr1 ($p=0.035$) and Atox1 ($p=0.002$), suggesting that these proteins play a key role in regulating human brain copper levels. ATP7A levels were significantly greater in the cerebellum compared to other brain regions investigated ($p=0.004$), supporting an important role for ATP7A in cerebellar neuronal health. **Conclusion:** This data will be critical to understand the mechanisms of disrupted brain copper levels in multiple disorders where brain metal levels are altered.

ORAL-22-06

NK1 RECEPTOR ANTAGONISTS DO NOT PREVENT TUMOUR CELL EXTRAVASATION AND SUBSEQUENT BRAIN METASTATIC GROWTH

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Purpose: The blood-brain barrier (BBB) should prevent tumour cells from entering the brain; despite this 20-40% of cancer patients develop secondary brain tumours. Preliminary data from our laboratory showed increased albumin immunoreactivity in the brain, indicative of BBB opening, together with increased substance P (SP) immunoreactivity, at 3 days post systemic injection of tumour cells and prior to the detection of tumour masses at 9 days. Given that SP acts on NK1 receptors to increase BBB permeability, we hypothesized that SP may be a mediator of tumour cell extravasation. We therefore assessed the efficacy of the NK1 receptor-antagonists, N-acetyl L-tryptophan (NAT) and EMEND, in reducing tumour cell extravasation and metastatic brain tumour growth. **Methods:** Walker 256 rat breast tumour cells (Cell Resource Centre for Medical Research at Tohoku University) were injected into the internal carotid artery of male Wistar rats (N=18), with culture medium injected as sham controls (N=6). On days 0-3 following tumour inoculation, animals were treated I.P. with either EMEND (3mg/kg/day, MERCK & CO), NAT (7.5mg/kg/day, Sigma), or saline as vehicle control (N=6 per group). Animals were euthanized 9 days after tumour inoculation and the brains were assessed for tumour incidence and volume. **Results:** The NK1 receptor antagonists had no effect on the incidence of tumours developing in the brain when compared to vehicle controls. Furthermore, there were no significant differences in tumour volume between treated and saline injected groups. **Conclusion:** Tumour cell invasion into the brain is not dependent on a SP-mediated increase in BBB permeability. Other mechanisms must be operating in tumour-endothelial cells interaction in metastatic brain tumours.

ORAL-22-07

CHARACTERISING THE ROLE OF SUBSTANCE P IN THE GENESIS OF PERITUMOURAL OEDEMA

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Purpose: Cerebral oedema develops rapidly around brain tumours and contributes to many tumour-associated deficits. Currently, the standard treatment for peritumoural oedema is dexamethasone, however prolonged use of this drug is associated with deleterious side effects. Previous research has implicated the neuropeptide substance P (SP) in blood-brain barrier (BBB) disruption and development of cerebral oedema in brain pathologies such as traumatic brain injury and stroke. As SP is reportedly increased in cancer, this study examines whether it plays a similar role in the genesis of peritumoural oedema. **Methods:** A-375 human melanoma cells were injected into the right striatum of male nude mice to induce brain tumour growth, with culture medium injected animals serving as controls. Three weeks following tumour inoculation animals were treated for 7 days with an NK1 antagonist, dexamethasone or saline vehicle. Following treatment, animals were sacrificed to determine BBB permeability, brain water content and a variety of immunohistochemical markers. **Results:** Tumour-inoculated animals demonstrated an increase in SP and NK1 receptor immunoreactivity in the peritumoral area. Brain water content was significantly increased in tumour-inoculated animals when compared to medium-injected controls ($p<0.01$). Treatment with an NK1 antagonist reduced BBB permeability and brain water content when compared to vehicle treated tumour-inoculated mice. Mice administered dexamethasone showed similar results to those administered the NK1 antagonist. **Conclusion:** The increase in peritumoral staining for SP and the NK1 receptor coupled with the reduction in brain water content and BBB permeability observed following treatment with an NK1 receptor antagonist, suggest that SP plays a role in the genesis of peritumoral oedema in the brain, and warrants further investigation as a potential treatment.

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P2X7 IS A SCAVENGER RECEPTOR FOR APOPTOTIC CELLS IN THE ABSENCE OF ITS LIGAND EXTRACELLULAR ATP

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Purpose: Phagocytosis of apoptotic cells is essential during development and tissue remodelling. Our previous study has shown that the P2X7 receptor regulates phagocytosis of non-opsonized particles and bacteria (Gu et al, Blood, 2010). To study the cell biological mechanisms by which apoptotic neurons are removed, we investigated the role of P2X7 in clearance of apoptotic neuronal cells. **Methods:** The phagocytosis of apoptotic lymphocytes and neuronal cells by human monocyte-derived macrophages were analysed using multi-colour flow cytometry. Peptide screen was used to identify the extracellular epitopes of P2X7 which recognize apoptotic cells. **Results:** P2X7 transfected HEK-293 cells acquired the ability to phagocytose apoptotic lymphocytes. P2X7 was found accumulated at the point of attachment between captured apoptotic cells and the HEK-293 cells. Injection of apoptotic thymocytes into the peritoneal cavity of wild type mice (n=5) resulted in their phagocytosis by macrophages but injection of ATP prior to thymocytes markedly decreased this uptake. In contrast, ATP failed to inhibit phagocytosis of apoptotic thymocytes in vivo by P2X7 deficient peritoneal macrophages. The surface expression of P2X7 on phagocytes increased significantly during phagocytosis of apoptotic cells. A peptide screen library containing 24 biotin-conjugated peptides mimicking the extracellular domain of P2X7 was used to evaluate the binding profile to apoptotic cells. One peptide showed binding to all particles and cell membrane lipids. Three other cysteine-containing peptides uniquely bound the surface of early apoptotic cells but not viable cells while substitution of alanine for cysteine abolished peptide binding. Several thiol reactive compounds including N-acetyl-L-cysteine abolished phagocytosis of apoptotic SH-SY5Y cells by macrophages. **Conclusion:** These data suggest that the P2X7 receptor in its unactivated state acts like a scavenger receptor and its extracellular disulphide bonds play an important role in direct recognition and engulfment of apoptotic neuronal cells in a phosphatidylserine-independent manner.