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POS-MON-249

SYNAPTIC ORGANIZATION OF MSO PRINCIPAL NEURONS IN HEARING, DEAF AND COCHLEAR-IMPLANTED CATS

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The medial superior olive (MSO) is a key component of the central auditory pathway that has been implicated in the processing of interaural time differences (ITDs) used for sound localization. The deaf white cat is a proven animal model of congenital deafness and was utilized to examine how deafness and cochlear implantation affected the synaptic organization of MSO principal neurons. Synaptic inputs to the MSO were investigated using electron microscopic examination of postsynaptic density characteristics as well as an objective method for synaptic vesicle (SV) analysis in which synapses were classified as excitatory or inhibitory based on a quantitative assessment of SV size and roundness. Analysis revealed that the principal neurons in the MSO of deaf cats (n=2) have a decreased number of axosomatic inhibitory endings and axodendritic excitatory endings as compared to normal hearing cats (n=2). To examine the possible restorative effects of cochlear implant stimulation on the MSO, congenitally deaf cats aged 3 months (n=4) received unilateral cochlear implants that had been modified by Advanced Bionics Corporation for use in kittens. Implantation and stimulation strategies were virtually identical to those used for human recipients. Animals were stimulated 5 days a week over a period of 3 months. Results show that MSO principal neurons from the cochlear implant animals have an increased number of axosomatic inhibitory inputs as compared to deaf cats. There is also a return of excitatory inputs to MSO dendrites. These results support the hypothesis that early cochlear implantation can induce synaptic plasticity to restore auditory neurons to a more normal state.

POS-MON-250

α4/7-CONOTOXIN REGIIA SELECTIVELY TARGETS THE α3β2 NACHR

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Neuronal nicotinic acetylcholine receptors (nAChRs) play an important role in the central and peripheral nervous system and may have important implications in certain disease states including Parkinson's disease and schizophrenia. Structurally, they are either homopentamers such as α7 nAChRs, or heteropentamers of α2-6 and β2-6 subunits. α9α10 nAChRs are unusual heteromeric receptors that are composed of only alpha subunits. α-conotoxins are peptides isolated from the venom of cone snails that are constrained by two disulphide bonds (1-3, 2-4). α-conotoxins inhibit nAChRs, targeting diverse receptor subtypes. One subfamily of these α-conotoxins, the α4/7-subfamily, has a characteristic –CCX4CX7C- motif, and is the largest subclass of α-conotoxins that target neuronal nAChRs. Using size exclusion and reversed phase HPLC we isolated nanomolar quantities of a novel α4/7-conotoxin (RgIIA). RgIIA was isolated from the venom of Conus regius, a worm-hunting species from the Western Atlantic Ocean. Its sequence was directly determined by Edman degradation and confirmed by the cDNA sequence of its protein precursor, which also indicates that RgIIA belongs to the A-superfamily of conotoxins. nanoNMR of native RgIIA shows the chemical shift dispersion characteristic of a folded α-conotoxin. Milimolar quantities of RgIIA were produced using solid phase peptide synthesis based on Boc chemistry. Native and synthetic RgIIA samples were functionally tested using voltage clamp electrophysiology on nAChRs expressed in Xenopus laevis oocytes. RgIIA is a selective inhibitor of the α3β2 nAChRs (n=3), and its potency surpasses MII, the most selective and potent α-conotoxin known to target the α3β2 nAChR. RgIIA exhibits sequence differences when compared to the α3β2 subunit-specific α4/7-conotoxins such as MII and PnIA isolated from a fish-hunting and mollusk-hunting cone snails, respectively. Thus, while structurally related to other α4/7 conotoxins, RgIIA has an exquisite balance of shape, charges, and polarity exposed on its structure to selectively and potently block the α3β2 nAChR.

POS-MON-251

GLYCATED PROTEINS INHIBIT K^+ CHANNELS IN ISOLATED VASCULAR SMOOTH MUSCLE CELLS

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Fibronectin (FN) has been shown to enhance K^+ channel activity via an integrin-mediated mechanism. As vascular smooth muscle (VSM) K^+ channels mediate vasodilation, we investigated whether advanced glycation of fibronectin (as occurs in diabetes and renal failure) alters the normal stimulatory effect of this matrix protein on these channels. Under sterile conditions, FN (1mg/ml) was glycated (gFN) for 5 days in the presence of methylglyoxal (50mM) or glycolaldehyde (50mM). Albumin, a non-matrix protein, was similarly glycated as a control. VSM cells were enzymatically isolated from rat cerebral arteries for K^+ channel activity studies via whole cell patch clamp. The inhibitors, iberiotoxin (0.1μM) and 4-aminopyridine (1mM), were used to identify contributions of large conductance, Ca^2+-activated, K^+ channels and voltage-gated K^+ channels, respectively. While native FN enhanced whole cell K^+ current (1.8 fold), gFN caused a 56% inhibition of current compared to baseline. Furthermore, native albumin did not enhance basal K^+ current but when glycated caused inhibition (61%; $p < 0.05$). Inhibitor studies indicated a predominant effect of gFN on the Kv component of total K^+ current. These studies provide a potential mechanism by which advanced glycated proteins impair VSM function and adversely impact arteriolar vasodilation.

POS-TUE-001

PHYSICAL EXERCISE ACTIVATES ENDOGENOUS NEURAL PRECURSOR CELLS FOLLOWING IRRADIATION OF THE AGING MURINE BRAIN

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Given the rapid increase in age-related neurodegenerative diseases, including dementia, it is of critical importance to identify strategies to slow, prevent or even reverse age-associated cognitive decline. Although it is well established that normal aging correlates with decreases in neurogenesis and cognitive function, little is currently known regarding the effects of aging on the endogenous neural precursor cell population. We recently revealed that within the ventricular neuraxis of the mouse there is a decrease in the number of neural precursor cells with age. This decrease was evident at 6 months (~40% decrease), culminating in an approximate 90% decrease at 24 months of age. Given our recent finding that physical exercise can stimulate precursor cells within the subventricular zone (SVZ) we sought to investigate if voluntary exercise can augment the regenerative capacity of the aging SVZ. Utilising a single dose of irradiation (3.5Gy) to ablate dividing cells we assessed the regenerative capacity of the SVZ in 12m old animals. In non-running animals neurosphere numbers decreased from naive levels (905±65, N=3), resulting in incomplete repopulation of the SVZ at both one and two weeks (597±14 and 528±19 respectively, N=3) post-ablation. This is in sharp contrast to animals that were provided access to a running wheel. In these animals neurosphere numbers in the SVZ were restored to naive levels within one week (935±39, N=3). Similar results were also obtained for 18m old animals, demonstrating that physical exercise following ablation/injury is able to augment regenerative capacity within the aged brain. Studies are currently focusing on the potential mechanisms involved in the activation of these endogenous precursor cells.

POS-TUE-002

TROPOMYOSIN ISOFORMS DIFFERENTIALLY IMPACT ON THE BRANCHING OF PROCESSES IN DIFFERENTIATED B35 NEUROBLASTOMA CELLS

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During early neuronal development the actin cytoskeleton is instrumental to support morphological changes. Tropomyosin proteins are a family of proteins that associate with and regulate the dynamics and function of actin filaments. The association of tropomyosins with the actin filaments can either prevent or facilitate, in an isoform dependent manner, the access of a number of actin-associated proteins. In eukayotic cells more than 40 isoforms are generated by alternative splicing from four different genes (α-, γ- and δ-gene) which are developmentally and spatially regulated. Products from three of the tropomyosin genes, the α- (Tm5a/b, TmBr1, TmBr2, TmBr3) γ- (Tm5NM1-11) and δ-tropomyosin (Tm4) gene, are found in neurons. We have recently shown that increased levels of Tm5NM1 result in an extension of neurite length and degree of branching. We have now investigated the effect of increased protein levels of the brain specific isoforms TmBr1-3, on process formation using the B35 neuroblastoma derived cell line. We show that these isoforms differentially impact on the branching pattern of processes. While increased levels of TmBr1 result in almost complete elimination of branching, overexpression of TmBr2 and TmBr3 leads to more branching as compared to control (n=3). Our results demonstrate that the actin cytoskeleton has a critical role in generating a specific branching pattern during process outgrowth. This suggests an important role for tropomyosins when neurons establish their complex network of highly branched neurites.

POS-TUE-003

Cancelled

POS-TUE-004

THE ROLE OF NEDD4-2 IN NEURITE DEVELOPMENT

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Nedd4-2 is an ubiquitin protein ligase that binds to the consensus motif L/PPXY in target proteins and catalyses the transfer of ubiquitin, thereby regulating protein turnover and degradation. Nedd4-2 is a developmentally regulated transcript in the mouse brain and has recently been identified as regulator of a number of neuronal receptors and transporters. We have found that silencing of Nedd4-2 results in an increased number of neurites per cell in differentiated PC12 cultures and that when Nedd4-2 is over expressed there is a 50 % ± 4 ($p<0.001$; n=4) reduction in differentiation efficiency. We hypothesized this is due to Nedd4-2 ubiquitinating neurogenic proteins involved in neurite outgrowth. An *in silico* screen for neurogenic proteins containing the Nedd4-2 recognition motif indentified the Microtubule Associated Protein 2 (MAP2) as a putative binding candidate. We show here that Nedd4-2 binds to and co-localises with the high molecular weight isoform of MAP2. Additionally in the Nedd4-2 null mouse expression of MAP2 is increased in the embryonic brain. Preliminary data shows that MAP2 is also ubiqutinated in PC12 cells. Furthermore inhibition of the proteasome in these cells results in a 4-fold increase in the abundance of MAP2 compared with controls (< 0.0001; n=4). MAP2 is known to determine the availability of tubulin subunits for polymerization into microtubules. We report here that silencing of Nedd4-2 results in a 3-fold increase in both alpha and acetylated tubulin fluorescence density. These data suggest Nedd4-2 is a negative regulator and a rate-determining factor in neurite outgrowth due to its ability to sequester alpha tubulin subunits through its regulation and ubiquitination of MAP2.

POS-TUE-005

THE SERINE PROTEASE INHIBITOR NEUROSERPIN REGULATES DENDRITIC PROTRUSION NUMBER AND DENDRITIC SPINE MORPHOLOGY

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It is thought that the cellular basis for learning and memory involves long-lasting changes at synapses occurring through morphological and functional plasticity. Dendritic spines are the postsynaptic components of most excitatory synapses in the brain. These dynamic structures are associated with plasticity of neural function as they are constantly changing in number and morphology throughout development and in the adult brain. Understanding the mechanisms of spine plasticity may provide insight to how learning and memory occurs at a cellular level. Neuroserpin, a member of the serpin superfamily, is principally expressed by neurons in the central and peripheral nervous system. Transgenic mice over-expressing neuroserpin show improved spatial learning, supporting a role for neuroserpin in synaptic plasticity. To investigate the cellular effect of neuroserpin on neuronal morphology, primary embryonic rat hippocampal neuronal cultures were transfected with a rat neuroserpin cDNA along with a plasmid expressing humanized *R. reniforms* GFP (hrGFP). In general, neurons overexpressing neuroserpin and hrGFP showed a similar global morphology to neurons expressing hrGFP alone. However, higher magnification images showed that there was an increase in dendritic protrusions in neuroserpin-overexpressing cells compared to controls ($p < 0.001$). In addition the overexpression of neuroserpin altered dendritic spine morphology. Increased neuroserpin levels resulted in a 52% increase in the density of thin spines ($p = 0.007$) and a 34% reduction in the density of mushroom spines ($p = 0.03$). These results suggest an underlying mechanism for neuroserpin's effects on learning and memory.

POS-TUE-006

SPATIAL LEARNING DEFICITS IN TEN_M KNOCKOUT MICE

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Purpose: Internal and external connections of the hippocampal formation (HCF) are topographically organised. One important function of these brain regions is thought to be spatial learning. The maintenance of topographic relationships aids in the association of neural representations between related brain regions. The HCF and the areas it connects with express mRNA of each member of the Ten_m family of transmembrane proteins in discrete but overlapping patterns during development. Studies from our lab indicate that Ten_m2, 3, and 4 are functionally important axonal guidance molecules in the visual system in vivo. **Methods:** Performance in the hidden-platform Morris water maze was compared between wildtype (WT; n=12) and Ten_m2, 3, and 4 knockout (KO; n=11, 12, 11) groups. During 7 days of acquisition mice had to locate a hidden platform using environmental cues. On the 8th day, mice were probed by relocating the platform to the opposite quadrant. Escape latency was compared for all groups and swim trajectories were recorded. **Results:** All groups of mice acquired the task, however all KO groups performed significantly worse compared to their WT littermates ($p < 0.05$). All groups of mice learned the spatial reversal probe ($p < 0.05$), but there was no difference between WTs and KOs ($p > 0.05$). Qualitative analysis suggested that groups navigate using different strategies. **Conclusion:** These data suggest that spatial learning is defective in Ten_m KO groups, consistent with the suggestion that Ten_m proteins contribute to the development of the circuitry underlying spatial learning.

POS-TUE-007

TEN-M3 IS EXPRESSED IN MATCHING GRADIENTS IN THE DEVELOPING VISUAL SYSTEM OF THE WALLABY, *MACROPUS EUGENII*

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Purpose: Ten-m3, a transmembrane glycoprotein, influences the mapping of ipsilaterally-projecting retinal axons to central targets in the mouse. The aim of this study was to determine the pattern of expression of Ten-m3 in the retina and superior colliculus (SC) of the Tammar wallaby during development of the retinocollicular projection. **Methods:** Real-time qualitative polymerase chain reaction for Ten-m3 mRNA was performed on whole RNA isolated from animals aged P15, P30, P45, P65, P95, and adults (n=4 per group). Samples were compared between dorsal and ventral halves of the retina, and medial and lateral areas of the SC. *In situ* hybridisation for Ten-m3 mRNA was performed on cryosectioned retina and SC in animals aged P15, P30, and P45 (n=2 per group). **Results:** Ten-m3 was detected in all age groups. In ages P15-P95, expression of Ten-m3 was significantly higher in ventral compared to dorsal retina, and medial compared to lateral SC (all groups; $p<0.05$, Student's t-test). These differences were greatest at P30 (retina; $p<0.01$, SC; $p<0.01$, One-way ANOVA). At P15-P45, expression was in an increasing dorsoventral gradient in the ganglion cell layer of the retina and a decreasing mediolateral gradient in the retinorecipient layers of the SC. **Conclusion:** Ten-m3 is expressed during development of the retinocollicular projection and takes the form of matching gradients in the retina and SC: regions that connect with each other express Ten-m3 at similar relative levels. The wallaby's protracted *ex utero* development will allow manipulation of Ten-m3 at early developmental stages not attainable in placental mammals.

POS-TUE-008

MECHANISMS UNDERLYING THE ROLE OF TEN-M3 IN VISUAL DEVELOPMENT

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Purpose: Ten-m3 knockout (KO) mice display profound abnormalities in mapping of ipsilateral retinal projections to the dorsal lateral geniculate nucleus (dLGN) and superior colliculus (SC) at maturity, which are associated with visual deficits. The aim of this study was to determine the potential mechanisms underlying this by examining the trajectory of retinal axons in Ten-m3 KO and wildtype (WT) mice during development, and to identify causal molecular mediators of the mismapping phenotype. **Methods:** Bulk-fill retinal cholera-toxin B injections were used to trace retinal axons during development at P0, P3, P6 and P10 (n=3 per group). Expression of genes with established roles in development of the retinofugal pathway were examined using *in situ* hybridisation (n= 2 per group), realtime PCR on total RNA from P0 mice (n=3 per group), and wholemount preparations of neonate SC (n=3 per group) incubated with EphA and ephrinA-AP probes. **Results:** In Ten-m3 KOs ipsilateral retinal axons enter the ventrolateral corner of the dLGN, unlike WTs where axons remain confined to the optic tract until they reach the dorsal part of the nucleus. EphA receptor expression in the developing SC is disrupted in Ten-m3 KOs as revealed by lower ephrinA-AP binding, and reduced EphA7 mRNA levels. Realtime PCR confirmed EphA disruption in KOs: with downregulation of EphA7 (19%, $p\leq0.05$, 35%, $p\leq0.05$), and EphA5 mRNA (21%, $p\leq0.05$, 30%, $p\leq0.05$) in SC and retina, respectively. Zic4 mRNA expression was downregulated in KO SC (25%, $p\leq0.0001$). **Conclusion:** Retinofugal axon guidance is disrupted in Ten-m3 KO mice. Alterations in EphA expression pose a potential mechanism for the retinofugal mismapping phenotype.

POS-TUE-009

TEN_M3: ROLES IN THE DEVELOPMENT OF THE RODENT STRIATUM

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Ten_m3 is a transmembrane glycoprotein which regulates cell adhesion and axonal guidance. We found that Ten_m3 has a patchy distribution within the striatum of neonatal mice. Comparison of the in situ hybridisation signal for Ten_m3 and immunohistochemistry for the μ-opioid receptor (μOR1, a striosomal marker) suggests that Ten-m3 is expressed in a subregion of the matrix. A potential role for Ten_m3 in the compartmentalisation of the striatum into the striosomes and matrix was investigated using double staining for Wisteria floribunda agglutinin (WFA) and μOR1, in P7 and P21 wild type (WT) and Ten_m3 knock out (KO) mice. Preliminary evidence indicates that a previously described transition in WFA labeling occurs identically in the two groups (n=3), suggesting that striatal compartments develop largely normally in Ten_m3 KOs. To examine a potential role for Ten_m3 in controlling the targeting of thalamostriatal axons, one of the major sources of input to the striatum, stereotaxic injections of biotinylated dextran amine were made into the parafascicular thalamic nucleus in adult mice. The area occupied by parafascicular terminals was increased in KOs, although this change was not significant (p=0.116, t-test). Interestingly, however, terminals were significantly denser in KOs than in WTs (WT: 125.36 Â± 12.82 (arbitrary units, mean Â± SEM), n = 6; KO: 166.66 Â± 11.58 (arbitrary units, mean Â± SEM), n = 5; p = 0.040, t-test). Qualitatively, this change was associated with a more uniform distribution of terminals in KOs, compared to a more â€˜patchyâ€™ arrangement in WTs. This study suggests a novel role for Ten_m3 in regulating the targeting of thalamostriatal projections to a specific subregion of the matrix.

POS-TUE-010

SEMAPHORIN3A IS INVOLVED IN THE AREAL DEVELOPMENT OF THE NONHUMAN PRIMATE VISUAL CORTEX

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The nonhuman primate comprises multiple visual areas that have specific cytological boundaries, and that develop in an inside-out manner. However, the spatiotemporal mechanism by which these specific nuclei develop is poorly understood. For this present study, we demonstrate the role of the secreted guidance cue Semaphorin3A (Sema3A), a factor which has previously been demonstrated to guide the migration of newborn neurons in the cortex. Sema3A influences the polarisation of pyramidal neurons by inducing the axonal growth cone collapse and the attraction of the apical dendrite. In the developing marmoset monkey (*Callithrix jacchus*), we have established the immunohistochemical expression pattern of Sema3A from the end of cortical lamination (E130) until P14 (n=5), a temporal stage when the primary visual cortex (V1), second visual area (V2) and the middle temporal area (MT) are mature. At ED130, Sema3A is expressed across the 6 layers of the visual cortex, except in V1 where Sema3A is restricted around $NeuN^+$ cell bodies located in layers 5 and 2. At later stages (P7), as V2 and MT mature, Sema3A expression in MT is localised around $NeuN^+$ cell bodies located in infra- and supra-granular layers as observed in V1 at E130. In V2, Sema3A is expressed around cell bodies only in infragranular layers and is still extensively expressed throughout the extracellular matrix of the subgranular layers. At P14, Sema3A overall expression decreases and is restricted around cell bodies. As maturation occurs in V1, MT then V2, Sema3A expression evolves from an extracellular to a pericellular pattern, in the infragranular prior to the subgranular layers. Our results suggest that Sema3A plays a role in controlling the spatial and temporal development of visual cortical areas, in addition to its role in cortical lamination. Furthermore, it is one of the specific factors involved in the early maturation of area MT at the same temporal stage as V1.

POS-TUE-011

STRUCTURAL CHANGES IN DENDRITIC ARBORS OF STRIATAL MEDIUM SPINY NEURONS COINCIDES WITH KEY POSTNATAL DEVELOPMENTAL EVENTS IN THE MOUSE

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Discipline of Physiology & Bosch Institute, University of Sydney, Sydney, NSW 2006.

Medium spiny neurons (MSN), the main output cells of the striatum, integrate corticostriatal and nigrostriatal inputs, thus mediating motor learning and reward-associated behaviours. Their maturation is considered vital for the establishment of basal ganglia function. Of key interest is a developmental period when major changes occur in neostriatal circuitry at around the second postnatal week, which correlates with the emergence of motor behaviours. Whilst increases in the number of spines and changes in the electrophysiological properties of MSNs within this developmental window have been reported previously, the gross changes occurring in dendritic morphology are less known. We used blind whole cell patch clamp technique to fill individual MSNs with neurobiotin in brain slices obtained from C57BL/6J mice at postnatal day (P)6-7, P9-11, P13-14, P21, P28-30, and P40+ (adult), then reconstructed 64 MSNs (6-10 from each age group). μ-opioid receptor immunohistochemistry was performed on these same sections to localize the MSNs to either the matrix or striosomes. Among matrix MSNs, we found a significant increase in the mean length of dendrites between p6-7 and p9-12 and older (154-190%), despite a significant decrease (27-50%) in the number of branch points ($p<0.05$, post-hoc multiple-comparisons test). These data suggest both pruning and growth processes lead to the dramatic dendritic restructuring of these important neurons during a time in development that coincides with major circuitry and behavioural changes linked to basal ganglia function.

POS-TUE-012

STIM1 REGULATES SOCE, AND IS NECESSARY FOR GROWTH CONE TURNING

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Calcium is a ubiquitous second messenger within cells, and is a crucial mediator of growth cone motility. Store-operated calcium entry (SOCE) is a necessary process for the repletion of intracellular calcium stores, after depletion. STIM1, a calcium sensing protein, is located on the endoplasmic reticulum membrane, and signals to Orai proteins. Orai proteins are the main components of calcium release activated channels (CRAC), on the plasma membrane, and STIM1 is known to regulate CRAC channel formation after calcium store depletion. The hypothesis of this study is that STIM1 and Orai1/2 are present within embryonic rat dorsal root ganglionic (DRG) neurons, and that STIM1 is necessary for the regulation of SOCE and hence growth cone turning. This was examined with the use of an in vitro growth cone turning assay, morpholino knockdown, immunofluorescence analyses and western blot analyses. STIM1 was found to be present within DRG growth cones. STIM1 knockdown induced a switch in growth cone turning responses, from a chemoattractive response to BDNF (10.64 ± 1.62°; n=24) into a chemorepulsive response (-7.02 ± 2.64°; n=16; $p<0.0001$). A normal chemorepulsive response to sema3a (-7.90 ± 2.94°; n=16) was abolished by STIM1 knockdown (0.95 ± 2.48°; n=19; $p<0.05$). STIM1 and Orai1/2 staining suggested that different staining patterns, diffuse and punctate, occur within growth cones depending upon the calcium store status, with areas of probable co-localisation. This data suggests that the two proteins work together to regulate SOCE in growth cones. These data provide evidence that SOCE, mediated by STIM1 and Orai is necessary for growth cone motility and accurate axon pathfinding. This work has significant implications for development and nerve regeneration.

POS-TUE-013

AXON GUIDANCE BY GROWTH RATE MODULATION DOMINATES OVER BIASED TURNING IN SHALLOW GRADIENTS

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Guidance of axons by molecular gradients is crucial for wiring up the developing nervous system. It is often assumed that the unique signature of such guidance is immediate and biased turning of the axon tip towards or away from the gradient. However, here we show that such turning is not required for guidance. First, we analyzed neurite growth from dorsal root ganglion (DRG) explants grown in precisely controlled shallow gradients of nerve growth factor (NGF). Although there was more growth up the gradient than down the gradient (Mortimer et al, PNAS, 106:10296-301, 2009), there was little neurite turning (n ~ 66 explants per condition). Second, we showed that the asymmetry in outgrowth was not due to trophism, by showing that neurites directed up the gradient grew more than neurites directed down the gradient, even when the former were at a lower absolute NGF concentration than the latter (n=93-95 explants per condition). Third, we showed that the lack of observable turning was not due to turning having already occurred within the explant (n = 60-120 explants per condition), or to subsequent straightening of initially curved trajectories (n = 6 trajectories). Fourth, we showed that a computational model based on modulating the speed of neurite growth depending on gradient direction fit our data, whereas a computational model based on biased turning did not (n ~ 66 explants per condition). Lastly, we showed that such growth-rate modulation does not occur in the steep gradients of the pipette assay. Thus, biased turning dominates in steep gradients while growth-rate modulation dominates in shallow gradients. These results reveal a previously unidentified mechanism for the directed development of neural connections.

POS-TUE-014

A UNIFYING MODEL OF RETINOTECTAL MAP FORMATION REVEALS A CRITICAL ROLE FOR AXON-AXON INTERACTIONS

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Retinal axons form a topographic map in the optic tectum / superior colliculus during development. Data from both surgical and genetic manipulations across a range of species show that chemospecificity and competition play essential roles in the correct formation of the map. Here we present a simple but realistic computational model of retinotectal / retinocollicular map formation, based on multiple activity-independent influences, that provides a unifying explanation for the results of both surgical and genetic manipulations. First, we show that the model is consistent with several typical features of normal development, such as realistic ingrowth and axonal trajectory shape. Second, we show that surgical or 'systems level' manipulations may be explained in the model using only a chemoaffinity rule combined with competition, but that the model is also able to reproduce the retinotopy seen in sparse conditions where there is little or no competition between axons. Thirdly, we demonstrate that to explain the map duplication and collapse observed in *Isl2* EphA knock-in experiments it is necessary to add axon-axon interactions based on receptor ratio evaluations. Overall the model shows that simple mechanistic rules are sufficient to unify a wide range of apparently disparate data in retinotectal map formation, including different classes of experiment in different model species.

POS-TUE-015

CHANGES IN LYSOSOMAL-LIKE ACTIVITY DURING DEVELOPMENT OF HEARING IN RAT AUDITORY BRAINSTEM

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The onset of hearing in rodents occurs during a sensitive period in postnatal development. In the auditory brainstem, the sensitive period is characterized by changes in the pattern of electrical activity and an increased expression of extracellular matrix components (perineuronal nets). Previous studies also demonstrated pruning of the inhibitory synaptic connection between the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO), which form part of a brainstem circuit involved in sound localization. Previous data from our lab indicate that MNTB afferents undergo structural pruning during postnatal development (Rodriguez-Contreras et al., 2008), suggesting that synapse elimination is another correlate of the sensitive period. What are the cellular mechanisms that underlie synapse elimination in auditory circuits? To begin to address this question we performed vital staining experiments in brainstem slices of P09-17 rats (n=17). We used a lysosome-specific fluorescent probe (LysoTracker Red) to explore a link between phagocytic activity and synaptic pruning in the MNTB. We observed an increase in the density of LysoTracker-stained particles as a function of age (P09-11 = 4.67±1.26; P12-14 = 6.82±0.96; P15-17 = 1.68±0.21). Lysosome-like labeling was also observed in the region of the LSO at P12, suggesting a link between synapse elimination and increased phagocytic activity. Using a combination of LysoTracker staining, neural tracing and immunohistochemistry allowed us to track LysoTracker-stained particles to neuropil regions and to S100β+ cells, which we presume are glial cells. These results suggest that axonal refinement in the auditory brainstem is driven by autophagic processes similar to those recently described at the neuromuscular junction (Song et al., 2008).

POS-TUE-016

REGULATION OF MOTONEURON SURVIVAL AND INNERVATION DURING EMBRYONIC DEVELOPMENT

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Purpose: Morphological and functional evidence shows that mice lacking either GABAergic (GAD67-/-) or glycinergic (Gephyrin -/-, Banks et al 2005) transmission do alter motoneuron survival in a region specific manner across during embryonic development. Here we provide evidence that the combined loss of GABAergic and Glycinergic synaptic transmission (VGAT-/- mice) produces exaggerated aberrance in motoneuron survival, muscle innervation and neuromuscular synaptic number, when compared to mice missing glycinergic and mice missing GABAergic transmission. **Methods:** Embryonic VGAT-/- and wild-type+/+ mice at E13, E15 and E18 had their spinal cords and skeletal muscle (diaphragm, latissimus dorsi and gluteus maximus) dissected out and processed for histology and whole-mount immunohistochemistry respectively. **Results:** At E15 and 18 respiratory spinal motoneurons (XIIn) was shown to have decreased survival compared to wild type (n=3) and decreased innervation of the diaphragm at these ages (n=4). Motoneuron count and bifurcation number was 1014 and 3.1 for mutant and 1278 and 3.8 for wild-type E18. By contrast, we observed increase numbers of lumbar motoneuron and hind limb innervation in VGAT-/- mice at E15 and E18 (n=-4-6). Motoneuron count and bifurcation number was 4146 and 5.9 for mutant and 1744 and 4.4 for wild-type E18. **Conclusion:** The results of this study indicate that there are additive effects of the loss of GABAergic and glycinergic on the regulation of motoneuron numbers and their innervation of their target muscles, but that these effects are exerted in a regional dependent manner.

POS-TUE-017

EXPRESSION OF COMPLEMENT FACTORS IN THE SOD1G93A MOUSE MODEL OF MOTOR NEURON DISEASE

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Purpose: The complement system has recently been implicated in pathogenesis of motoneuron disease (MND). Our previous studies in $SOD1^{G93A}$ rat model of MND demonstrated a role for the complement factors in motoneuron death. The current study aimed to determine the expression and localization of the receptors for C5a (CD88 and C5L2) and other complement components (C1qB, C3, C5, C9, C3aR and CD55) at mRNA and protein level, in $SOD1^{G93A}$ mouse model of MND. **Methods:** C57BL/6J $SOD1^{G93A}$ and wild-type (WT) mice were examined at 3 different post-natal (P) ages: P30 (pre-symptomatic); P70 (onset); and P160 (end stage) of MND. At each age, mRNA expression levels of various complement factors were investigated by RT-PCR or qPCR (n=3/age). Protein levels of CD88 and C5L2 were also determined using immuno-blotting. Localization of CD88 and C5L2 mRNA and protein within the spinal cord was determined by in-situ hybridisation and immuno-histochemistry (n=3/age). **Results:** CD88 mRNA and protein levels were increased at P160 in $SOD1^{G93A}$ mice compared to WT littermates. CD88 mRNA and protein were expressed on motoneurons and proliferating microglia throughout disease progression, whereas CD88 was present only on motoneurons in WT mice at all ages. By contrast, C5L2 protein expression was significantly higher in $SOD1^{G93A}$ mice when compared to WT mice only at P30. C5L2 mRNA and protein was localized to motoneurons in both WT and $SOD1^{G93A}$ mice at all ages. **Conclusion:** These results indicate that the expression of complement factors including C5a and its receptors, is increased in $SOD1^{G93A}$ mice, and may therefore play a role in the progression of MND in the $SOD1^{G93A}$ mouse.

POS-TUE-018

PROTEOMIC ANALYSIS OF SPINAL CORD IN RESPONSE TO INJURY AT DIFFERENT DEVELOPMENT STAGES IN MONODELPHIS DOMESTICA

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Spinal cord (SC) injury affects sensory and motor functions, the degree of which depends on the site of trauma. The immature SC has been shown to functionally recover from injury but mature SC does not (1). The marsupial, Monodelphis domestica has the ability to recover functionally and morphologically from SC injury but only if the injury occurs in the first two weeks of life (2). Newborn pups were subjected to complete spinal transection under isofluorane anesthesia at 7 or 28 days of age. Cords were removed 1 and 7 days after operation and proteomic analyses performed for the segment caudal to the injury site. Extracted proteins were fractionated based on isoelectric point and separated to subunit molecular weights by SDS PAGE. Gels were analyzed by gel analysis software, Genetools (Syngene). Results from injured animals were compared to age matched controls. Mass spectrometry was performed on P7+1days (P8 control) and P28+7days cords (P35 control). Several differentially expressed proteins were identified. These belong to: cytoskeleton networks; signaling, apoptotic or protein degradation pathways or are involved in regulation and stress response, neurite growth and protein synthesis. 14-3-3 proteins, cofilin and ubiquitin, implicated in apoptosis regulation and axonal growth guidance, showed age related differential expression. Current efforts include cellular localization of identified proteins and further analysis as more results become available. (1) Fry, E. & Saunders, N. (2000). Clin Exp Pharm Physio, 27, 542-547. (2) Fry, E., Stolp, H., Lane, M., Dziegielewska, K. & Saunders, N. (2003). J Comp Neurol, 466, 422-444.

POS-TUE-019

DIFFERENTIALLY EXPRESSED GENES IN THE INJURED SPINAL CORD OF EPHA4 KNOCKOUT MICE

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Mice lacking the developmental axon guidance molecule EphA4 show extensive axonal regeneration and functional recovery following spinal cord injury. Alterations in the level of astrocytic gliosis and the vascular response to injury have previously been identified in EphA4 knockout mice. In this study we have examined differentially expressed genes which may be important for the axonal regeneration observed. **Methods:** Adult EphA4 knockout and wild-type mice given a lumbar spinal cord hemisection were compared to those with sham injury (laminectomy only). RNA from injury epicentre taken at 4 days post-injury or equivalent tissue in control mice (n=3 per group) was individually hybridised to Affymetrix Mouse All-Exon Array 1.0 chips. Microarray data was analysed with Partek Genomics Suite to identify differentially expressed genes. Histological examination of genes of interest was conducted at 4 days post-injury (n=3 per group). **Results:** A two-way ANOVA identified 90 genes differentially expressed in response to injury in EphA4 knockout compared to wild-type mice ($p \leq 0.01$). These included inflammatory phospholipid-related genes LPA1 ($p=0.002$), a receptor for lysophosphatidic acid, and alkaline ceramidase 2 (ACER2, $p=0.006$), a regulator of sphingosine production. By histological examination, at 4 days post-injury LPA1 was localised to reactive astrocytes and ACER2 was localised to a subset of reactive astrocytes, microglia and macrophages. RT-PCR indicated LPA1 and ACER2 gene expression in postnatal astrocyte cultures, suggesting lysophospholipid responsiveness may play a role in the altered astrocytic gliosis observed in EphA4 knockout mice.

POS-TUE-020

LONG TERM REDUCTIONS IN GLUTAMATERGIC NMDA AND DOPAMINERGIC D1 AND D2 RECEPTORS IN THE RAT BRAIN FOLLOWING ADOLESCENT, BUT NOT PERINATAL, MK-801 TREATMENT

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The glutamatergic NMDA receptor plays a key role in perinatal and adolescent brain development. Hypofunction of NMDA receptors, especially during key periods of brain development, is hypothesized to play a role in schizophrenia aetiology and pathophysiology. The aim of this study was to investigate the long-term effects of perinatal and adolescent NMDA receptor antagonism on glutamatergic and dopaminergic receptors in the rat brain. **Methods:** Sprague-Dawley rats were treated with the highly specific NMDA receptor antagonist MK-801 (0.3-0.5mg/kg) or saline on postnatal days 7, 9, and 11 (the perinatal period) or on postnatal days 42, 44, and 46 (the adolescent period). Rats were sacrificed at 12 weeks of age (adulthood) and brains collected (n=5/group). Receptor autoradiography was used to measure glutamate NMDA receptor and dopamine D1 and D2 receptor levels in the prefrontal cortex, hippocampus and striatum. **Results:** NMDA receptor binding density was significantly reduced in the prefrontal cortex (13.1%; $p<0.05$) and hippocampus (14.4%; $p<0.05$) of adolescent MK-801 treated rats compared to saline controls, but was unchanged following perinatal MK-801 treatment. D1 receptor binding density showed small (<10%) but significant ($p<0.05$) reductions in the prefrontal cortex and striatum of adolescent MK-801 treated rats compared to controls, while D2 receptor binding density was also reduced in the striatum of adolescent treated rats ($p<0.001$). D1 and D2 receptor binding density was unchanged following perinatal MK-801 treatment. **Conclusion**: These results show that adolescent, but not perinatal, NMDA receptor antagonism induces long-lasting changes in NMDA, D1 and D2 receptors in the rat brain. This suggests differing sensitivities of the perinatal and adolescent brains to NMDA receptor antagonism and highlights a vulnerability of the adolescent brain to NMDA receptor hypofunction.

POS-TUE-021

BINGE DRINKING AND THE ADOLESCENT BRAIN

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Adolescence is a critical period for brain development, with active rewiring of circuitry necessary in successful development of adult adaptive patterns of behaviour. Binge drinking amongst adolescents is of deep concern considering the capacity for interference with the development of these important circuits. The available evidence suggests that heavy adolescent alcohol consumption disrupts cortical development in a manner that promotes continued impulsive behaviour, alcohol abuse and risk of alcohol dependence. However, there are few studies of the brain targeted to binge drinking effects in adolescent humans. We obtained binge-drinking histories, cognitive data (STROOP, emotional face recognition, depression and anxiety measures) and magnetic resonance imaging (structural, diffusion tensor and spectroscopy) on a group of adolescents (N ≥ 20; males and females aged 16-17 years). This group included non-drinking adolescents (controls). Binge drinkers show significant impairments on the STROOP task with slower responses and greater errors ($P < 0.03$), poorer emotional recognition (FACES: angry/afraid $P < 0.05$) compared to non-drinkers. These neuro-cognitive changes were positively correlated with binge drinking episodes and alcohol consumption. Drinkers had significantly elevated dorsolateral prefrontal cortex glutamate (Glu) levels compared to non-drinkers, and these correlated with depression and anxiety scores ($r2 = 0.7$, ($P = 0.05$) and 0.91 ($P = 0.001$)) respectively. Our data indicated a non-linear response of the DLPFC to alcohol, with increased Glu relative to alcohol consumption in males (amount of binge/length of time since first binge) until a point is reached when there are signs indicative of brain damage. An extreme binger (17 drinks per session twice per week) had NMR spectra indicative of damage, with significantly less (> 2 SD) NAA, Cre, MI and Glu, and more choline than the average for males as well as visible lactate, a compound rarely seen in normal adolescent brain.

POS-TUE-022

FUNCTIONAL RECOVERY AND MORPHOLOGICAL REPAIR FOLLOWING SPINAL CORD INJURY IN THE DEVELOPING OPOSSUM *(MONODELPHIS DOMESTICA)*

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Complete spinal cord injury (SCI) usually results in permanent loss of motor and sensory function below the lesion. Previous work in neonatal opossums has shown that, following SCI, projection of axons through the injury site and functional recovery are possible, but these abilities decline with age (Fry et al., J. Comp. Neurol., 466, 422-444, 2003). A key question is to what extent functional recovery is possible in older animals and how this relates to axonal growth. METHODS: The spinal cords of opossum pups at 7- or 28-days of age (n=6 per group), attached to the anaesthetised mother, were completely transected in mid-thoracic region under sterile conditions. Three months post-injury the animals' locomotion was assessed using random rung ladder, BBB and swimming tests. Following these tests bilateral injections of Fluororuby were made below the lesion to label descending axons. RESULTS: Opossums injured at P28 scored significantly lower on the BBB scale than the P7-injured (12±0.3 vs 17±1.4) and control animals (21±0). In the ladder test P28-injured animals made more footslips than P7-injured animals and in the swimming test P7-injured animals regained some ability to use their hindlimbs but P28-injured animals did not. Morphological examination of P7-injured cords revealed the formation of a tissue bridge across the lesion site. This bridge was absent in P28-injured animals. Backlabelled neurons were abundant in brainstems of control and P7-injured animals, but were nearly absent in P28-injured animals. DISCUSSION: In this study both P7- and P28- injured opossums could walk with hindlimb-forelimb co-ordination, but only P7-injured animals could swim using their hindlimbs. The swimming test and axon tracing results indicate that the locomotor function of P28-injured animals did not involve supraspinal input to regions below the lesion and presumably involved changes in local spinal circuits. Supported by the Victorian Neurotrauma Initiative (Project DPO48).

POS-TUE-023

CONCENTRATION OF CORTISOL IN HUMAN HAIR UNDER REST AND PAIN CONDITIONS

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Four investigations were conducted into the concentration and responsivity of cortisol in adult human hair. Concentration across body sites (n=10 males) varied significantly, with highest values in the arms, followed by legs, with the scalp being lowest. However, concentrations within-shaft from a single site were significantly correlated in longer female (n = 12) hair. Two studies of concentration changes following 1 min immersion in ice water (00C to 40C) were also conducted. The first study (n =3 males) showed immediate, brief and localized increases in cortisol from hair on the immersed forearm but not from hair on the opposite lower leg. The second study (n = 5 males) showed further localization of hair cortisol changes along the forearm, with independent responses being observed in areas only 250mm apart. These results are considered within a model of localized anti-inflammatory hair cortisol responses to trauma and add to our knowledge of the peripheral cortisol synthesis system in human hair.

POS-TUE-024

TEMPORAL PATTERNS OF BONE FORMATION DURING FETAL GROWTH IN *PTEROPUS POLIOCEPHALUS*

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Histology is being used to analyse the transitions from a cartilage model, to osteoid, then to calcified bone matrix during skeletal development. The aim is to determine the timecourse of bone development in the fetus, and eventually design a staging system for fetal development, comparable to the Carnegie stages for embryo development. ImageJ was used to measure Goldners or alcian blue stained wax sections of decalcified bone. The proximal epiphysis and mid-diaphysis were examined in the humerus, radius, femur, and tibia from 17 *Pteropus poliocephalus*, greyheaded flying-fox, fetuses that had been sourced from stored collections. A hyaline cartilage model of the mid-diaphysis of the tibia was present in the very early fetal specimens, but had already been replaced by osteoid in the humerus, radius and femur; the tibia was the last of the four bones to develop. Osteoid and some calcified bone matrix was present in the humerus, radius, femur and proximal epiphysis of the tibia for all stages of development monitored. Total cross-sectional areas of the epiphyses grew faster in forelimbs than hindlimbs ($P<0.01$ Students T-test) as did bone cavities during subsequent remodelling ($P<0.01$ Students T-test). In the diaphysis, growth of the humerus proceeded fastest and that of the tibia was slowest ($P<0.01$ Students T-test), but there was no difference between bones in the rate of remodelling of the diaphyses. The typical antero-posterior sequence of development was confirmed. A cartilage model provided a scaffold for osteoid, which was replaced by calcified bone: all three tissues were present early in fetal life. These results provide the necessary information for interpretation of computed tomography images of dense regions and cavities that are being used to assess skeletal development.

POS-TUE-025

CHARACTERISATION OF THE AROMATASE-EGFP TRANSGENIC MOUSE

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Aromatase (Cyp19a1) is a cytochrome P450 enzyme that converts androgens to estrogens. It is expressed mainly in the granulosa cells of the ovary but is also expressed in other tissues such as testis and brain. This suggests that estrogens can be produced by various tissues in male and female animals. Due to the high structural similarity the protein shares with other members of the cytochrome P450 superfamily, and the lack of a readily available and specific antibody, our study of aromatase protein expression was carried out using the Enhanced Green Fluorescence Protein (EGFP) tagged aromatase transgenic mouse model from from the Mutant Mouse Regional Resource Centers, USA. This transgenic mouse carries a mouse genomic bacterial artificial chromosome which contains the coding sequence for EGFP, followed by a polyadenylation signal which is inserted at the ATG translattion initiation codon of the Cyp19a1 gene. The expression of the reporter mRNA/protein is thus driven by the promoters of the mouse Cyp19a1 gene. We have reported previously that the promoter regions of the Cyp19a1 gene is fairly complex with tissue-specific promoters and untranslated first exons. In this study, we have characterised the Aromatase-EGFP mouse, and showed both male and female transgenic mice have normal gross anatomy and reproduced normally with the Mendelian distribution of the transgene. Similar to previous reports, we have observed EGFP in tissues that expressed aromatase, such as the granulosa cells in the ovaries. We have also detected the expression of EGFP by immunostaining in brain regions (eg hypothalamus) which have been previously demonstrated by in situ-hybridisation to express aromatase. In addition, EGFP immunostaining in the cortex of both male and female adult brains were also observed. In summary, the EGFP expression is driven by the Cyp19a1 regulatory sequences to express specifically in the ovary, testis and brain of the Aromatase-EGFP mice.

POS-TUE-026

INTERSTITIAL CELLS OF CAJAL IN THE MOUSE REPRODUCTIVE TRACT

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The pacemaker mechanism activating spontaneous contractions in the uterus and hence the expulsion of the foetus remains poorly understood. A recent finding that could advance understanding of this has been the discovery that cells resembling pacemaker cells in the gastrointestinal tract termed Interstitial Cells of Cajal (ICCs) are also present in the uterus. However, it is not yet clear whether these cells play a role in uterine pacemaking. The present research addresses this issue by comparing the presence and functional properties of ICCs-like in the uterus to that in the cervix and vagina of non-pregnant mice. Female Swiss mice (6-10 weeks) were euthanased by overexposure to the inhalation anaesthetic isoflurane (5-10%), a procedure approved by the Animal Care and Ethics Committee at the University of Newcastle. ICCs-like and smooth muscle cells were labelled by fluorescence immunohistochemistry using a rat anti-CD117 antibody visualised with donkey anti-rat FITC and an alpha-smooth muscle actin conjugated with Cy3, respectively. Contractions were measured from tissues mounted under 0.5g tension in baths containing physiological solution at 37°C. The uterus, which always exhibited spontaneous contractions, contained a layer of ICCs-like and the well-reported relatively thick muscle layers (n=7). The cervix was only spontaneously active in approximately 40% of tissues (n=5); it did not exhibit a significant amount of ICCs-like and had a relatively low density of smooth muscle cells. Vaginal tissue, while exhibiting ICCs-like, was not spontaneously active and had only a thin bundle of smooth muscle (n=5). These results indicate that the presence of ICCs-like might not necessarily correlate with spontaneous activity but cell density may be a factor here.

POS-TUE-027

MOLECULAR DELIVERY TO THE BRAIN USING ENDOGENOUS PROTEIN TRANSPORT

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The highly vascularised choroid plexuses within the ventricles of the brain are comprised of epithelial cells connected by tight junctions. The tissue is involved in cerebrospinal fluid (CSF) production and secretion, as well as transfer of molecules between the blood and CSF. During early development, when the brain is poorly vascularised, the choroid plexus is the main route of entry for molecules from blood into CSF. This route of entry has been shown to be transcellular and localised in a specific population of plexus epithelial cells (Liddelow et al., 2009) The present study investigated the effect changes in protein content of blood would have on the plexus protein transferring cells, together with resulting modification in CSF composition. This was achieved by increasing levels of circulating plasma protein at different stages of development and by introducing an exogenous foetal protein, fetuin. Three ages of *Monodelphis domestica* (opossum) pups were used in this study: P9 (early stage of brain and choroid plexus development), P65 (juvenile) and P110 (adult), *n*=6 at each age and time point. Animals were injected intraperitoneally with either adult *Monodelphis* plasma (250µg protein/g body weight) or bovine fetuin (CalBiochem, 250µg/g body weight). At the end of the experimental period the animals were terminally anaesthetised (inhaled isoflurane) and CSF, blood and brains collected. Brains processed for histology. Results show that introducing an inappropriate protein into the circulation lead to changes in the transfer of proteins across the blood-CSF barrier, reflected in the changed protein composition of the CSF and the number of protein transferring cells of the choroid plexus. In addition, once in the CSF, fetuin was readily taken up by some cortical neurons.

POS-TUE-028

EXPRESSION AND FUNCTION OF GHRELIN AND RECEPTORS IN HUMAN ENDOMETRIAL CANCER CELL LINES

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Purpose: Endometrial cancer is the most common malignant tumour in female reproductive tract. This study has examined the expression and function of two new facets of the growth hormone axis, the growth hormone secretagogue receptor (GHS-R) and its endogenous ligand ghrelin, in endometrial cancer cells. **Methods:** Four human endometrial cancer cell lines with different differentiation were used (Ishikawa, HEC1A, HEC1B and KLE). Ghrelin and its receptors GHS-R 1a and GHS-R 1b mRNA expression was detected by RT-PCR and quantified with qPCR by normalising to 18s rRNA. The protein expression of GHS-R1a was also detected by immuno-blotting with specific antibodies. Effect of ghrelin on endometrial cancer cell proliferation was determined by using the MTS dye method. Endometrial cancer cell lines were cultured in presence or absence of human n-octanoylated ghrelin at concentrations ranged from 0.1 to 1,000 nM for 24, 48 or 72 hours (n=3/time point). **Results:** Ghrelin, GHS-R1a and GHS-R1b gene expression was detected in all cell lines. Quantification of mRNA level demonstrated that both receptor isoforms gene expression is highly and positively associated with the differentiation level of the cell lines. GHS-R1b gene expression of poorly differentiated KLE endometrial cancer cell line is 3.9-fold higher than that of well differentiated endometrial cancer cell line Ishikawa. Protein expression of GHS-R1a was detected in all four endometrial cancer cell lines. Ghrelin treatment significantly increase the cell proliferation of Ishikawa, HEC1B and KLE cell lines by 32%, 29% and 28% above untreated controls after 72 hours incubation. **Conclusion:** This study demonstrates the expression of the GHS-R and ghrelin in endometrial cancer cell lines and circulating ghrelin may promote cancer cell proliferation.

POS-TUE-029

GHRELIN AND APPETITE REGULATION IN THE SPINIFEX HOPPING MOUSE

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Ghrelin is a hormone released from the gut that stimulates food intake, and is important in the control of energy balance. The water-deprived Spinifex hopping mouse, *Notomys alexis*, exhibits a natural cycle of fasting, followed by sustained food intake that is greater than animals with access to water. Food intake is increased to generate metabolic water in order to maintain fluid homeostasis during water deprivation (WD). We hypothesised that an important driver of the increased appetite is ghrelin. Five groups (n = 5) of hopping mice were subjected to WD with unlimited food availability over a time course of 29 days. Food intake and body weight were determined each day and compared to a control group with access to water. Plasma and tissue samples were collected at five time points (days 0, 2, 5, 10 and 29), and the level of plasma ghrelin and brain ghrelin receptor (GHSR1a) mRNA were determined by ELISA and real time-PCR, respectively. Plasma ghrelin concentration mirrored the decreased food intake during the first five days of WD, rising significantly above control (Day 0) at day 10 ($p<0.05$) and then decreasing markedly in the second phase of WD. Brain GHSR1a mRNA expression peaked at day 2 of WD when plasma ghrelin levels were lowest, then decreased to control levels for the remainder of the experiment. The data suggest ghrelin is important in stimulating the increase in food intake in hopping mice during the first phase of WD. However, ghrelin signalling appears to be down-regulated in the sustained appetite drive of the second phase of WD, suggesting that other signalling system are involved in appetite regulation during this phase.

POS-TUE-030

A FUNCTIONAL ROLE FOR CANNABINOID RECEPTORS IN THE KIDNEY PROXIMAL TUBULES

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The current obesity epidemic has lead to an increased rate of many obesity related comorbidities such as Type 2 diabetes. This places considerable pressure on the health care system with increased rates of diabetes and obesity leading to numerous medical complications such as end stage renal failure. Endocannabinoids bind to a small number of identified receptors including Cannabinoid receptor 1 (CB1) and Cannabinoid receptor 2 (CB2), Transient receptor potential cation channel subfamily V member 1 (TRPV1) and the putative cannabinoid receptor, GPR55. The cannabinoid receptors although initially thought to be predominantly located in the brain and central nervous system, are located in a number of peripheral tissues. Importantly, despite their abundance in kidneys, the functional role of the endocannabinoid system in the renal system has largely been overlooked. This study aims to establish which cannabinoid receptors are expressed in rat kidney tissue and specifically in the proximal tubule via the cell line Human Kidney-2 (HK2), as well as the functional role of the cannabinoid receptors in HK2 cells. Using RT-PCR analysis of mRNA extracted from rat kidney and HK2 cells, it was found that all four cannabinoid receptors CB1, CB2, TRPV1 and GPR55 were expressed in these samples. Further, western blot analysis of protein samples determined that all receptors were expressed in kidney and proximal tubule samples. In addition, using HK2 and assessing cell viability using the MTT assay, it was found that the cannabinoid receptors significantly influenced cell viability in proximal tubule cells. In summary, we have characterised expression of cannabinoid receptors in kidney tissue and proximal tubule cells which will improve our understanding of how cannabinoid receptors influence normal kidney function.

POS-TUE-031

ZUCKER OBESE (FA/FA) RATS, COMPARED TO SPRAGUE-DAWLEY RATS, HAVE FEWER EPISODES OF BROWN ADIPOSE TISSUE (BAT) THERMOGENESIS

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Purpose: In Sprague-Dawley rats, brown adipose tissue (BAT) temperature suddenly increases in an episodic burst-like fashion approximately every 80-100 min during the dark active phase of the circadian cycle. This ultradian rhythm contributes to a highly correlated increase in body and brain temperature that occurs during periods of behavioral activity (1). Obese fa/fa Zucker rats (no functioning leptin receptors) have reduced behavioral activity (2). We investigated the dark-active phase ultradian rhythms in BAT, brain and body temperatures, and their correspondence with behavioral activity, in Zucker rats (n=10, aged 11-18 weeks, weight 435-660 g). **Methods**: Temperature variables were measured and analyzed as previously described (1). Behavioral activity was measured electronically using a grid of laser light beams. **Results**: During the dark active period increases in BAT temperature, occurring together with highly correlated increases in brain and body temperature and behavioral activity, occurred every 124±5 min (mean±SEM), significantly greater $P<0.001$) than 86±3 min, the corresponding values for Sprague Dawley rats in the same experimental conditions (data from ref 1). Amplitude of the BAT temperature increases in Zucker rats (1.22±0.04°C) was significantly greater ($P<0.05$) than 1.10±0.03°C, the corresponding value in Sprague Dawley rats (data from ref 1). **Conclusion**: Our study demonstrates that obese Zucker rats have larger, less frequently occurring episodic ultradian increases in BAT, body and brain temperature, and that these increases are still coordinated with corresponding increases in behavioral activity. (1) Ootsuka et al., Neuroscience 2009. (2) Towa et al. Exp Animal, 2004.

POS-TUE-032

LEUCINE SUPPLEMENTATION IMPROVES GLUCOSE METABOLISM IN OFFSPRING FROM OBESE DAMS WITHOUT AFFECTING FEEDING REGULATION

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The hypothalamic fuel sensor mammalian target of rapamycin (mTOR) is critical for appetite regulation, through its inhibition of the potent appetite stimulator neuropeptide Y (NPY). Previous studies in our lab showed that maternal obesity caused reduced hypothalamic mTOR expression in offspring during the suckling period, accompanied by increased milk intake during suckling and greater energy intake immediately after weaning. The known mTOR agonist leucine can cross the blood brain barrier. We hypothesized that using L-leucine in drinking water to activate hypothalamic mTOR could ameliorate the hyperphagic phenotype induced by maternal obesity. Method: Female Sprague Dawley rats were fed chow or high-fat-diet (HFD) for 5 weeks before mating, throughout gestation and lactation. At 20 days, male pups from obese dams were weaned onto either chow or HFD diet. Within each dietary cohort, half of the pups had leucine in the drinking water. The pups from the lean dams were weaned onto the chow diet only with normal water. At 13 weeks, leucine supplementation led to a non-significant 9% reduction in both 24h energy intake and body weight in chow-fed offspring only; whereas it did not affect the weight gain of HFD-fed rats. L-leucine significantly reduced the blood glucose levels in HFD-fed rats during the entire glucose tolerance test and halved the insulin level in chow-fed rats. Hypothalamic NPY and Y2 receptor mRNA was downregulated in offspring from obese dams independent of post-weaning diet. L-leucine treatment did not affect these markers. Therefore, leucine may impact on pathways other than appetite circuitry to improve glucose metabolism.

POS-TUE-033

CENTRAL MECHANISMS OF LEPTIN RESISTANCE IN HYPERPHAGIC OBESE MICE WITH METABOLIC SYNDROME

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Diet-induced obesity is associated with hyperphagia and increased serum leptin, a central satiety regulator. We studied hypothalamic changes related to leptin resistance in Alms1 mutant (foz/foz) mice, a murine model of Alström syndrome, a form of monogenic obesity associated with cilia disorder, diabetes and metabolic syndrome. Serum leptin was measured in gps of foz/foz and wildtype (WT) mice at 3wk (weaning), 8 and 18wk of age (n>5), fed either rodent chow or high-fat (HF) diet. Hypothalamic proteins were estimated by western blot, mRNA expression by semi-quantitative real-time PCR. The role of neuronal primary cilia was assessed by counting number of ciliated cells. Serum leptin levels were not significantly different in weanling foz/foz mice compared to WT littermates. By 8wk, serum leptin increased in chow or HF-fed foz/foz and HF-fed WT mice, with greatest elevation in HF-fed foz/foz mice; levels continued to increase with time. Foz/foz mice exhibited decreased numbers of hypothalamic ciliated neurons. Recently, it has been proposed that neuronal cilia express leptin receptor (Ob-R). While leptin hypothalamic Ob-R protein did not change, there was a significant increase in SOCS3 at 8wk and PTP1B protein at 18wk in foz/foz mice; both proteins inhibit leptin receptor signaling. Induction of hyperleptinemia by HF feeding is exacerbated in this genetic model of obesity in association with decreased neuronal cilia and increased expression of molecular inhibitors of the Ob-R pathway. These data indicate that obesity in foz/foz mice may be driven by two different pathways of leptin resistance, one structural and the other molecular. Further studies in this model should clarify central mechanisms of leptin resistance.

POS-TUE-034

NOVEL ACTIN FILAMENTS REGULATE GLUCOSE CLEARANCE, INSULIN SENSITIVITY AND INSULIN SECRETION

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The onset of Type 2 diabetes is associated with alterations in both glucose uptake and insulin secretion. Glucose uptake involves a shift of Glut-4 vesicles from intracellular stores to the cell surface, whilst insulin secretion involves the fusion of insulin-containing granules with the pancreatic β-cell surface. We have identified novel actin cytoskeletons defined by the cytoskeletal tropomyosin (Tm) isoform Tm5NM1. Experimental analysis using Tm5NM1 transgenic (Tg) mice suggests these filaments play a role in both glucose uptake and basal insulin secretion. Tg mice have increased glucose clearance in part due to increased insulin sensitivity. The molecular events facilitating Glut4 translocation include activation of the PI3-kinase pathway and also major rearrangements of cytoskeleton components. Tm5NM1 Tg mice showed no change in insulin-stimulated Akt phosphorylation suggesting Tm5NM1 is acting downstream of insulin signalling. Using gene expression profiling with a dedicated microarray, an increase in genes involved in GLUT4 trafficking and actin filament turnover was detected in adipose tissue from Tg mice. The gene expression of genes involved in Glut-4 trafficking was examined by quantitative real-time PCR analysis (n=10/gp). Two genes Myo1c and Sec8 were increased in Tg adipose tissue ($P<0.05$ and $P<0.005$ respectively), Myolc and Sec8 were also increased at the protein level (Western blot). We propose that Tm5NM1 induces more stable cortical actin filament network in adipocytes leading to the accumulation of GLUT4 trafficking machinery and enhancing glucose uptake.

POS-TUE-035

DEPLETION OF BRAIN NORADRENERGIC NEURONS, INCLUDING LOCUS COERULEUS NEURONS, REDUCES THE FREQUENCY OF THE EPISODIC ULTRADIAN OCCURRENCES OF BROWN ADIPOSE TISSUE (BAT) THERMOGENESIS

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Purpose: Brown adipose tissue (BAT) temperature suddenly increases in an episodic manner, approximately every 95 min during the active phase of the circadian cycle. This ultradian rhythm contributes to a highly correlated increase in brain temperature that occurs during activity associated with brain arousal [1]. The locus coeruleus noradrenaline-synthesizing neurons are important regulators of arousal state [2]. We investigated whether lesioning these neurons with a monoclonal antibody to dopamine-β-hydroxylase-coupled saporin toxin (anti-DBH-saporin) affects ultradian rhythms in BAT temperature. **Methods**: Either anti-DBH-saporin (5 µg in 10 µl, Advanced Targeting Systems) or saline-vehicle was injected into the CSF of a lateral ventricle of the brain. Two weeks after the injection, temperature variables during the dark active period were measured and analyzed as previously described [1]. Elimination of noradrenergic neurons in locus coeruleus and their axonal processes was assessed using DBH immunohistochemistry [3]. **Results**: In rats treated with anti-DBH-saporin, increases in BAT temperature occurred every 106±10 min (mean±SEM), significantly greater (n=6, $P<0.05$) than 85±7 min, the corresponding value for vehicle-treated rats. Amplitude of the BAT temperature increases in the two groups was not significantly different ($P>0.05$). **Conclusion**: Our study demonstrates that after depletion of locus coeruleus neurons, rats have less frequent episodic ultradian temperature increases, suggesting that the locus coeruleus contributes to episodic ultradian BAT thermogenesis. (1) Ootsuka et al., Neuroscience 164:849, 2009. (2) Berridge, Brain Res Rev, 2008. (3) Blessing et al., Neurosci. Lett., 1998.

POS-TUE-036

GHS-R MRNA EXPRESSION LEVELS ARE INCREASED IN THE VMH OF DIET-RESISTANT MICE

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Purpose: Hypothalamic growth hormone secretagogue receptor (GHS-R) is a key receptor for ghrelin, which regulates body weight via pituitary growth hormone. Previous study reported a negative association between an upper regulated GHS-R mRNA and body fat accumulation in mice. This study examined if the GHS-R mRNA is differentially expressed between the diet-induced obese (DIO) and diet-resistant (DR) mice as well as these mice on various dietary intervention. **Methods**: Forty-five C57Bl/6 male mice were fed a high-fat diet for 8 weeks and then divided into DIO and DR mice (n=15/group), according to the highest and lowest body weight gainers, respectively. The DIO and DR mice were then randomly divided into three groups (n= 5/group) and either continued on their high-fat diet (HF), changed to a low-fat diet (LF) or an energy-restricted pair-feeding diet (ER) for a further 6 weeks. **Results**: The DR mice had higher levels of GHS-R mRNA expression in the ventromedial hypothalamic nucleus (VMH) than the DIO mice on high-fat diet (~28%, $p<0.05$). After energy-restricted pair-feeding diet for 6 weeks, the DIO mice had their body weight and fat mass reduced to normal level, which is accompanied by a significant reduction of the GHS-R mRNA expression (DIO vs DIO-ER, -40%). Interestingly, DR-ER mice still had a significantly higher GHS-R mRNA expression than DIO-ER mice although they had the same body weight and diet following 6-week energy- restricted diet. **Conclusion**: The DR mice had a higher level of VMH GHS-R mRNA expression than the DIO mice. The body weight reduction program using energy-restricted diet is effective to lose body weight, which is accompanied by a reduction of VMH GHS-R mRNA. This study has provided evidence of central involvement in response of an elevated ghrelin after energy-restricted diet induced weight loss program.

POS-TUE-037

CURCUMIN REDUCES HEPATIC AND RENAL TOXICITY OF ACETAMINOPHEN IN RATS

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Acetaminophen is one of the world's most popular analgesics that can be obtained over the counter. Overdose of acetaminophen, however, can cause severe damages to liver and kidneys. In this study we investigated the effects of curcumin, derived from Curcuma Longa, on the acetaminophen toxicity, and the possibility of combining therapy of curcumin and N-acetyl cysteine (NAC) to treat the damage caused by acetaminophen. The experiments were conducted on 72 male Sprague-Dawley rats randomly divided into 12 groups. Control group was left without treatment, and the other groups were treated with different combinations of acetaminophen, curcumin and NAC. Blood levels of AST Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Blood Urea Nitrogen and Creatinine were determined 18 and 42 h after acetaminophen injection. One week later, left kidney and the caudate lobe of liver were harvested to assay Glutathione Peroxidase, Catalase and Malondialdehyde. Right kidney and the remaining lobes of liver were used for histopathology. Analysis of organ function and oxidation parameters showed that curcumin significantly reduced toxic effects of acetaminophen on liver and kidneys in a dose-dependent manner and significantly potentiated the protective effects of NAC. These findings were confirmed by histopathology. It is concluded that curcumin can protect liver and kidney from the damage caused by acetaminophen overdose. Moreover, curcumin has the potential to be used in a combination therapy with NAC, significantly decreasing the therapeutic dose of NAC and therefore its side effects.

POS-TUE-038

CHARACTERISATION OF THE KYNURENINE PATHWAY IN ASTROGLIOMAS

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The kynurenine pathway (KP) is a major route of L-tryptophan catabolism leading to the production of nicotinamide adenine dinucleotide (NAD^+), the anti-tumoral and neuroprotective agent, picolinic acid (PIC), and a number of other neuroactive metabolites. Evidence suggests that the induction of indoleamine 2,3- dioxygenase-1 (IDO-1) in tumour cells facilitates tumour evasion from T-cell responses. The intermediate α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) plays a key role in tryptophan catabolism by enzymatically driving the production of PIC. Furthermore, NAD^+ is an important contributor to energy (ATP) production and plays a key role in the regulation of DNA repair, genomic stability, replication, and cell division. Most cancer cells have significantly higher energy consumption compared to non-transformed cells. The KP has been fully characterised in human neuroblastoma cells. However, no literature exists characterising the KP in the most aggressive form of brain cancer, astrogliomas. Here we report the first characterisation of the KP in astroglioma cells using astroglioma cell lines (U87, U251 and SVG) and primary cultures of human foetal astrocytes (HFA) stimulated or not with IFN-γ (100IU/ml). Reverse transcriptase (RT)-PCR revealed that astroglioma cell lines (n=6) expressed significantly lower ACMSD but higher IDO-1 compared to HFA. We also observed that astroglioma cell lines (n=6) produced significantly higher intracellular NAD^+ concentrations compared to HFA. These findings suggest that altered KP metabolism in astroglioma cell lines may promote tumour cell viability and ultimately contribute to tumour cell persistence. This study provides the foundation for the identification of novel therapeutic strategies that exploit the differences in KP metabolism we observed.

POS-TUE-039

KYNURENINE PATHWAY METABOLISM IS INVOLVED IN THE MAINTENANCE OF INTRACELLULAR NAD CONCENTRATIONS IN NEURONS AND ASTROCYTES

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NAD+ (the parent pyridine nucleotide) is involved in a number of essential cell metabolic processes from electron transport and ATP production to nuclear repair and regulation of DNA transcription. Maintenance of NAD+ levels are therefore crucial to cell viability in both astrocytes and neurons with some suggestion that glia may also serve as a supplier of NAD+ to neurons during times of stress. Unfortunately little detail of the pathways(s) by which these cells maintain their intracellular NAD+ is available in the literature. The aim of this study was therefore to investigate the relationship, between kynurenine pathway (KP) metabolism and *de novo* NAD+ synthesis in the two major cells types of the central nervous system (CNS), astrocytes and neurons. We show that inhibition of selected enzymes of the KP, results in a significant decrease in both intracellular NAD+ levels and cell viability in primary human astrocytes and neurons. We also show that up-regulation of the KP by IFN-γ, results in an increase in intracellular NAD+ levels in the neuroblastoma cell line SK-N-SH, but a decrease in intracellular NAD+ levels in primary astrocytes. Reduced viability and intracellular NAD+ levels of primary astrocytes can be restored by supplementation with either tryptophan, a precursor of the *de novo* pathway, or via metabolism of either nicotinic acid or nicotinamide by the salvage pathway. NAD+ depletion is becoming increasingly recognised as a cause of cell death in neuroinflammatory and degenerative disorders. Information from this study, showing the dependence of CNS cells on KP metabolism for NAD+ synthesis, suggests the KP as a pathway of potential clinical significance.

POS-TUE-040

PROPERTIES OF ISOLATED SKINNED FAST-TWITCH FIBRES FROM α-ACTININ-3 KNOCKOUT MICE

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α-Actinin-3 is found in the Z-disks of fast glycolytic skeletal muscle fibres, where it cross-links the actin filaments of the contractile apparatus. About 1 billion people worldwide are completely deficient in this protein. In this study we used individual skinned fibres from the EDL muscles of wild-type and *Actn3* knockout mice to examine possible mechanisms for the slowing of relaxation observed in α-actinin-3-deficient whole muscle. Animals aged 9 to 10 months were sacrificed with an overdose of halothane (ethics approval UNSW). Mechanically skinned fibres were first placed in K^+-HDTA solution containing low Mg^{2+} (0.25 mM) and 30 mM caffeine, to deplete the SR of endogenous Ca^{2+}, and 0.25 mM EGTA to chelate all released Ca^{2+} and prevent SR Ca^{2+} reaccumulation. The fibre was then reloaded with Ca^{2+} for predetermined periods of time by exposure to a highly buffered Ca^{2+} solution (pCa 6.57). Loading was rapidly terminated at the end of each loading period by a brief exposure to a relaxing solution, after which the fibre was washed in a K^+-HDTA solution to remove excess EGTA. The fibre was then reexposed to the caffeine solution and the force response was recorded. The area under the force response curve was used as a measure of the amount of Ca^{2+} released, and hence of the amount of Ca^{2+} loaded. For all loading periods, the amount of Ca^{2+} loaded by the SR, expressed as a percentage of the maximum amount it could load in our solution, was lower in knockout fibres than in wild-type fibres. This suggests that in knockout fibres the SR resequesters Ca^{2+} at a slower rate than in wild-type fibres. This result provides one possible reason for the slowing of relaxation observed in whole *Actn3* knockout muscle. Following the SR loading experiments the fibre was chemically skinned and the properties of the contractile filaments were examined. Force-pCa and force-pSr curves were obtained by exposing the fibres to a series of increasing [Ca^{2+}] and [Sr^{2+}]. No differences were found between wild-type and knockout fibres in their pSr_{50}-pCa_{50}, indicating that the slowing of relaxation was not due to any shift in myosin heavy chain isoforms from fast types to slow-type. However, the knockout fibres had significantly steeper force-pCa curves than wild-type fibres (Hill coefficient 3.31 ± 0.17 n=18 KO vs 2.68 ± 0.07 n=17 WT, p=0.002). The impact of this on whole muscle relaxation times is unclear, but it does indicate that loss of α-actinin-3 leads to subtle changes in the sensitivity of the contractile proteins to Ca^{2+}.

POS-TUE-041

EXPLORING THE SPREAD OF EXCITATION THROUGHOUT THE TUBULAR NETWORK IN MAMMALIAN SKELETAL MUSCLE USING SUPERFAST CONFOCAL MICROSCOPY

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In skeletal muscle, the rapid spread of excitation across the sarcolemma and throughout the tubular (t-) system is essential for uniform Ca^{2+} release and subsequent force production. The longitudinal spread of excitation within the t-system network has been reported in spontaneously active mechanically skinned fibres. In such a large cell, every transverse tubule may not be excited following depolarization at the cell surface. Any longitudinal spread of excitation between sarcomeres where transverse tubules fail to depolarize cannot be easily measured with conventional imaging techniques. By imaging Ca^{2+} transients with Oregon Green Bapta 5N at 15.5 μs $line^{-1}$ on a Zeiss 5 LIVE confocal system, we a) tracked the longitudinal spread of excitation along the t-system from the subsequently released Ca^{2+} and b) also resolved the Ca^{2+} release waveform with the highest temporal resolution to date. Following field stimulation of skinned fibres, we observed that in areas where transverse tubules failed to be excited by the initial stimulus, Ca^{2+} release propagated in from the adjacent regions at a rate of ~16μm ms^{-1}(n=6). The rise time of the Ca^{2+} transient showed two phases. It initially rose rapidly for 1ms and then continued at a slowing rate for a further 0.5ms until the peak of the transient. Nav1.4 immunostaining identified a complex subsarcolemmal t-system network which may help ensure the synchronous spread of excitation throughout the fibre from the surface membrane. However, uniform calcium release in skeletal muscle also requires longitudinal tubules deep within the t-system network to pass action potentials between excited and 'failing' transverse tubules.

POS-TUE-042

OVEREXPRESSION OF HSP72 ATTENUATES SKELETAL MUSCLE PATHOPHYSIOLOGY IN *MDX* DYSTROPHIC MICE

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An absence of dystrophin in muscle fibres results in fragility, membrane tears, Ca^{2+} influx and an elevated cytoplasmic [Ca^{2+}], resulting in the activation of degenerative pathways. Chronic degeneration and ineffective regeneration results in fibrotic infiltration leading to functional impairments in DMD patients and in muscles from dystrophin-deficient *mdx* mice. Heat-shock protein 72 (HSP72) has potential to protect contractile function and improve Ca^{2+} handling in cardiac muscle. We tested the hypothesis that HSP72 overexpression would ameliorate the pathophysiology of skeletal muscles of *mdx* dystrophic mice. Contractile properties of isolated diaphragm muscle preparations from *mdx* mice overexpressing HSP72 (*mdx*^{HSP72}) and *mdx* littermate control mice (n≥5) were determined according to methods we have described previously. HSP72 overexpression improved normalised force of isolated diaphragm muscle strips ($P < 0.05$), reduced collagen infiltration ($P < 0.05$), and improved the minimal Ferets variance coefficient, indicative of a reduced dystrophic muscle fibre pathology ($P < 0.05$). Serum creatine kinase levels were significantly lower in *mdx*^{HSP72} mice compared with *mdx* littermate controls ($P < 0.05$), indicating a general reduction in muscle degeneration. The findings reveal that overexpression of HSP72 protein improved the dystrophic muscle pathology.

POS-TUE-043

ANTI-MUSK AUTOANTIBODIES CAUSE MUSK ACTIVATION AND INTERNALIZATION LEADING TO DISASSEMBLY OF ACETYLCHOLINE RECEPTOR CLUSTERS

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Purpose: Muscle Specific Kinase (MuSK) is a key component of a postsynaptic signaling complex required for induction of postsynaptic differentiation by agrin. MuSK has been recently identified as the auto-antigen in a subset of myasthenia gravis patients. Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular synapse that causes impaired neuromuscular transmission and muscle weakness. Recently, we have shown that injection of anti-MuSK-positive patient IgG into mice caused myasthenia gravis. Here we aimed to define the mechanisms by which anti-MuSK antibodies interfere with neuromuscular transmission. **Methods:** Immunoprecipitation was used to assess the effect of anti-MuSK antibodies on tyrosine phosphorylation of MuSK and AChR β-subunit.To investigate the effect of anti-MuSK antibodies on pre-existing AChR clusters, cultured mouse C2 myotubes were pre-treated with agrin (1nM, 4h) to form AChR clusters and then exposed to control human IgG or IgG from an anti-MuSK-positive MG patient. Confocal optical sections of control and experimental myotubes were used to compare the number and size of AChR clusters. To assess the effect of anti-MuSK antibodies on the subcellular distribution of MuSK, C2 myoblasts transfected with MuSK-GFP were imaged live on a confocal microscope. **Results:** Anti-MuSK antibodies caused tyrosine phosphorylation of MuSK and the AChR β-subunit, and internalization of MuSK (n=3). When added to cultured cells, anti-MuSK antibodies caused a significant reduction in number and size of AChR clusters compared with cells treated with control human IgG (n=3). **Conclusion:** anti-MuSK IgG may interfere with neuromuscular transmission by depleting MuSK from the postsynaptic membrane scaffold, leading to disassembly of AChR clusters.

POS-TUE-044

ORIGIN OF THE LOW-LEVEL EMG DURING THE CORTICAL SILENT PERIOD

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The cortical silent period refers to a period of up to 300 ms of near silence in the electromyogram (EMG) after transcranial magnetic stimulation (TMS) over the motor cortex during voluntary contraction. Despite the name, there are often small amounts of EMG present. The origin of this activity is not known. We hypothesized that it arises through spinal reflex mechanisms, in which lengthening of the muscle during relaxation caused by the cortical silent period could cause muscle spindle firing and facilitate motoneurones. **Purpose**: The current study tested whether low-level EMG in the silent period depended on muscle lengthening. **Methods**: Subjects (n=8) performed maximal isometric, shortening and lengthening contractions of the elbow flexors during which TMS (90-100% stimulator output) was delivered over the motor cortex. The rate of elbow flexion during the shortening contraction (225 degrees/s) was chosen to offset the estimated muscle lengthening caused by muscle relaxation. Surface EMG activity was recorded from biceps brachii and brachioradialis muscles, and the low-level EMG during cortical silent periods produced by TMS was measured. **Results**: The low-level EMG activity in the cortical silent period was reduced by up to 60-65% in the shortening contraction compared to all other contraction conditions ($p<0.05$). There was no difference between the contraction conditions for the pre-stimulus EMG and the duration of the cortical silent period in both muscles was similar across the contraction conditions. **Conclusion**: Muscle lengthening contributes to the low-level EMG activity in the cortical silent period, probably through spinal reflex facilitation by muscle spindle afferents.

POS-TUE-045

REGENERATION EFFICIENCY IN AGED/SENESCENT SKELETAL MUSCLE DEPENDS ON THE TYPE OF INJURY

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Studies have shown that regeneration is impaired in muscles of aged mice. Regeneration improves in aged muscle exposed to a young circulatory system indicating that muscle progenitor or satellite cells are intact (Conboy et al. 2005). We hypothesised that the efficiency of repair/regeneration in aged muscles may be due to the injury method and in particular, the extent of nerve and vascular damage. We subjected the extensor digitorum longus (EDL) muscles of young (3 months; n=7), aged (22 months; n=8) and senescent (27 months; n=10) female mice to myotoxin (notexin) injury, which leaves the basal lamina, associated blood vessels and nerves intact. We subjected young (n=6) and aged (n=7) EDL muscles to Denervation-Devascularization (DD) injury, which detaches the associated nerves and blood vessels. Notexin injury resulted in full regeneration with hyperplasia in young and hypertrophy in old and senescent muscles. In vitro contractility measurements showed comparable absolute and specific forces, and active stiffness between the untreated and regenerated young and senescent muscles. Both young and senescent muscles showed a significant increase in passive stiffness ($p<0.01$ One-Way ANOVA) and shift towards slow-twitch characteristics ($p<0.05$ student's t-test). With DD injury, there was a severe hypotrophy of myofibres and decrease in muscle size/weight in both young and aged regenerated muscles. The results clearly demonstrate that the nature of muscle injury determines the efficiency of muscle regeneration in both young and aged muscles.

POS-TUE-046

ISOTONIC FORCE DEPENDENT MUSCLE ACTIVITY IN THE MULTI-JOINT MOVEMENTS

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To investigate how the muscular activity affects the dynamic properties during knee-hip extension movements, electromyography (EMG) activity was obtained from major working muscles in isometric and isotonic (force clamp) conditions. Nine healthy subjects performed various loads of concentric and isometric knee-hip extensions at their maximal effort on a servo-controlled dynamometer. EMGs were simultaneously recorded by using surface electrodes from seven muscles: the gastrocnemius (Gas), vastus medialis (VM), rectus femoris (RF), vastus lateralis (VL), bicep femoris (BF), semitendinosus (ST), and gluteus maximum (Glut). Also, EMGs of maximal voluntary isometric contractions for each muscle were measured and used for the normalization of the muscle activity during knee-hip extensions. The EMG was full-wave rectified, integrated and averaged with respect to time (mEMG). The force-velocity relation of the knee-hip extension movement was well described with a linear function ($r=-0.996$). The EMG activities of VM and VL during maximum isometric knee-hip extension movement as compared with isolated muscle contractions were larger while RF was same; on the other hand, GA, BF, ST and GM were lower. In all muscles studies, mEMG did not change significantly with force in the isotonic condition. In VL, however, it was significantly smaller in isometric condition than in the lower forces of isotonic condition ($P<0.05$). In conclusion, the coordination between knee extensor and hip extensor muscles changes with force in the knee-hip extension movement. In particular, decreased activation of knee extensor relative to hip extensor under the large force may strongly affect the nature of force-velocity relation.

POS-TUE-047

ROBUST STORE-OPERATED CALCIUM ENTRY IN AGED MAMMALIAN SKELETAL MUSCLE

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Store-operated Ca^{2+} entry (SOCE) is a specialized mechanism in muscle, involving extracellular Ca^{2+} entry in response to depleting intracellular Ca^{2+} stores during work. Although a report recently suggested SOCE is compromised in aged muscle (Zhao et al, 2008, *Aging Cell*), we reassessed this with our novel, sensitive techniques (Launikonis & Rios, 2007, *J.Physiol*). Young (8-20 weeks) and aged (25 months) C57BL/10 mice from the same colony were compared for SOCE functionality and relevant protein abundances. Fluo-5N trapped in the tubular system of skinned fibres was imaged with confocal microscopy. Substitution of the standard intracellular solution with low Mg^{2+} solution induced Ca^{2+} release. There was initial Ca^{2+} uptake in sealed t-tubules, followed by depletion due to SOCE. SOCE deactivation followed Ca^{2+} reuptake into sarcoplasmic reticulum (SR) and reduction in myoplasmic Ca^{2+}. Robust SOCE was observed in all fibres (n=8) from aged mice. In some fibres, subsequent Ca^{2+} waves were observed with defined onset of SOCE, allowing determination of SOCE activation kinetics. Whilst SOCE activation was delayed in aged (38±3.1 ms, n=4) compared to young (27±3.6 ms, n=6, p=0.044) muscle, SOCE deactivation was robust. Of note, rate of SR refilling compared to rate of SOCE deactivation varied between aged fibres (n=8). Furthermore, Orai1 and Stim1 protein levels were also varied suggesting the need for physiological and biochemical measurements on the same aged fibres. We conclude that SOCE continues to work in aged muscle but its deactivation and activation thresholds, as well as the integral SOCE protein levels may vary.

POS-TUE-048

MODULATION OF HUMAN CORTICOMOTOR EXCITABILITY BY SENSORY, MOTOR AND NOXIOUS STIMULI

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Background: Electrical stimulation has been used as a means of inducing cortical plasticity in both healthy and pathological populations. However, the parameters used vary considerably and are often not consistent with those used in the clinic. In addition, it is unclear which parameters are the most beneficial for inducing directionally specific plastic change. Here, we aim to examine the effect of five clinically relevant electrical stimulation paradigms on corticomotor excitability. **Methods:** Transcranial magnetic stimulation was used to measure corticomotor excitability of the biceps brachii and triceps brachii muscles before and after 30 minutes of electrical stimulation. Ten healthy individuals received five different electrical stimulation paradigms, applied in a random order, to the right biceps brachii. Each electrical stimulation paradigm was delivered at least 3 days apart. Responses evoked by TMS were normalised to M-wave amplitudes. **Results:** Electrical stimulation (10Hz) delivered at sensory or noxious intensity produced a decrease in corticomotor excitability ($p < 0.03$). In contrast, motor stimulation (30Hz) to create a functional muscle contraction resulted in a significant increase in corticomotor excitability ($p = 0.04$) while stimulation at 10Hz sufficient to produce a motor twitch resulted in no significant change ($p = 0.37$). Changes in cortical excitability were consistent across the agonist and antagonist muscles in all conditions ($p > 0.19$). **Conclusion:** These results suggest that clinically relevant electrical stimulation paradigms can induce transient inhibitory and facilitatory changes in corticomotor excitability. The direction of excitability change may be dependent on the balance between positive and negative drives.

POS-TUE-049

A NOVEL REHABILITATION TOOL TO PROMOTE FUNCTIONAL RECOVERY AFTER STROKE

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More than half of those who survive a stroke are profoundly disabled in activities of daily life. Because there is no cure for stroke, rehabilitation remains the only option to recover functional movement. In Australia there is no standardised approach to rehabilitation and no single proven therapy. We used the Nintendo Wii game system as an intensive therapy protocol. Seven stroke patients (mean age 65.3 years, 1-38 months post-stroke) and five healthy controls (mean age 59.0 years) undertook one hour of formal therapy on 10 consecutive weekdays. Additionally, the stroke patients gradually increased additional home practice to three hours per day. Functional testing was performed immediately pre- and post-therapy. Functional movement ability significantly improved for all seven patients. A significant decrease in the performance time for the Wolf Motor Function Test ($p=0.006$) was matched by a significant improvement in Fugl-Meyer Assessment scores ($p=0.013$). The improved functional test scores were reflected in activities of daily living, assessed using the Motor Activity Log ($p=0.008$) and represents a direct transfer of the gains achieved in therapy to real-world tasks. The range of motion for upper limb joints increased on average by 12.7° and 7.2°, for passive and active movements respectively. No significant change was seen in any measure for the control subjects, despite an improvement in their skill level for the Wii therapy games. These results suggest that an intensive 2 week protocol using the Nintendo Wii is sufficient to induce significant and clinically relevant improvements in functional motor ability after stroke. Moreover the gains achieved in therapy translated to improved functional movement in activities of daily living.

POS-TUE-050

FEASIBILITY OF MEASURING VOLUNTARY ACTIVATION AFTER STROKE USING CORTICAL STIMULATION

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Maximal voluntary activation is a measure of the neural drive to produce maximal force. Impaired motoneurone drive is revealed by peripheral stimulation while cortical stimulation reveals impaired cortical output. Activation scores are calculated using the resting twitch which must be estimated for cortical stimulation. This study was designed to determine the feasibility of using cortical stimulation to study voluntary activation in stroke patients. Voluntary activation was studied in the elbow flexor muscles in 5 stroke patients (mean age 67 years; 1-16 years post-stroke) using both peripheral and cortical stimulation. Elbow flexion force was measured with an isometric myograph while electromyographic activity was recorded from biceps and triceps brachii. Stimuli were delivered to the peripheral nerve (0.1 ms pulse width) or to the motor cortex using transcranial magnetic stimulation (1 ms pulse) while subjects performed 5 sets of brief maximal and submaximal voluntary contractions (MVC). Peripheral voluntary activation scores for stroke patients in this study (median 77%) were lower than those reported in the literature for young, healthy subjects (97% Todd et al 2003). Similarly, the pattern of cortical activation in stroke patients was different and more inconsistent to that of healthy controls. Motor evoked potentials (MEPs) could not be evoked at rest in any stroke patient. In healthy controls the biceps normalised MEP was largest in contractions at 50% MVC, while in stroke patients this occurred most frequently at 75% MVC with a maximum occurring at 50% MVC in only 2 subjects. These results suggest that the methods used to measure voluntary activation using cortical stimulation in healthy subjects cannot be used with stroke patients.

POS-TUE-051

EXCITABILITY OF MOTOR CORTICAL OUTPUT TO HUMAN SCALENES MUSCLES IS ALTERED BY LUNG VOLUME

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Pulmonary afferents are known to inhibit inspiratory output from the medulla with increasing lung volume. **Purpose**: The aim of this study was to assess the effect of pulmonary afferent feedback on the excitability of motor cortical output to the respiratory muscles. **Methods**: In 8 subjects lying supine, motor evoked potentials (MEPs) were recorded from the right scalenes muscles in the neck (obligatory inspiratory muscle) and from biceps (non-respiratory muscle). Pulmonary afferent feedback was altered by changing lung volume. Subjects performed two manoeuvres (10 trials each): (1) incremental inspiration from functional residual capacity (FRC) to total lung capacity (TLC) and (2) incremental exhalation from TLC to FRC. High-intensity transcranial magnetic stimulation (75-95% stimulator output) was delivered over the motor cortex during relaxation at three lung volumes; FRC, FRC + 40% inspiratory capacity, and FRC + 90% inspiratory capacity. Prior to stimulation, the breathing apparatus was closed so that subjects could relax at each volume. **Results**: The amplitude and area of the MEPs recorded from the scalenes muscles were ~ 50% greater at a high lung volume compared to lower lung volumes ($p < 0.001$). However, there was no difference in MEP size for the same lung volume in inspiratory and expiratory manoeuvres. In the control muscle biceps, the size of MEPs was similar at all lung volumes and in the two manoeuvres ($p \geq 0.2$). **Conclusion**: The results suggest that unlike their effect at the medulla, pulmonary afferents activated at high lung volume increase the excitability of the motor cortical output to inspiratory muscles.

POS-TUE-052

EFFECTS OF HIGH FREQUENCY AND THETA BURST TRANSCRANIAL MAGNETIC STIMULATION ON GRIP STRENGTH

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Transcranial magnetic stimulation (TMS) involves a brief but strong magnetic field capable of activating cortical elements. With the introduction of repetitive TMS (rTMS) it has been possible to study the modulatory effects of various stimulation paradigms on the excitability of motor systems. The outcomes are representative of the diverse effects of rTMS dependant on both the intensity and frequency of stimulation (Houdayer et al. 2008). Most studies investigate these effects on the motor evoked potentials of resting or slightly active muscles. Although the influence of magnetic stimulation on maximum voluntary contraction force of quadriceps femoris muscle in healthy individuals have been reported (Urbach & Awiszus 2001), the upper extremity muscles have not been studied with different stimulation paradigms. In this study we examined the effects of 'simple' rTMS at 5Hz and theta burst stimulation paradigm (30Hz 3 pulse burst at 5Hz) on grip strength of healthy 18-35 yo individuals. An adjustable dynamometer (Jamar) was used to measure the grip strengths on Position-2 and Position-3 separations. Measurements were carried out before and after rTMS intervention with 300 pulses delivered to the forearm representation of motor cortex at 80% active motor threshold. Each participant was also subjected to sham stimulation in their first session. The preliminary results suggest that there is no consistent change in the grip strength with either stimulation paradigm. Houdayer, E., Degardin, A, Cassim, F. et al. Exp Brain Res (2008), 187: 207-217. Urbach, D. & Awiszus, F. Exp Brain Res (2001), 142: 25-31.

POS-TUE-053

ARE RAT MODELS OF RUBROSPINAL TRACT INJURY ANY GOOD TO ASSESS RECOVERY OF HAND FUNCTION?

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The rubrospinal tract (RST) has recently been the focus of considerable attention in the field of spinal cord injury and repair. Lesions damaging variable extents of the LF but that consistently disrupt the RST support the view that the RST is involved in the control of the paw/digit movements while reaching. Such lesions, however, also damage other pathways within the LF that possibly contribute to reaching. The present study was designed to isolate more precisely the contribution of the RST to skilled movements of the forelimb. Rats (N=21) were trained on the skilled reaching task and were subsequently subjected to either 1) large lesions of the LF that extend below the central canal (CC), 2) medium lesions of the LF, restricted to its dorsal part and that stop at the level of the CC or 3) small lesions of the LF, restricted to its dorsal part with considerable sparing above the CC. All lesions included the full extent of the RST and care was taken in all instances to leave the dorsal roots intact (Wu et al., 2009). Detailed movement analysis revealed that, although all operated animals were still able to reach, the three types of lesions differently affected some components of the reach. Most interestingly, however, the pronation and arpeggio movements were impaired or missing to the same extent in all groups of lesions, suggesting that these components of the reach are under the control of the RST. The results are discussed in terms of translation of animal models of cervical spinal cord injury to clinically relevant therapeutic scenarios to improve the quality of life of people living with quadriplegia.

POS-TUE-054

NEURONS IN THE LOCUS COERULEUS AND SUBCOERULEUS NUCLEUS THAT PROJECT TO SPINAL CORD ARE NOT NORADRENERGIC

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The locus coeruleus and subcoeruleus nucleus play an essential role in arousal. About 95% of the noradrenaline in the brain is in the neurons of the locus coeruleus. Descending projections from the nucleus locus coeruleus and subcoeruleus to the spinal cord have been demonstrated in a number of mammals, and it has been assumed that the spinal projecting neurons are noradrenergic. We studied this projection in the C57BJ6 mouse by injecting the retrograde tracer Fluoro-gold into the cervical spinal cord. We found that spinal projecting neurons in the nucleus locus coeruleus were confined to the ipsilateral side of the injection, but the subcoeruleus nucleus had bilateral labelling with an ipsilateral predominance. Double labelling with anti-tyrosine hydroxylase (TH) revealed that not all of the spinal projecting neurons were TH positive. We have concluded that the TH-negative projecting neurons in the locus coeruleus and subcoeruleus nucleus are not noradrenergic. This suggests that there are at least two distinct cell populations in the locus coeruleus - a finding which is consistent with the involvement of this nucleus physiological functions other than arousal and sleep modulation.

POS-TUE-055

BEHAVIOUR OF HUMAN GENIOGLOSSUS SINGLE MOTOR UNITS (SMU) DISCHARGE PROPERTIES IN QUIET BREATHING, CO2 AND CPAP

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The genioglossus is a primary muscle involved in dilating the upper airway. Two primary stimuli that contribute to genioglossal control are CO2 and negative pressure which can modify the behavior of respiratory drive through chemoresponsiveness and mechanoreceptor activation respectively. We examined genioglossus SMU discharge properties to quantify neural drive during periods of quiet breathing, elevated ETCO2 and CPAP (2cmH2O increments until 10cmH2O) to reduce negative pressure influences on muscle activity. 15 subjects studied awake lying supine, breathing through a nasal mask. 3 fine-wire electrodes were inserted after ultrasound. We measured onset time, onset and peak firing frequency relative to respiration for 96 SMUs, tracked throughout 8 conditions. Genioglossus SMU activity increased with CO2, there was an increased discharge rate of inspiratory units (19Hz to 21Hz, $p<0.05$) with activation earlier in the inspiratory cycle (7.5%TI to -9.1%TI, advancement $p<0.05$,), and firing for a longer period of the respiratory cycle (80.5%TI to 105.7%TI, $p<0.05$). An additional 33.3% of distinguishable SMUs within the selective electrode recoding area were recruited. CPAP led to a progressive inhibitory response on the number of motor units active. At ~6cmH2O a similar number of motor units were active as in baseline conditions, with peak frequencies of the inspiratory units returned to baseline 19.3Hz. At 10cmH2O the number of active units was 36.1% less than baseline conditions. Genioglossus SMU activity is altered in response to chemical and mechanical stimuli. Inspiratory Phasic and Inspiratory Tonic SMUs have earlier pre-activation and increased peak firing frequencies during inspiration in response to CO2, and this increase in activity is terminated by CPAP.

POS-TUE-056

THE BOUNDARIES AND CONTENTS OF THE MAMMALIAN ISTHMUS REVEALED BY FGF8 GENE EXPRESSION

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The isthmus is a distinct part of the vertebrate brain, situated between the midbrain and the first rhombomere. The signature component of the vertebrate isthmus is the trochlear nucleus. Transient Fgf8 expression in the isthmic region plays a crucial role as a secondary organizing center in the embryonic brain. In avian and mammalian embryos, Fgf8 is expressed immediately caudal to the junction of Otx2 and Gbx2 expression. The isthmus is recognized as a substantial part of the hindbrain of adult birds and reptiles, but most studies on mammals ignore its existence entirely. **METHOD** This study has defined the contents and boundaries of the mammalian isthmus by examining the distribution of cells of the Fgf8 lineage in postnatal mice (n=4) possessing an Fgf8-cre-lacZ transgene (stained sections kindly provided by Dr Tomomi Shimogori of RIKEN). **RESULTS** The neuron groups expressing Fgf8 correspond well to the predictions based on avian studies; the trochlear, dorsal raphe, caudal linear, and parabigeminal nuclei are all intensely labelled with lacZ. A large part (perhaps 50%) of the substantia nigra (SN) appears to be derived from the isthmus. Only a small portion of SN can therefore be considered to be mesencephalic, since the rostral parts of SN belong to the diencephalon. The Fgf8-cre preparations also indicate that the whole cerebellar vermis is derived from the isthmus. **CONCLUSION** This study demonstrates the isthmic character of many structures at the midbrain-hindbrain junction, and point to the need for a re-evaluation of the regional anatomy of the substantia nigra. These results demand recognition of the existence of the isthmus as a major component of the mammalian hindbrain.

POS-TUE-057

AFFERENTS TO THE MESENCEPHALIC RETICULAR FORMATION

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Studies on the mesencephalic reticular formation (mRt) in the 1970s and 1980s indicated that this structure was involved in many behaviors, including eye movement, eating, sleep-walking, urination, defecation and sexual activity. However, the anatomy of this region has not been well defined. We found a distinct group of calbindin positive neurons in the central mRt. In order to map input to these neurons, we injected the retrograde tracer (FluoroGold; 20 nanolitres per rat) into the mRt. The mRt receives afferents from diverse regions in the brain. The major sources of forebrain afferents are the internal pyramidal layer (V) of the motor cortex (the primary and secondary), the primary somatosensory cortex, the insular cortex, the amygdala, the medial preoptic nucleus, the zona incerta, the ventromedial hypothalamic nucleus, the paraventricular hypothalamic nucleus and the lateral hypothalamic area. The mRt also receives substantial input from the periaqueductal grey, the superior colliculus, the substantia nigra, the dorsal tegmental nucleus, the superior olivary complex, the spinal trigeminal nucleus, and the lateral paragigantocellular nucleus. The most of the structures that send input to the mRt are related to motor activity, which is consistent with the physiological literature on the role of the mRt in patterned motor activity.

POS-TUE-058

THE ARCUATE NUCLEUS OF THE HINDBRAIN IS A DISPLACED INFERIOR OLIVARY COMPONENT

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The arcuate nucleus is a prominent cell group in the human hindbrain, and is considered to be a precerebellar nucleus. It is not commonly found in other mammals, and it has not been previously identified in the mouse. To further investigate whether the arcuate nucleus issues mossy fibers to the cerebellum like the lateral reticular nucleus, or whether it can be grouped developmentally and functionally with the cells of the inferior olive, the only source of climbing fibers in the cerebellar cortex, we examined the cytology, gene expression, immunohistochemistry, and cerebellar projections of the arcuate nucleus, the inferior olive, and the lateral reticular nucleus in the mouse (n=8). Gene expression was examined using the AGEA tool (http://mouse.brain-map.org). We found that the arcuate nucleus is a distinct but inconstant group of neurons, which lie either on the ventral surface of the pyramid or in a stream continuous with the ventrolateral part of rostral inferior olive, the principal nucleus. The neurons of arcuate nucleus and of inferior olive share three characteristics: they both stain strongly for calbindin; they both express a number of genes not seen in the lateral reticular nucleus, such as Htr5b, Tmem16B, and Stac; they both project to the contralateral cerebellum. We conclude that the arcuate nucleus is a subgroup of the inferior olive, issuing climbing fibers to cerebellum, but that during the caudal extension of the pyramidal tract, its cells are separated from the remaining inferior olive.

POS-TUE-059

BISTABILITY PERMITS RAPID TRANSITIONS BETWEEN SPATIOTEMPORAL WAVES AND SYNCHRONOUS SPIKING ACTIVITY IN A COMPUTATIONAL MODEL OF CORTEX

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Rapid changes in behaviour must be driven by rapid changes in dynamic brain states, yet the brain must also remain stable in the face of noise and uncertainty. The neural mechanism for satisfying these competing demands is unknown and presents a fundamental problem to computational neuroscience. We modelled a small region of motor cortex as an ensemble of phase-coupled neural oscillators arranged in a two-dimensional sheet. The strengths of the lateral connections between neurons (synaptic kernels) in this sheet varied periodically with distance. The model induced either phase-matched synchronous firing or spatiotemporal wave-like firing patterns across the entire neural ensemble. Increasing the baseline strength of neural connections favoured synchronous firing patterns whereas increasing the amplitude of the periodicity favoured wave patterns. Manipulating both parameters, we found that certain combinations of baseline strength and periodicity gave rise to bistability wherein the neural ensemble converged to either synchrony or waves depending on initial conditions. We argue that wave patterns and synchronous firing patterns represent distinct stable brain states and that switching between these brain states is consistent with switching between behavioural states. We suggest that very rapid transitions between otherwise stable brain states is most efficient within the region of bistability and that motor cortex may exploit this property to achieve rapid transitions between motor behaviours.

POS-TUE-060

PATHOGENIC ROLE OF GENE MUTATIONS IN ALS

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BACKGROUND: ALS (amyotrophic lateral sclerosis) is an adult-onset neurodegenerative disorder that causes degeneration of both upper and lower motor neurons. The principal pathology of ALS is the presence of ubiquitin positive protein aggregates in the cell body of the motor neurons. TDP-43, principally a nuclear protein (encoded by TARDBP gene) is a major component of the Ubiquinated inclusions in ALS. Several TARDBP and FUS mutations have recently been reported in ALS cases. Mutations in FUS are the second most common known gene abnormality in familial ALS after SOD1. OBJECTIVES: The purpose of this study is to screen for additional mutations in TARDBP and FUS genes among familial ALS cohort (n=124) and sporadic ALS (n = 247). Another aim is to establish neuronal cell models expressing mutantTDP-43 and FUS. The effects of TARDBP mutations are also being investigated in lymphoblasts. RESULTS: Three TARDBP gene mutation were found in the ALS cohort. One new FUS mutation R521H was found in the extended FALS cohort. Our preliminary immunohistochemistry and immunofluorescence results show that there is presence of aggregates and an abnormal redistribution of TDP-43 from nucleus to the cytoplasm in cells tranfected with TARDBP mutations when different cellular stresses are induced. These phenotypic changes under different stresses implicate pathways and mechanisms through which TDP-43 plays a pathogenic role. Preliminary results of induced cellular stresses on patient lymphoblasts show an increase in proteolytic cleavage and also redistribution of TDP-43 from nucleus to the cytoplasm.

POS-TUE-061

EXPRESSION OF VOLTAGE-GATED SODIUM CHANNEL β-SUBUNITS IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND: Motoneuron hyper-excitability and increased persistent Na^+ current (I_{NaP}) is an early feature of amyotrophic lateral sclerosis (ALS) in both humans and transgenic mouse models of ALS ($hSOD1^{G93A}$). We previously reported that expression of voltage-gated sodium channel (Na_V) α-subunit genes were up-regulated in $hSOD1^{G93A}$ mouse motor cortex during the first postnatal week. Na_V channel activity can also be modulated by associated β-subunits. Here, we investigated levels of gene expression of Na_V β-subunits in motor cortex, brainstem and lumbar spinal cord of wild type (WT) and $hSOD1^{G93A}$ mice aged P0, P7, P16, P28 and P71. **METHODS:** Samples were dissected from these three brain regions of mice anaesthetised with sodium pentobarbitone (100 mg/kg i.p.) (n=3-9 for each age and genotype), followed by total RNA extraction and reverse transcription. mRNA abundance was measured by real-time PCR using commercial TaqMan probes for mouse Na_V β1, β2, β3 and β4. Data were normalized to the geometric mean of multiple reference genes (Ubc, Hprt1 and Sdha). **RESULTS:** In WT mice, Na_V β1, β2 and β4 expression levels were very low at P0 in cortex, brain stem and lumbar spinal cord. β1 expression increased for three weeks after birth and then stabilized, while β2 and β4 expression remained at low levels throughout the postnatal period. β3 expression was highest at birth in all areas, relative to expression of other β-subunits, then declined rapidly to stable moderate levels at P16. In $hSOD1^{G93A}$ mice, expression patterns of β-subunits in all regions were similar to WT littermates; only β3 expression was significantly increased by 1.36 fold at P7 in $hSOD1^{G93A}$ cortex and by 1.75 fold at P21 in $hSOD1^{G93A}$ spinal cord, compared with WT controls. **CONCLUSION:** β3 subunit gene expression levels were up-regulated postnatally in some $hSOD1^{G93A}$ mouse brain areas; this may contribute to neuronal hyper-excitability due to increased I_{NaP}.

POS-TUE-062

CO-ORDINATING CONTRACTION IN THE PREGNANT UTERUS

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Uterine contractions require Ca^{2+} influx through voltage-gated Ca^{2+} channels, raising the critical question as to events mediating the depolarization necessary for opening these channels. In gut, Interstitial cells of Cajal (ICC) play a key role in pacemaking. We investigated the possibility that ICC-like cells underpin uterine contractions. ICC were stained with vimentin and smooth muscle (SM) cells localized with SM actin in uteri from 20 women undergoing caesarean delivery. Uteri from 23 pregnant mice were also studied. Contractions were recorded simultaneously with membrane potential, using intracellular microelectrodes. Ionic currents were recorded via conventional patch clamp, the characterized cells were then subjected to single cell PCR. In human tissue, ICC occupied 1.3±0.3% of SM bundles, but occupied 2.6±0.4% of the space between bundles (p=0.01). Ca^{2+}-sensitive K^+ channels were rare in ICC (maximum current 58±10pA) (facilitating excitability), but abundant in SM cells (904±163pA) (facilitating quiescence). This was confirmed using single-cell PCR. In mouse uterus, ICC staining was absent from longitudinal (LSM) layer, was present in circular (CSM) layer and abundant between the two layers. Vimentin-staining co-localized with c-Kit staining. LSM cells had membrane potentials of -68±1mV and were quiescent, while CSM had membrane potentials of -55±1mV (p=0.008) and had spontaneous contractions. The c-Kit antagonist imatinib did not abolish spontaneous activity in CSM but it blocked the spread of activity, generated in CSM, to LSM. We conclude that ICC are not responsible for generating contractions in uterus but may be involved in propagation of activity. Human uterus consists of an intricate network of thin bundles of SMC. Efficient communication between bundles is critical for coordinated organ contraction. Targeting uterine ICC could have therapeutic possibilities.

POS-TUE-063

NEURAL SST_1 AND SST_2 RECEPTORS DECREASE CHLORIDE SECRETION IN THE GUINEA-PIG SMALL INTESTINE

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Purpose: Somatostatin (SOM) inhibits secretion. We have found that vasoactive intestinal peptide (VIP) secretomotor neurons display inhibitory postsynaptic potentials that are mediated by SOM acting on SST_1 and SST_2 receptors. This study investigated the role these SST receptors play in secretion in guinea-pig small intestine. ***Methods:*** Mucosa-submucosa preparations were dissected and mounted in Ussing chambers to measure Cl^- secretion across the mucosa. All drugs were added serosally. Veratridine (1μM) was applied to stimulate neurons and provide a robust secretory response, reflected by an increase in short circuit current (ΔI_{sc}). Quantitative-PCR (qPCR) was performed on stripped guinea-pig submucous plexus and mucosa to provide a relative quantification of SST_1 and SST_2 receptor gene expression, using ribosomal 18S as the endogenous house-keeping gene. Results were considered significant if $P<0.05$. ***Results:*** SOM (50nM) induced a tetrodotoxin (1μM)-sensitive (n=6), decrease in basal secretion (n=9). SOM also significantly reduced veratridine-induced ΔI_{sc} (n=8). The effects of SOM were significantly reduced by blocking SST_1 (SRA 880 3 μM; basal secretion n=8, veratridine-induced ΔI_{sc} n=9) and SST_2 receptors (CYN 154 806 100nM; basal secretion n=10, veratridine-induced ΔI_{sc} n=10) individually, or in combination (basal secretion n=6, veratridine-induced ΔI_{sc} n=7). Blocking SST_1 receptors were more effective than blocking SST_2 receptors. q-PCR demonstrated that SST_1 (n=6) and SST_2 (n= 6) receptors were highly expressed in the submucous plexus compared to the mucosa, and SST_1 receptor expression was significantly higher than SST_2. ***Conclusions:*** SOM exerts its antisecretory effects indirectly by suppressing the excitability of VIP secretomotor neurons via SST_1 and SST_2 receptors. SST_1 receptors appear to play a more prominent role in secretion.

POS-TUE-064

INNERVATED MYOFIBROBLASTS IN THE URINARY BLADDER? FUNCTIONAL AND ULTRA-STRUCTURAL EVIDENCE

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We have shown that neurokinin A (NKA) and other agents are able to contract isolated mucosal strips from porcine bladder (Sadananda et al., 2008). Dense smooth muscle actin staining on the discrete population of porcine suburothelial myofibroblasts suggested that these cells may be contractile, and mediate the contraction to NKA, hypothesised to be originate from suburothelial afferent nerves. The purpose of this study was to examine the ultrastructure of the porcine bladder mucosa and characterize the relationship of the suburothelial myofibroblasts with adjacent cells. Pig bladders (n=2) were obtained from an abbatoir, fixed in glutaraldehyde and processed for electron microscopy. The suburothelial population of porcine bladder myofibroblasts were morphologically similar to the deep myofibroblasts of human detrusor, in that they have prominent rough endoplasmic reticulum and elongated processes. Extensive nerve bundles with varicosities were located below the urothelium and in close proximity to myofibroblasts. These nerve bundles contained individual varicosities with discrete regions (bare of Schwann cells) containing accumulations of synaptic vesicles, which faced the potential neuroeffector cell. This is in accordance with the definition of neuroeffector junctions, as neurotransmitter release sites. Further, the dense-cored synaptic vesicles in these nerves were 100-250 nm in diameter, consistent with a primarily peptidergic content. In low resolution studies (n=4), synaptophysin and tachykinin immunoreactivity indicated that nerve fibres containing tachykinins were located within the suburothelial nerve plexus and in association with myofibroblasts. The strategic location of suburothelial myofibroblasts between the urothelium and afferent nerve plexus suggests that they are involved in sensory processing and cross talk between the two cell layers. Data suggest that peptides such as NKA are released from these dense-cored synaptic vesicle-containing varicosities, to act directly on suburothelial myofibroblasts, resulting in contraction of the porcine bladder mucosa. Subsequent studies will further characterise this hypothesis. Sadananda P, Chess-Williams R & Burcher E. (2008). Br J Pharmacol 153, 1465-1473.

POS-TUE-065

EFFECTS OF A GHRELIN RECEPTOR AGONIST AT THE SPINAL CORD LEVEL ON URINARY BLADDER CONTRACTILE ACTIVITY

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We have previously shown that ghrelin receptor (GhrR) agonists can act on the spinal cord to selectively stimulate autonomic outputs to blood vessels and the colorectum, whilst not affecting small intestine or heart. We have now investigated whether spinal pathways controlling the urinary bladder could also be affected by GhrR agonists. **Method:** A new potent GhrR agonist, capromorelin, was injected either intravenously (10mg/kg) or intrathecally (250µg) into Sprague Dawley rats (n=10) undergoing continuous infusion cystometry under urethane anesthesia. Two rats were used to test the effect of capromorelin directly on detrusor muscle with 6 strips set up in organ baths. A series of rats (n=14) was perfused following stimulation of bladders for 30 minutes to induce c-Fos expression within the spinal cord that was later co-localized with GhrR gene expression. **Results:** Both i.v. and i.t. capromorelin caused increased, but disrupted, contractile activity, without improving efficiency of voiding. The effect was blocked by both hexamethonium and atropine. Direct application of capromorelin (up to 100µM) had no effect on bladder contractility. Immunohistochemistry revealed the early gene product c-Fos in neurons of the autonomic intermedio-lateral cell columns at L5 to S2, with 20% also expressing the GhrR gene transcript. **Conclusions:** We conclude that ghrelin receptors are expressed in lumbosacral autonomic preganglionic neurons that control excitatory pathways to the detrusor muscle.

POS-TUE-066

c-FOS IDENTIFICATION OF SPINAL NEURONS ACTIVATED BY THE URETHRO-GENITAL REFLEX IN FEMALE GUINEA-PIGS

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To examine spinal circuits underlying the urethro-genital reflex (UGR), we visualised neurons activated by the reflex with c-Fos immunoreactivity in the lumbar and sacral spinal cord of female guinea-pigs. In anaesthetised female guinea pigs (200-250g), a balloon was inserted into the urethra and inflated for 30s every 10 minutes for 90 minutes to activate UGR. A second balloon was inserted into the vagina and uterus to record reflex contractions. One control group had no balloon; a second control group had a vaginal recording balloon only. In some animals, the spinal cord was transected acutely at L4 or T12 segments. Animals were left for 90 minutes after eliciting the UGR and were then processed for c-Fos immunoreactivity in L3 to S2 segments of the spinal cord. Using multivariate analysis of variance, there was no significant difference in spinal c-Fos expression between the two control conditions (n=5 each). The UGR significantly increased the number of c-Fos-expressing neurons by 100-200% throughout dorsal and intermediate laminae at S2 but only by about 100% in superficial laminae at L3 (n=5). Transection at T12 had little consistent effect on c-Fos expression in response to UGR at either spinal level (n=5). However, transection at L4 significantly increased the number of c-Fos-expressing neurons in intermediate laminae by about 100% at S2 and by about 50% at L3 in response to UGR (n=5). These data suggest that the UGR apparently activates local spinal inhibitory circuits that suppress expression of c-Fos in neurons likely to include preganglionic outputs to the pelvic viscera.

POS-TUE-067

DISRUPTED GASTRIC SLOW WAVE ACTIVITY AND NEUROMUSCULAR TRANSMISSION IN A MURINE MODEL OF TYPE 2 DIABETES

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Gastric motor function is frequently disordered in patients with type 2 diabetes mellitus (T2DM) and can be associated with symptoms such as nausea and bloating, impaired absorption of nutrients and medications, and disordered blood glucose control. Delayed gastric emptying has traditionally been attributed to autonomic neuropathy however a weak correlation between autonomic nerve damage and the extent of disordered motility suggests other mechanisms are affected. In addition, acute variations in blood glucose have major effects on gastric emptying but the mechanisms are poorly understood. In this study, slow wave activity and postjunctional neural responses were compared in non-diabetic and T2DM (db/db) gastric tissues in normoglycaemic and hyperglycaemic conditions. In normoglycaemic conditions db/db antral slow waves were of smaller amplitude (14.1±1.0mV) and higher frequency (10.5±0.6 events.min^{-1}) than in non-diabetics (22.9±2.2mV and 5.1±1.0 events.min^{-1} respectfully; n=7). In non-diabetic antrum acute elevation of glucose concentration to 33mmol produced a decrease in slow wave rate (to 4.0±1.0 events.min^{-1}) and increased the variability of slow wave intervals. In contrast, the amplitude, frequency and variability of slow waves in db/db antrum were unchanged by acute hyperglycaemic conditions. Inhibitory junction potentials, evoked by multiple pulses of electrical field stimulation (0.5ms pulse duration, 5-20Hz) were significantly attenuated in db/db fundus. Kit-immunohistochemistry revealed reduced densities of both intramuscular ICC (ICC-IM) and NOS-positive inhibitory neurons in db/db gastric fundus compared to non-diabetic fundus. These pathophysiological changes to gastric slow wave rhythm and neuromuscular transmission likely contribute to the disrupted gastric motility patterns and impaired accommodation to stomach filling associated with T2DM.

POS-TUE-068

SLOWLY PROPAGATING MOTOR ACTIVITY IN THE ISOLATED RABBIT SMALL INTESTINE

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In addition to spontaneous myogenic activity, generated by the pacemaker net of interstitial cells of Cajal, and neurally mediated content dependent movements, in conscious animals spontaneous neural activity generates slowly migrating motor complexes. It has not been possible to establish how these three separate mechanisms of motor activity interact to produce the complex adaptive intestinal movements, because they do not occur together in isolated preparations. Purpose: To investigate if migrating motor complexes can be generated in isolated segments of intestine. Methods: We investigated the spatio-temporal features of motor activity in isolated segments of intestine taken from 8 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: Spontaneous pendulum activity of the longitudinal muscle generated aborally propagating contractions readily visible in the spatio-temporal maps (speed 21.11mm/s ± 7.9 SD; frequency 12.9/min±1.8 SD; n=9). Erythromycin lactobionate (10-6 M), shown to trigger migrating motor complexes (2), initiated irregular rings of circular muscle contractions, which slowly propagated aborally at speed of 1.9 ± 0.4 mm/s (SD; n=7). Within the slowly propagating area of circular muscle contractions, the short rings of muscle contraction propagated at the speed of the myogenic contractions. Hexamethonium 100µM blocked the slowly propagating contractions (n=3). Conclusions: Thus the isolated rabbit small intestine can be used to investigate the interaction between myogenic, propulsive movements and migrating complexes. References: 1. Hennig et al (1999), J. Physiol., 517, 575-590; 2. Marzio et al (1994) Peptides, 15,10067-1977.

POS-TUE-069

RECEPTORS TO DETECT SHEAR IN THE WALL OF THE COLON

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Extrinsic sensory nerves to gut give rise to gastrointestinal sensations and reflexes. We aim to characterize systematically major classes of gut afferents according to responses and morphology. Biotinamide labeling was combined with extracellular recordings from colonic nerves, in isolated specimens of colon taken from humanely killed guinea pigs. Recordings with all layers present (n=9) revealed a population of sensory axons that could be strongly activated by probing or stroking with light von Frey hairs (0.1 – 10mN, maximum instantaneous firing: 83±12, n=7) and by isotonic circumferential stretch (10mN – 100mN loads, maximum instantaneous firing: 59±12, n=7). They therefore correspond to "muscle-mucosal" afferents previously reported. Removal of the muscularis externa (n=11) had no detectable effect on their excitability, suggesting that they have transductive endings in the submucosa or mucosa. These primary afferents fired in synchrony with spontaneous contractions of the muscularis mucosa and of the muscularis externa (when present). They did not respond to locally applied capsaicin (n=5) and their action potentials had larger amplitude (283±123 vs 133±48, P<0.05, n=5) and trended towards shorter half duration (440±120 vs 1250±1005, n=5, NS) than capsaicin-sensitive units in the same preparations. Their mechanosensitivity was not blocked by application of low calcium solution (n=5), suggesting that they are intrinsically mechanosensitive rather than relying on release of mediators from other cells. Dye filling revealed a single type of ending that corresponded to their receptive fields. These axons branched extensively in the sub-epithelial plexus, close to the base of the colonic glands. This class of receptor has the ideal characteristics to detect shear stimuli combined with small distension - of the sort probably caused by propulsion of solid faecal pellets.

POS-TUE-070

MECHANISM UNDERLYING STRETCH-ACTIVATION OF COLONIC MIGRATING MOTOR COMPLEXES

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It is well known that colonic distension can evoke a premature colonic migrating motor complex (CMMC), but the mechanism underlying this process is unknown. Studies have suggested that distension of the gut wall releases 5-HT from the mucosa that is necessary for the activation of intrinsic nerve endings in the mucosa which then initiate propulsive motor patterns, such as the CMMC. In this study, we investigated whether removal of the mucosa, submucosa and submucosal plexus prevents colonic distension-evoked CMMCs in isolated mouse colon. The entire colon was removed from C57BL/6 mice and a longitudinal incision made along the full length of colon. Results: Graded increases in m/s) applied to the mid/distal region ofμcircumferential stretch (30% at 100 colon reliably evoked a premature CMMC in the mid/distal colon and the proximal colon. Complete removal of the mucosa, submucosa and submucosal plexus did not prevent spontaneous or evoked CMMCs, and actually decreased the stretch threshold required to evoke premature CMMCs (control: 2.1 ± 0.1mm, n=5; mucosa off: 1.6 ± 0.04mm, n=5, P<0.01), with no effect on the amplitude or duration of stretch-evoked CMMCs (n=5). Real time amperometric recordings confirmed that all dynamic release of 5-HT was prevented in preparations devoid of mucosa, but where stretch reliably evoked CMMCs. Hexamethonium (100μM) abolished spontaneous and stretch-evoked CMMCs (n=5). Conclusions: Neither the release of 5-HT from the mucosa, nor even the presence of the mucosa or submucosal plexus is necessary for the mechanism underlying distension-evoked CMMCs. We suggest that circumferential stretch evokes propagating CMMCs by activating a population of intrinsic sensory neurons that lie in the myenteric plexus and that stimulation of nerve endings in the mucosa is not required for this process.

POS-TUE-071

DOWNREGULATION OF CANNABINOID CB1 RECEPTORS IN COLONIC MUCOSA BUT NOT MUSCLE, IN FEMALES WITH SLOW TRANSIT CONSTIPATION

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Slow transit constipation (STC) is an example of extreme dysmotility of the human gastrointestinal tract. STC is a disorder of unknown aetiology; it affects mainly women and is characterised by the inability to defecate more than once every 2-3 weeks. Endocannabinoids are important mediators in regulating intestinal function. The cannabinoid CB1 receptors are predominantly localised to myenteric neurons and submucosal neurons where they mediate intestinal motility and water exchange, mainly through an inhibitory action on cholinergic enteric neurons. In this study, we aimed to determine whether CB1 receptor expression is altered in STC. Sigmoid colon segments were obtained from age-matched female patients (aged 23-76 years) undergoing resection for STC (n=12), or for carcinoma (control, n=8). Segments were dissected into smooth muscle and mucosa layers for separate RNA extraction and real time PCR. In STC mucosa (containing submucosal plexus), there was a 25-fold down-regulation of CB1 receptor mRNA (P< 0.01, Mann Whitney test) compared to control. In contrast, no change was observed in muscle (containing myenteric plexus). The expression of CB2 receptor mRNA was very low in both mucosa and muscle without difference between STC and control. Similar to CB1, mRNA level for synaptophysin, a neuronal marker, was reduced in STC mucosa (6-fold reduction compared to control, P<0.01), but with no differential expression in muscle. These results suggest that secretomotor neuron dysfunction may occur in STC, which is manifested by marked changes in CB1 and synaptophysin gene expression. Thus changes in function may occur in the colonic mucosa, which is currently overlooked in research on pathophysiology of STC.

POS-TUE-072

THE EFFECT OF PLANT-DERIVED CHEMICALS ON HISTAMINE EVOKED CONTRACTION OF SMOOTH MUSCLE

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The plant-derived chemicals 1,8-cineole, α-pinene, *cis*-3-hexen-1-ol, lavender, linalool and *trans*-2-hexenal are commonly found in many household products and are widely used in complementary medicine. However, the effect of these plant-derived chemicals on histamine evoked contraction of smooth muscle has not been well characterised. **Purpose:** The present study examined the effect of 1,8-cineole, α-pinene, *cis*-3-hexen-1-ol, lavender, linalool and *trans*-2-hexenal on histamine evoked contraction of guinea pig ileum. **Methods:** A guinea pig ileum was mounted in an organ bath and the contraction evoked by histamine in the presence of 1,8-cineole (n=6), α-pinene (n=6), *cis*-3-hexen-1-ol (n=6), lavender (n=6), linalool (n=6), *trans*-2-hexenal (n=6) or no plant-derived chemical (control) (n=12) was recorded. A concentration-response curve was generated by adding increasing concentrations of histamine ranging from 1×10^{-9} M to 3×10^{-4} M. All plant-derived chemicals were examined at a concentration of 0.03% (vol/vol). **Results:** Lavender, linalool and *trans*-2-hexenal induced an extremely significant reduction (p<0.001) in histamine evoked contraction from 1×10^{-7} M to 3×10^{-4} M, 1,8-cineole induced a significant reduction (p<0.05) in histamine evoked contraction from 3×10^{-7} M to 1×10^{-6} M and an extremely significant reduction (p<0.001) in histamine evoked contraction from 3×10^{-6} M to 3×10^{-4} M and α-pinene and *cis*-3-hexen-1-ol did not significantly reduce (p>0.05) histamine evoked contraction. **Conclusion:** Our present findings indicate that lavender, linalool, *trans*-2-hexenal and to a lesser extent 1,8-cineole reduce histamine evoked contraction of smooth muscle.

POS-TUE-073

PYY AVAILABILITY IS UNCHANGED IN RAT SMALL INTESTINE DURING A HIGH-FAT DIET

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Purpose: A high fat diet (HFD) is associated with subtle changes in gastrointestinal (GI) function and in the levels of intestinal hormones secreted from enteroendocrine cells. One such hormone is peptide-tyrosine-tyrosine (PYY) which has a role in central appetite control and in local GI function. How local PYY synthesis is changed in a rat HFD model of obesity is unknown. Methods: Rat ileum was taken from control (age-matched, chow-fed, CON: 450-550g; n=20) and high-fat diet rats (HFD; 700-800g; n=28). The mRNA levels of PYY were determined by quantitative RT-PCR and normalised against GAPDH, β-actin and the brush border protein villin. Full-thickness, intact ileum was compared against tissue composed of mucosa-lamina propria. The mRNA level was expressed as fold change = 2-ΔΔCt. Unpaired, non-parametric data were compared using a Mann-Whitney test ($P<0.05$). Results: Intact ileum showed a pronounced decrease in PYY mRNA in HFD compared to control using GAPDH (CON: 0.68±0.32 n=8; HFD: 0.10±0.03 n=14; $P<0.05$) and β-actin. However, when mucosa-lamina propria was analysed separately, there was no change in PYY mRNA levels using GAPDH (1.49±0.74 n=8; 1.39±0.38 n=11) or β-actin. Similarly, when villin was used as a loading control, PYY was unchanged in the intact ileum (0.31±0.14 n=8; 0.27±0.07 n=15) and in the mucosa-lamina propria. Intact ileum showed reduced villin versus GAPDH in HFD, but no difference in mucosa-only samples. Changes in the non-mucosal compartment were tested using markers of smooth muscle cells. Intact ileum showed no change in either marker in HFD rats using calponin (2.00±0.78 n=10; 1.70±0.62 n=10) or smoothelin (1.45±0.45 n=10; 1.70±0.44 n=9). Conclusions: Our data shows that PYY mRNA levels are not changed in a rat model of obesity but suggest that other structural changes are taking place in the non-mucosal compartment. Overall, our data predict that PYY availability during a high fat diet is unchanged.

POS-TUE-074

NEURONAL ACTIVITY IN DEVELOPING ENTERIC NEURONS

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The enteric nervous system arises from neural crest cells that migrate into the gastrointestinal tract during development. A sub-population of enteric neural crest cells expresses pan-neuronal markers at early stages of development (E10.5 in the mouse). However, when developing enteric neurons become electrically active is unknown. To study this, we used calcium imaging to examine enteric neurons that were dissociated from embryonic gut and then cultured overnight. Sharp increases in intracellular calcium ion concentration ($[Ca^{2+}]_i$) follow action potentials in mature enteric neurons, and can be elicited by electrical field stimulation (EFS). We found that a sub-population of enteric neurons isolated from E11.5 mice responded to EFS (20mA for 2 seconds at 20 Hz) with a peak in $[Ca^{2+}]_i$ similar to that of adult neurons. The proportion of responding neurons and the amplitude of their responses increased dramatically between E11.5 and E12.5, and further increased in preparations from older embryos. The calcium transients were dependent on voltage-gated sodium channels as they were abolished or diminished in the presence of tetrodotoxin (TTX). Several types of voltage-gated calcium channels were involved, but their roles differed with embryonic age. Furthermore, the majority of neurons from E11.5 embryos responded to DMPP (an agonist of nicotinic cholinergic receptors), and to ATP. Thus, neuronal activity is present very early during the development of the enteric nervous system.

POS-TUE-075

AXONAL DAMAGE AND PLASTICITY FOLLOWING INTESTINAL INFLAMMATION

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The **aim** of this study was to investigate the early stages following the induction of inflammation and mechanisms triggering intracellular changes in the subpopulations of enteric neurons which can lead to long-term pathological changes in neuronal properties. **Methods:** Inflammation was induced by injecting TNBS (30mg/kg in 30% ethanol) into the guinea-pig ileum. Segments of inflamed ileum were examined at 3, 6, 12, 24 hours and 7 days post-TNBS injection or sham operation and compared to control ileum. Inflammation was quantified histologically and immunochemically. Nerve fibre bundles were labeled with anti-βTubulin, anti-GAP-43, anti-tau and anti-phospho-Tau antibodies followed by double-staining with anti-calbindin antibody specific to Dogiel type II (DII) neurons. The number of nerve bundles was visualized and quantified in mid-villi sections. Changes in the electrophysiological properties of enteric neurons were investigated by intracellular recording technique followed by morphological identification of the neuron type. **Results:** This study provides evidence that significant axonal damage occurs at 3-24 hours after induction of inflammation in the guinea-pig ileum. Increase in the number of nerve fibers projecting to the mucosa, possibly due to axonal sprouting, occurs at later stages (day 7 post-TNBS). Neurons projecting to the mucosa that were affected by inflammation include DII neurons from the myenteric plexus which functionally are the intrinsic primary afferent neurons. Significant increases in excitability of DII neurons recorded at 3 (n=18) and 24 hours (n=20) after the induction of inflammation. **Conclusion:** This is the first functional study of the very early changes in enteric neurons following an inflammatory challenge. Intestinal inflammation causes damage to the neuronal processes in the mucosa and rapidly increases neuronal hyperexcitability.

POS-TUE-076

HISTOLOGICAL CHANGES IN THE ILEUM AFTER INTESTINAL ISCHEMIA AND REPERFUSION

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A period of ischemia followed by reperfusion (I/R) of the intestine causes long-term changes in motility. We have recently found degenerative changes in enteric neurons and glia after I/R that could cause motility changes. In the present work, we have investigated changes in muscle and other cell types that could also contribute to functional deficits. **Purpose:** The aim was to provide a detailed analysis of the histological changes that occur in the mouse ileum following I/R. **Methods:** I/R was achieved in the ileum of anaesthetised mice by occluding a branch of the superior mesenteric artery for 1hr. Reperfusion was allowed to occur from 1hr to 7days. Ileum samples (from 20 animals) were taken from within the occluded area and from a non-occluded area proximal to the occlusion. The samples were prepared for haematoxylin and eosin histology. **Results:** At 1hr the epithelium was broken and sloughing off into the lumen; at 3hr the villi appeared flattened but the epithelium had regrown. Numerous intra-epithelial inclusions were present. At 24hr cells of the longitudinal muscle layer were separated and contained vacuoles. By 48hrs they were rounded and pale staining, but still contained normal nuclei. By 7 days the morphology of the muscle appeared normal. There were no changes in the epithelium or muscle in the non-occluded region. In comparison, neuronal and glial cell damage was observed in both regions. **Conclusion:** The motility disorders that are observed after I/R probably involve damage to both neurons and muscle. The muscle cells appear to de-differentiate to a synthetic phenotype and then return to a contractile phenotype. This needs to be confirmed by functional studies.

POS-TUE-077

IDENTIFICATION OF A NOVEL NEURO-NEURONAL PATHWAY THAT UNDERLIES PERISTALSIS AND FECAL PELLET PROPULSION IN GUINEA-PIG DISTAL COLON

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The mechanism by which distension of the colon activates peristalsis and the propulsion of colonic contents is poorly understood. In this study, we used video imaging and spatio-temporal mapping techniques to investigate the neuronal mechanisms underlying peristalsis in isolated guinea-pig distal colon. In contrast to previous studies, we found that hexamethonium (100μM–1mM) or mecamylamine (20μM) never abolished peristalsis or fecal pellet propulsion. In hexamethonium or mecamylamine, further addition of PPADS (10μM), ondansetron (1μM) and SR 142-801 (300nM) had no inhibitory effect on the propagation velocity of fecal pellets. During the initiation of peristalsis, the intraluminal propulsive force applied to an inserted fecal pellet was significantly reduced by hexamethonium 100μM (control of 8.9 ± 1.1 to 4.1 ± 0.25mN in hexmethonium; n=5; P<0.05). Although, the rate-of-rise of tension developed by the non-nicotinic pathway on the fecal pellet was not significantly different in the presence of hexamethonium (control: 0.49 ± 0.2 g.sec-1 to 0.42 ± 0.1 g.sec-1 in hexamethonium; n=5; P>0.05). The reduction in propulsive force in hexamethonium was not associated with a reduction in propagation velocity of fecal pellets. When the distal colon was removed from guinea-pigs (with endogenous fecal pellets present) and placed immediately into hexamethonium (100μM), peristalsis and endogenous fecal pellet propulsion along the colon was abolished, so that all endogenous pellet movement ceased. However, in these same preparations whilst in hexamethonium, local acute stimulation of the colon, such as insertion of exogenous fecal pellets triggered peristalsis and induced propulsion of fecal pellets. Taken together, nicotinic transmission plays an essential role in the natural propulsion and expulsion of endogenous fecal pellets. However, a novel non-nicotinic pathway, can also be activated, by local acute stimulation, which can generate peristalsis and fecal pellet propulsion at normal propagation velocities, but does not require purinergic (P2), serotonergic (5-HT3) or tachykinergic (NK3) receptors. The non-nicotinic pathway appears to require acute stimulation for its activation, and is not sufficiently activated by maintained distension by multiple endogenous pellets.

POS-TUE-078

NICOTINIC PATHWAYS AND THEIR CONTROL OVER CYCLICAL MOTOR PATTERNS UNDERLYING COLONIC PROPULSION

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Propulsion of pellets in the colon involves both acute distension activation of enteric circuits and cyclic motor complexes (1). Nicotinic transmission may be not essential for the propulsion of single pellets (2). Purpose: To investigate the role of nicotinic transmission in distension evoked cyclic motor complexes and pellet propulsion in the same preparation. Methods: segments of distal colon from 5 adult guinea-pigs killed humanely, were placed in organ bath with Krebs at 370C. Video spatio-temporal maps of changes in length and diameter were constructed (3) during short and long fixed balloon distensions and during interrupted and uninterrupted artificial pellet propulsion. Results: short balloon distensions (20-30s) elicited oral contraction of the circular muscle and longitudinal shortening over the entire segment, which were reduced but not abolished by Hexamethonium (100μM). Distensions of 15-20min elicited similar muscle contractions in cycles at frequency of 0.27±0.03 cycles/min SEM). Hexamethonium reduced the amplitude of cyclic contractions but did not affect their frequency (0.34±0.15 cycles/min SEM; n=5). These cyclic contractions exerted of propulsive force on held pellets, which was significantly reduced by hexamethonium (7.31±1.18g to 2.31±0.80g SEM, n=5). However, after being held fixed, pellets cut free to move were still propelled in hexamethonium at a similar speed as in controls (2.73±1.37 vs 2.56±1.28mm/s SEM; n=5). Conclusions: propulsion of single pellets in the guinea-pig distal colon occurs independently from cyclic motor activity and requires minimal propulsive force that does not involve nicotinic enteric pathways. References: 1. Costa and Furness (1976). Naunyn Schmied Arch Pharmacol. 16, 294:47-60. 2. Gregory and Spencer, J Physiol (submitted). 3. Hennig et al (1999), J. Physiol., 517, 575-590.

POS-TUE-079

ELECTROPHYSIOLOGICAL CHANGES IN ENTERIC NEURONS FOLLOWING ISCHEMIA/REPERFUSION INJURY

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Damage following ischemia and reperfusion (I/R) injury is common in the intestine, and can be caused during abdominal surgery, in several disease states and following intestinal transplantation. I/R results in changes in motility, implying that properties of motility-controlling neurons are altered. Previous investigations in this laboratory have shown that a brief period of ischemia, followed by reperfusion, causes sustained changes in specific subtypes of enteric neurons. Purpose: There have been no reported electrophysiological investigations in enteric neurons following ischemia. Therefore, this study was designed to determine whether there is a functional correlate of the structural changes that were observed in neurons following I/R. Methods: A branch of the superior mesenteric artery of anaesthetised guinea-pigs was occluded for one hour. Tissues from the occluded and non-occluded regions (5 cm oral from the occlusion site) were taken for in vitro investigation 24 hours later. Myenteric neurons were investigated using intracellular microelectrodes and the neurons were filled with marker dyes from the recording electrode to determine their morphologies. Results: The majority of the neurons that have been electrophysiologically classified as AH neurons (n=11) and a small proportion of S neurons (n=4) became hyperexcitable (average of 15 spikes in response to a depolarisation step of 0.3nA, 500ms). These neurons also exhibited anodal break action potentials (after hyperpolarisation of -5mV, 100ms from the resting membrane potential), and some of these neurons showed spontaneous action potentials in both the occluded and non-occluded regions. Some S neurons appeared to be unaffected. Conclusion: We conclude that I/R injury causes hyper-excitability of particular subtypes of enteric neurons. These changes may contribute to the dysmotility that is observed after intestinal I/R.

POS-TUE-080

TRANSABDOMINAL ELECTRICAL STIMULATION INCREASES COLONIC ACTIVITY IN CHILDREN WITH CHRONIC CONSTIPATION

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Background: All neurobiologists recognise that electricity activates nerves. Physiotherapists specialise in applying transcutaneous electrical stimulation (TES). TES used to treat bladder overactivity produces diarrhoea, suggesting that TES speeds up bowel transit. In a pilot study, TES increased defecation in 5/8 children with chronic constipation. Chronic slow transit constipation (STC) is resistant to medical management and is marked by soft stools that accumulate in the proximal colon and soiling. Aim: Determine if TES improves colonic function in pediatric STC. Methods: 46 children (8-18 yr) with STC (confirmed by radionuclear transit study) were randomly assigned to active(A) or sham(S) stimulation (20 min, 12 sessions, 3/wk). Two (4 x 4 cm) adhesive electrodes were placed paraspinally and 2 on the abdomen near the belly button (T9-L2). Stimulation was just below sensory threshold (<40mA, carrier-frequency 4kHz, beat frequency 80-150Hz). Daily diaries recorded defecation and soiling for 1mth baseline, 1 mth during and 2mths after stimulation. Quality of life (PedsQL) and colonic transit (scintigraphy) were compared before and 2 mths after treatment. 5 children had 24-hr colonic manometry before and 2 mths after active stimulation. 11 children also received daily stimulation for 2 mths, 12 mths after completing the RCT. Results: 42 children (8-18 yr, 20 male,) completed the RCT. Active stimulation resulted in faster colonic transit times (Geometric centre at 48 hrs, mean±SEM: A4.28±0.03 vs S3.22±0.05, p=0.007), less abdominal pain (0.60±0.16 vs 1.8±0.47 days/wk, p=0.0002), less soiling (1.92±0.43 vs 2.63±0.56 episodes/wk, p=0.002), and improved self-perceived physical quality of life (83.9±3.0 vs 76.7±3.5, p=0.01, A). There was no change in defecation in children stimulated 3 times/wk, but with stimulation daily, defecation increased into the normal range (2.5±2.1 to 6.7±4.4 episodes/wk, p=0.008). Conclusion: Colonic transit, soiling, QOL and defecation improved in children given TES. The effect is dose-dependent. TES could provide a treatment for chronic constipation.

POS-TUE-081

INVESTIGATING THE CONTRIBUTION OF ENTEROCHROMAFFIN CELL DISTRIBUTION TO LOCAL 5-HT AVAILABILITY

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Local availability of 5-HT at the mucosal surface of the intestine is related to the amount of 5-HT released by enterochromaffin (EC) cells, the number of EC cells in the area and the subsequent re-uptake of 5-HT. Our previous data showed that the local availability of 5-HT increases dramatically with inflammation or during obesity, and is coupled with increased numbers of EC cells. We aimed to determine the relationship between the concentration of 5-HT at the mucosal surface and the number of EC cells within small regions of colon. Distal colon was taken from control (n=9) and high-fat diet rats (n=11). 5-HT concentrations near the mucosal surface were measured electrochemically during steady state (SS) and peak evoked (compression) release. Eight spots from each preparation were tested with 5-8 repetitions (n=6) and showed overall peak 5-HT of 16.1μM and SS 5-HT of 4.7μM. Individual spots showed a high (27%) or low variability (73%) in 5-HT levels which were well correlated between peak and SS measurements. EC cell numbers were determined by counting immunohistochemically labelled 5-HT cells in cross-sections. The variability over 1,973 EC cells in 2542 crypts (n=13) also showed high (n=5) and low variability (n=7). Our data suggests that the variability in the electrochemical recordings between spots is of the same order of magnitude as seen in the EC cells counts. However, the electrochemical recordings also varied widely within single spots. This suggests that the function of the EC cells also varies or that the distribution of EC cells is important on a smaller scale than can be assessed electrochemically.

POS-TUE-082

NEURONAL DEATH AND GLIAL CELL DAMAGE OCCURS IN MOUSE ILEUM FOLLOWING INTESTINAL ISCHEMIA AND REPERFUSION

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Neuronal changes following ischemia reperfusion (I/R), in the guinea pig ileum, have previously been documented by this laboratory. However, the extent of neuronal damage/death has not been determined. Interestingly, the effects of intestinal I/R on enteric glia remains unexplored despite the knowledge that glia contribute to neuronal maintenance and survival. Purpose: The aim of this study was to assess damage to enteric glia and quantify the neuronal loss following intestinal ischemia reperfusion (I/R) in mouse ileum. Method: A branch of the superior mesenteric artery in anaesthetised C57/Blk6 or S100-GFP mice was occluded for 1 hour and the animal was allowed to recover for a period of 1 hour to seven days. Tissue was taken from both occluded and non-occluded regions of the ileum. Immunohistochemical methods were used for the investigation of specific neuronal and glial cell markers, including indicators of cell death (TUNEL) and protein nitrotyrosylation (3-NT). Results: There were significantly more neurons ($p<0.05$) and glial cells ($p<0.01$) containing 3-NT in the occluded regions from 6-24 hours following I/R compared to non-occluded and control regions (no 3-NT). A small percentage of neurons displayed TUNEL immunoreactvity 6 hours after I/R. Glial degradation was visualized using the S100-GFP mice and glial fibrillary acidic protein (GFAP) immunohistochemistry. GFAP re-organisation and ablation of the S100-GFP protein was evident 3 hours following I/R. Conclusion: The data suggests that glial cell damage precedes neuronal loss; thus the glial damage may contribute to neuronal degradation.

POS-TUE-083

SOMATO-ADRENAL REFLEX AND UPPER CERVICAL SPINAL CORD COMPRESSION – A PILOT STUDY

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Purpose: The aim of this study was to test whether applying transient (range 10-60 min) compression to the upper cervical spinal cord modulates somatic evoked reflex activity in the adrenal nerve. **Methods:** Experiments were performed on spontaneously breathing adult Wistar rats (n=5; 380-430g) initially anaesthetized with urethane (1.3g/kg i.p.) and supplemented (i.v.) to maintain absence of withdrawal and palpebral reflexes. Venous and arterial canulas provided fluids and a record of arterial blood pressure. Averaged adrenal nerve activity was recorded in response to electrical stimulation (1Hz, 5 X 0.5ms square wave pulses) of the ipsilateral sciatic nerve at ≥ 1.5X threshold (T) for muscle twitch, while static compression was applied using a probe (2.3 X 2.8 mm) placed on the dorsal surface of the exposed, dura intact, upper cervical spinal cord. **Results:** High intensity (≥ 15T) stimuli evoked a reflex response (onset latency range 50-100ms; duration ~120ms) in each rat's stimulus-triggered averaged (n=500) adrenal nerve recordings. Applied pressure ranging (1.13-3.92g) from that sufficient to compress the dura so it just contacted the dorsal surface of the cord to that necessary to occlude the vessels on the dorsal surface of the cord, induced a reduction (range 12-35%) in the amplitude of the somatic evoked adrenal nerve response. When tested up to 60 min after removing the probe, the somatic evoked responses were present but remained reduced in amplitude **Conclusion:** In the anaesthetized rat, static transient (< 60 min) compression of the upper cervical spinal cord can reduce somatic afferent induced activity in the adrenal nerve and it remains reduced for more than an hour after compression has been removed.

POS-TUE-084

NEUROVASCULAR TRANSMISSION IS IMPAIRED IN ARTERIES SUPPLYING SKIN OF DIABETIC RATS

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Abnormal neural control of skin blood flow is implicated in pathogenesis of diabetic foot. The mechanisms whereby diabetes affects the neural control of skin vasculature are not understood but it is believed to cause perivascular nerve loss. Here we investigated the effects of diabetes on sympathetic neurovascular transmission in two arteries supplying skin (tail artery, planter metatarsal arteries) and compared them with those in mesenteric arteries. Rats were made diabetic with streptozotocin (60 mg/kg, i.p.). Twelve weeks after the induction of diabetes, artery segments were removed under terminal anaesthesia with pentobarbitone (100 mg/kg i.p.) and mounted isometrically. Comparisons were made with arteries from vehicle-treated rats. In both tail artery (n=5) and planter metatarsal arteries (n=6), diabetes reduced nerve-evoked constrictions ($P < 0.01$ for both comparisons). In contrast, nerve-evoked constrictions of mesenteric arteries were unaffected by diabetes (n=6, $P=0.33$). In both cutaneous arteries, diabetes did not affect the sensitivity to α_1- and α_2-adrenoceptor agonists (phenylephrine and clonidine respectively; $P > 0.65$ for all comparisons). Furthermore, diabetes did not change the leftward shift in the concentration response curve for phenylephrine produced by blockade of the neuronal noradrenaline transporter with desmethylimipramine (30 nM; $P > 0.1$ for both comparisons). As sympathetic denervation reduces neuronal uptake of phenylephrine and thereby increases vascular sensitivity to this agent [1], these findings suggest reduced neural activation of these vessels cannot be attributed to nerve loss. Instead, the findings suggest neurovascular transmission is impaired in these vessels. In conclusion, diabetes impairs neurovascular transmission in arteries supplying skin and this change precedes nerve loss (if it occurs). 1. Tripovic et al. (2009) *Br J Pharmacol*. In press.

POS-TUE-085

DIFFERENTIAL CONTRIBUTION OF NEURONAL NITRIC OXIDE TO CEREBRAL ARTERIES OF THE RAT

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Nitric oxide (NO) released from parasympathetic nerves causes vasodilatation of large cerebral arteries and increases cerebral blood flow in vivo[1]. However, the relative importance of nitrergic nerves to control of cerebral vessels of different sizes has not been determined. The present study therefore aimed to compare nerve-mediated responses of pressurized vertebral arteries (351 ± 10µm maximal diameter) with those of caudal cerebellar arteries (193 ± 2µm maximal diameter) of the rat. Immunohistochemistry demonstrated a dense plexus of neuronal NO synthase-containing nerves surrounding the basilar and vertebral arteries, while the caudal cerebellar arteries showed sparse nitrergic innervation and nitrergic nerves were absent from smaller pial arterioles. Electrical field stimulation (10Hz; 5s trains) of both vessels elicited vasodilatation. In order to eliminate the influence of sensory nerves, experiments were conducted in the presence of capsaicin (10µM), which significantly reduced tone in the vertebral, but not in the caudal cerebellar artery. In the vertebral artery, effects of capsaicin in reducing nerve-mediated responses could be attributed to the loss of tone, while in the caudal cerebellar artery, nerve-mediated vasodilation was significantly reduced in amplitude. Inhibition of NO with L-NAME (10µM) significantly increased tone relative to control in the vertebral artery and eliminated nerve-mediated vasodilation (n=4) but not in the caudal cerebellar artery (n=3). We conclude that nitrergic nerves are the main contributor to vasodilator responses of the vertebral artery, while sensory nerves play a role in the smaller caudal cerebellar artery, along with other NO-independent vasodilatory neurotransmitters. [1]Toda N, Ayajiki K, Okamura T (2009) Cerebral blood flow regulation by nitric oxide: recent advances. Pharmacol Rev 61:62-97.

POS-TUE-086

MODULATION OF MUSCLE SYMPATHETIC NERVE ACTIVITY BY SINUSOIDAL GALVANIC VESTIBULAR STIMULATION IS LOWER WHEN DELIVERED AT THE CARDIAC FREQUENCY

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Purpose: Muscle sympathetic nerve activity (MSNA) is normally entrained by the arterial baroreceptors to occur as bursts phase-locked to the cardiac cycle. However, we have previously demonstrated that selective stimulation of vestibular inputs, via sinusoidal galvanic vestibular stimulation (sGVS), can modulate MSNA (Bent et al., 2006) and recently showed that the modulation is weakest at 0.8 Hz and greatest at 0.2 Hz (Grewal et al., 2009). Here we test the hypothesis that frequencies of sGVS delivered at the subject's cardiac frequency causes less modulation than frequencies delivered on either side of the cardiac frequency. **Methods:** MSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 7 awake seated subjects. Bipolar binaural sinusoidal GVS (±2 mA, 200 cycles) was applied to the mastoid processes at the cardiac frequency and at 0.1, 0.2, 0.3 and 0.6 Hz above and below this frequency. **Results:** Cross-correlation analysis revealed a cyclic modulation of MSNA at all frequencies, with a clear dip at the cardiac frequency (47.9±3.8%); by contrast, modulation was 57.9±3.6% when delivered 0.1 Hz lower, and 55.1±3.5% when delivered 0.1 Hz higher, than the cardiac frequency. **Conclusions:** We conclude that vestibular inputs compete with baroreceptor inputs operating at the cardiac rhythm, with vestibular modulation of MSNA being lowest when this competition with the baroreceptors is highest. Bent LR, Bolton PS & Macefield VG, Modulation of muscle sympathetic bursts by sinusoidal galvanic vestibular stimulation in human subjects. *Exp Brain Res* 2006 174: 701-711 Grewal T, James C & Macefield VG, Frequency-dependent modulation of muscle sympathetic nerve activity by sinusoidal galvanic vestibular stimulation in human subjects. *Exp Brain Res* 2009 197: 379-386.

POS-TUE-087

ASSYMETRICAL SOMATOSYMPATHETIC RESPONSES TO RIGHT AND LEFT SCIATIC NERVE STIMULATION

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We have previously shown that different patterns of hindlimb bloodflow can be evoked by left versus right sciatic nerve stimulation (L- and R-SN respectively).The aim of this study was to compare changes in left lumbar sympathetic nerve activity (LSNA) evoked by electrical stimulation of the L- and R-SN, and to investigate the central pathways responsible. In urethane anesthetized (1.3 g/kg, i.p.), vagotomized, paralyzed (pancuronium bromide 0.4 mg i.v.) and artificially ventilated Sprague-Dawley rats (N = 9) averages of LSNA responses to L- and R-SN stimulation (0.5 Hz, 0.6 ms, 1 mA) revealed two excitatory peaks (126 ms & 242 ms). Responses to L-SN stimulation were characterized by an inhibitory phase that preceded the first excitatory peak (onset 54 ms), which was absent when the R-SN was stimulated. Microinjection of muscimol (6 mM, N = 6) or kynurenic acid (50 mM, n = 6) into the right rostral ventrolateral medulla (R-RVLM) abolished the excitatory response to L-SN stimulation, leaving the inhibitory phase intact. L-RVLM Muscimol abolished the excitatory response to R-SN stimulation without affecting the response to L-SN stimulation. Cervical spinal cord transection abolished the inhibitory phase evoked by L-SN stimulation. These data suggest that somatic nerve stimulation evokes bilateral sympathoexcitation, which is mediated by glutamatergic transmission in the RVLM, and simultaneous unilateral sympathoinhibition ipsilateral to the sensory nerve. Such sympathoinhibition, generated by unknown supraspinal structures, has not been previously described. The physiological role of this mechanism may be related to the differential control of regional bloodflow in response to nociceptive stimulation and high states of arousal.

POS-TUE-088

INTRATHECAL OREXIN A INCREASES SYMPATHETIC OUTFLOW AND RESPIRATORY DRIVE AND MODULATE PHYSIOLOGICAL REFLEXES

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Orexin A and orexin B, two hypothalamic peptides, are important signalling molecules in feeding and sleep/wakefulness. Orexin containing neurons in the lateral hypothalamus project to numerous areas of the brain and spinal cord including the intermediolateral cell column that contain sympathetic preganglionic neurons. This study was undertaken to determine if orexin A modulates sympathetic output. Experiments were conducted on anesthetized, vagotomised and artificially ventilated Sprague-Dawley rats (n = 16). Intrathecal injections of orexin A caused dose-dependent hypertension, tachycardia and sympathoexcitation. The maximum effect was found at 10 nmol with increases in MAP, HR and sSNA of 30 ± 7 mmHg, 37 ± 6 bpm and 84 ± 18 % respectively. Orexin A also increased phrenic nerve amplitude by 69 ± 11 %. The effects of intrathecal orexin A (10 nmol) on baroreflex, chemoreflex and somatosympathetic reflex were also investigated. Orexin A caused no significant change in baroreflex. It also potentiated the pressor response to stimulation of chemoreflex with 100% nitrogen without significant change in splanchnic sympathetic nerve activity. Orexin A significantly reduced the 2nd peak of somatosympathetic reflex but the 1st peak was unaffected. These findings demonstrate that i) orexin A causes sympathetically mediated increase in MAP and HR and increases inspiratory drive and ii) differentially modulates the reflex activity.

POS-TUE-089

NON-L-TYPE CALCIUM CHANNELS CONTRIBUTE TO CEREBROVASCULAR FUNCTION

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Antagonists of L-type voltage dependent calcium channels (VDCCs) are widely used in the treatment of vasospasm following subarachnoid haemorrhage, however their effectiveness in improving patient outcome is questionable. Our aim was to determine whether non-L-type calcium currents contribute to cerebrovascular function, and to determine the selectivity of T-type VDCC antagonists. Quantitative PCR, immunohistochemistry and immunoelectronmicroscopy were used to define the subtypes and location of voltage-dependent calcium channels expressed in cerebral arteries. Patch clamp electrophysiology, pressure myography and pharmacology were used to classify calcium currents in isolated cerebrovascular SMCs and responses of cerebral arteries. MessengerRNA and protein for L- ($Ca_V1.2$) and T-type channels (Ca_V 3.1, Ca_V 3.2) were detected in cerebral arteries. In isolated SMCs, a high voltage-activated calcium current with L-type VDCC kinetics and sensitivity to the dihydropyridines, nifedipine and nimodipine, comprised 75% of the current, while the residual current had kinetics typical of T-type currents. Both the dihydropyridine-sensitive and insensitive components could be blocked by T-type blockers, but only the sensitive component was blocked by diltiazem. This component was larger in SMCs from smaller arteries. In large pressurised arteries, voltage-dependent constriction was abolished by L-type antagonists, while in smaller arteries, this required both L- and T-type antagonists. However, considerable overlap in the action of these antagonists was found. We suggest that a heterogeneous population of L-type and high voltage-activated T-type VDCCs contribute to vascular tone in resistance sized cerebral arteries, providing a novel therapeutic target for therapy-resistant vasospasm.

POS-TUE-090

CARDIAC BAROREFLEX DELAY: HOW IS IT REDUCED BY CLONIDINE?

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The delay between spontaneous rises in blood pressure and baroreflex bradycardia increases when vagal tone is low. Vagal tone is high after clonidine, and we investigated its action on baroreflex delay in humans and rats. In 8 male volunteers, baroreflex delay was assessed by both sequence and cross correlation methods before and after clonidine (6 µg/kg orally). Clonidine lowered heart rate and significantly reduced baroreflex delay ($P< 0.05$, both methods), increasing the proportion of sequences showing a delay of 0 vs.1 beat. In anaesthetised rats (urethane, 1.4 g/kg. i.v.), we assessed baroreflex delay from the inverse correlation between heart rate and systolic pressure during inflation of an aortic balloon catheter. Clonidine (100 µg/kg i.v.) lowered blood pressure and heart rate and reduced baroreflex delay ($n=5$, $p<0.05$). Eight cardiac vagal motoneurons, identified electrophysiologically by antidromic activation, showed ongoing activity linked to the arterial pulse; but clonidine caused no shift in its latency from the pulse wave. We then measured heart rate responses to brief supramaximal stimuli to the cervical vagus, and found that timing was critical. Efferent stimuli synchronous with the R-wave slowed the heart maximally after 1 beat, which became 0 beat after clonidine ($n=5$, $p<0.05$). When stimulus timing was varied, and cycle lengthening plotted vs. time after stimulus, clonidine changed neither the latency nor the time course of the cardiac response ($n= 5$ rats, $p=0.47$). In conclusion, central reflex proccessing time is unchanged by clonidine, but the longer cardiac period allows naturally timed CVM volleys to arrive in time to slow an earlier heartbeat.

POS-TUE-091

SYNERGY BETWEEN α1-ADRENOCEPTOR SUBTYPES AND WITH P2X RECEPTORS IN NERVE-INDUCED RESPONSES OF MOUSE MESENTERIC ARTERIES REVEALED BY DOUBLE AND TRIPLE KNOCKOUT STRATEGIES

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It is not known whether sympathetically-mediated constriction of resistance arteries involves all 3 α1-adrenoceptor (AR) subtypes because of the poor selectivity of antagonist drugs and the complexity of identifying the individual contributions of different receptors. We employed double and triple knockouts of the α1-AR to analyse responses to perivascular stimulation of isolated mouse first order mesenteric arteries using wire myography. In wild type arteries (n=6), antagonists of either α1A-ARs or α1D-ARs produced >90% blockade of constriction to low frequencies in the physiological range, suggesting these receptor subtypes act synergistically. The results in arteries with only α1A-ARs (n=6) or α1D-ARs (n=5) confirmed these observations. After knockout of all 3 α1-ARs (n=5), the small residual constriction was blocked by α,β methylene ATP, indicating it was mediated by P2X receptors. Analysis of knockouts with either α1A-ARs or α1D-ARs using antagonists revealed synergism between P2X receptors and α1A-AR but not between P2X receptors and α1D-AR. These data imply powerful synergism between noradrenergic and purinergic responses involving α1A-adrenoceptors, pointing to multiple post-receptor signalling interactions. Supported by British Heart Foundation (PG/05/140/20094 and FS/04/035).

POS-TUE-092

HEMORRHAGIC STROKE LESION AND THE ALTERATION OF CIRCADIAN BLOOD PRESSURE PATTERN

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Objective: Importance of domestic blood pressure values and 24 hour ambulatory blood pressure monitoring (24hABPM) has been discussed in the context of risk factor of cerebrovascular diseases. In this study, we evaluated the effect of stroke lesions for the alteration of circadian blood pressure pattern. **Methods:** Hemorrhagic stroke patients admitted to the hospital within 24 hr after the onset were enrolled in this study (n=34: 61.6±10.6 years-old). 24hABPM was performed every 30 min starting from 1 pm on admission and in following 3 weeks. All patients were classified into dipper and non-dipper types based on the ratio of average daytime and nighttime BP. Urine level of vanillylmandelic acid (VMA) was measured on admission and in following 3 weeks. The hematoma size was calculated based on the findings of brain computed tomography on admission. Lesions were classified into pons, left and right thalamus and left and right putamen (n=2, 6, 10, 7 and 9, respectively). **Results:** There was no significant correlation between the hematoma size and the measurement of blood pressure at the emergency room. However, size of the hematoma was significantly larger in the non-dipper type compared to that in the dipper type observed in 24hABPM in 3 weeks after the onset ($p=0.016$). VMA was significantly decreased in the patients of non-dipper type both on admission and in following 3 weeks ($p=0.015$). All right thalamic lesions showed dipper type in following 3 weeks. **Conclusions:** The size of lesion rather than the blood pressure at the onset can be a predictor of the prognosis of circadian blood pressure pattern. Moreover, the right thalamic hemorrhage may not affect the circadian blood pressure pattern.

POS-TUE-093

DORSOMEDIAL HYPOTHALAMUS AND MEDULLARY RAPHE MEDIATE RESPIRATORY AROUSAL RESPONSES IN RATS

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Alerting sensory stimuli elicit stereotyped tachypnoeic responses in conscious rats. We aimed to reveal which brain areas are involved in the generation of these responses. In adult male Wistar rats instrumented for telemetric ECG transmitters, respiration was recorded using whole body plethysmography. On different days, microinjection of either muscimol or vehicle was made into the dorsomedial hypothalamus (DMH, Group 1, n=6) or into the medullary raphe (MR, Group 2, n=6). Alerting stimuli were presented in the following sequence: tap (acoustic); sudden side move (proprioceptor/vestibular); turning on light for 30 secs (visual). Basal values of respiratory rate (90±5 and 88±8 cpm) and heart rate (377±10 and 354±10 bpm) did not differ between two groups. In Group 1 studied post-vehicle, respiratory rate raised transiently by 105±27, 150±27 and 183±15.03 cpm after tap, move and light stimuli respectively. Pharmacological inhibition of the DMH suppressed these responses by 92, 83 and 90% (tap/move/light). In Group 2 studied post-vehicle, respiratory rate raised transiently by 99±14, 118±23 and 165±20 cpm after tap, move and light stimuli respectively. Pharmacological inhibition of the medullary raphe suppressed these responses by 81, 75, and 71% (tap/move/light). Heart rate was not affected by alerting stimuli. Blockade of the DMH or raphe region had no effect on basal levels of respiratory or heart rate. We conclude that DMH and MR are not involved in the control of respiratory rate at rest, but their integrity is essential for mediating tachypnoeic arousal responses.

POS-TUE-094

BRAIN ACTIVATION ASSOCIATED WITH EVOKED COUGH IS LESS THAN THE SUM OF ITS PARTS

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PURPOSE: Cough occurs in response to airways irritation and can be initiated voluntarily in the absence of airways stimulation. It has been proposed that supramedullary brain regions are likely to contribute to control of evoked cough through the facilitation or inhibition of brainstem reflex pathways. Using functional brain imaging, we put this putative model to the test. METHODS: Blood oxygen level-dependent (BOLD) contrast images were acquired using a 3T Siemens scanner from healthy participants (n=15) after inhalation of saline, inhalation of capsaicin without cough (cough suppression), inhalation of capsaicin with cough (evoked cough), and voluntary cough after inhalation of saline. BOLD signals were analysed to identify changes associated with cough, capsaicin stimulation and their interactions. RESULTS: Contrary to the hypothesised effect, the positive interaction of cough and capsaicin stimulation did not implicate brain activation uniquely associated with evoked cough. However, the negative interaction revealed brain activations that may be requisite for the initiation of voluntary cough and suppression of evoked cough (inferior frontal gyrus, SMA, cingulate cortex) as well as ongoing sensations associated with capsaicin in the airways (insula, orbitofrontal cortex)($p_{corrected}$<0.05). CONCLUSIONS: The facilitation of evoked cough in response to airways irritation is not dependent on regional brain activation in addition to the activation that accompanies a voluntarily initiated cough. Indeed, voluntary cough and suppression of evoked cough are associated with brain activation in excess of evoked cough. This outcome suggests that evoked cough is a default motor pattern that does not require active facilitation by higher order centres, but is under higher order inhibitory influences.

POS-TUE-095

IMMUNOHISTOCHEMICAL COMPARISON OF NEURONS IN THE GUINEA PIG AND HUMAN NODOSE GANGLION

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PURPOSE: In guinea pigs, defensive coughing is mediated by mechanosensitive neurons (cough receptors) that originate in the nodose ganglia. These neurons are characterised by the expression of neurofilament, alpha3 Na+/ K+ ATPase, and the transporters NKCC1, VGlut1 and VGlut2. Whether cough receptors are unique to guinea pigs is unknown. As an initial investigation into this question we compared the characteristics of neurons in the guinea pig and human nodose ganglia. METHODS: Male Hartley guinea pigs (n=5, 230-330g) were perfusion fixed with 4% PFA prior to harvesting nodose ganglia. Ganglia from humans were removed from embalmed donor cadaveric specimens (n=3, 2M:1F, 57-92 years). Cryostat cut slide mounted sections (12-16µm) were processed for neurofilament, alpha3 Na+/ K+ ATPase, NKCC1, VGlut1 and VGlut2 immunofluorescence or immunoperoxidase. RESULTS: Guinea pig nodose cells could broadly be categorised as having small (50-90µm, mean 87.3±4.5), medium (100-150µm, mean 122.2±5.8) or large (160-190µm, mean 173.4±4.6) perimeters. NKCC1 and VGlut2 labelled cells of all somal sizes whereas each of the other markers labelled subsets of cells with a highest relative frequency in the medium or large somal size range. The size of cells was similarly distributed in human nodose ganglia (60-170µm). Robust immunostaining for neurofilament, VGlut1 and VGlut2 was evident in many small, medium and large cells whereas only faint (but detectable) staining for alpha3 Na+/ K+ ATPase and NKCC1 was observed. CONCLUSIONS: These data indicate that cells in human nodose ganglia display some characteristics similar to guinea pigs. Markers that characterise guinea pig cough receptors are expressed by human nodose neurons, providing initial evidence that a comparable afferent neuron may exist.

POS-TUE-096

DIGIGAIT, A USEFUL METHOD ASSESSING SUBTLE GAIT ABNORMALITIES IN NEDD4 TRANSGENIC MICE

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Introduction: Nedd4 (Neuronally Expressed Developmentally Down-regulated 4) is an E3 ubiquitin ligase that has an important role in the central nervous system (CNS). A recent study in embryonic Nedd4(-/-) mice found a decrease in size and number of motor neurons compared to controls[1]. However Nedd4 knockout (KO) mice are perinatal lethal, and thus assessment of motor function is only possible in Nedd4 heterozygous(+/-) mice. This may not be possible using traditional methods such as Rotarod, thus a novel method such as DigiGait may be more beneficial to investigate subtle motor changes. **Aim**: To identify if Nedd4 (+/-) display impaired motor function. **Methods**: Male Nedd4 (+/-) and wildtype (WT) mice were trained and then tested for the latency to fall of the rotarod. (2x2min accelerating and 1x5min from 4rmp-40rpm). For Digigait analysis mice ran on a motorized transparent treadmill at two speeds 15cm/s and 20cm/s. **Results**: There was no significant difference in latency to fall (s) between WT (162±42, n=9) and Nedd4 (+/-) (166±46, n=8). Digigait analysis showed that at 20cm/sec, hindpaw stance was significantly reduced in Nedd4 (+/-) compared to WT (0.15±0.005 and 0.17±0.006), as was the time spent in the propulsion phase (0.12±0.003 and 0.14±0.006). Nedd4 (+/-) paw angle was significantly reduced in both hindpaw and forepaw, at both speeds tested. **Conclusion**: This study has demonstrated the utility of DigiGait as an excellent tool in identifying subtle phenotypic changes in transgenic mice. 1.Liu Y, Oppenheim RW, Sugiura Y and Lin W. (2009). Developmental Biology, 330(1), pp153-166.

POS-TUE-097

RECEPTORS IN MOTION: RASTER IMAGE CORRELATION SPECTROSCOPY (RICS) ANALYSIS OF PEPTIDE RECEPTOR AND MEMBRANE MOBILITY

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Neuronal cell membranes are dynamic structures in which peptide receptors form distinct domains. Peripheral sympathetic neurons express different G-protein coupled receptors (GPCRs) regulating a common set of ion channels that probably co-exist in the same membrane domains. However, it is not known how domains enriched in functionally-linked receptors and channels are maintained in a dynamic membrane structure. We have begun to investigate this problem using analytical confocal imaging techniques with very high spatial and temporal resolution to examine cell lines expressing GPCRs. High resolution confocal images have been analysed with Raster Image Correlation Spectroscopy (RICS) and its derivatives. We have used CHO cells transfected with angiotensin II receptors (type 1A; AT-1AR) modified with enhanced green fluorescent protein (eGFP) on their intracellular C-terminus, combined with angiotensin II coupled with Alexa647 fluorophore. Images were collected in photon counting mode on a Leica SP5 confocal microscope or an Olympus FluoView 1000 and analysed with SimFCS 2.0. High resolution imaging revealed a surprising amount of mobility in the cell membrane in addition to processes associated with the internalisation of agonist bound to receptors. Membrane regions rich in AT-1AR-eGFP displayed rapidly moving filopodia with an effective diffusion coefficient for the receptors of 0.1-3μm^2/s (n=6), equivalent to a linear velocity of about 1μm/s. Our results to date suggest that the analysis of agonist-receptor interactions, at least in cell lines, will be significantly confounded by the consequences of membrane mobility.

POS-TUE-098

CAN VOLUME TRANSMISSION REALLY WORK? MODELLING EXTRACELLULAR DIFFUSION OF SUBSTANCE P

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The concept that neuropeptides can act non-synaptically via volume transmission has been debated for more than 20 years. Fluorescence correlation spectroscopy (FCS) combined with scanning laser confocal microscopy permits direct measurement of diffusion properties of neuropeptides labelled with a fluorescent tag. We have used FCS to examine diffusion of substance P (SP) labelled with Oregon Green-488 (SP-OG) in the neighbourhood of CHO cells expressing neurokinin-1 (NK1) receptors. We used a Leica SP5 confocal microscope fitted with dual avalanche photodiode detectors coupled to an ISS photon counting and FCS system. Our FCS data provide a diffusion coefficient for SP-OG of around 200 μm^2/s in balanced salt solution. We used the FCS data to model how SP might diffuse through the extra-cellular environment between neurons. Neurotransmitter diffusion was modelled in MatLab (R12) to solve differential equations with variables including the apparent diffusion coefficient of the peptide, the tortuosity of the diffusion path, the volume fraction available for peptide diffusion, the number of potential release sites and the amount of SP released. The models suggest that SP concentrations follow a complex spatio-temporal profile following neural release. After a single release event, SP concentration falls off rapidly in space and time making long range transmission would be unlikely. However, after multiple releases, even at frequencies below 10Hz, extracellular SP concentrations remain in the nM-μM range up to 20μm from the release sites for 20 seconds or more. This concentration range can activate neuronal receptors NK1 receptors, supporting the feasibility of volume transmission. The reliability of such transmission depends critically on the number and disposition of release sites.

POS-TUE-099

PREPULSE INHIBITION OF THE STARTLE RESPONSE PARADIGM IN NEDD4 MICE

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Introduction: Nedd4 (neural precursor cell-expressed developmentally downregulated gene 4) is a cytoplasmic E3 ubiquitin ligase responsible for the targeted degradation of proteins through the ubiquitin-proteasomal pathway. Alterations in the function of the ubiquitin-proteosomal system have recently been implicated in the pathology observed in many neurodegenerative disorders such as Alzheimer Disease (AD) and Parkinson Disease (PD). Prepulse Inhibition (PPI) is a measure of sensory gating. Deficits of PPI reflect an inability to filter out unnecessary sensory information. Previous animal studies have indicated that the hippocampus and entorhinal cortex structures affected in mild AD, are involved in the regulation of PPI. It has also been reported that PD patients exhibited a deficiency in sensorimotor gating mechanisms. We therefore examined whether the Nedd4 heterozygote knockout mice ($Nedd4^{+/-}$) exhibited a sensorimotor gating deficit in the PPI of the acoustic startle response paradigm. **Method**: Male transgenic $Nedd4^{+/-}$ (n = 12) and wild type (WT; n =8) 8-12 week-old mice were used. Prepulse startle responses were assessed using the SR-Lab Startle Response System. **Results:** No significant differences were found in PPI between $Nedd4^{+/-}$ and WT mice. **Conclusion:** These data suggests that $Nedd4^{+/-}$ mice do not display abnormalities of sensorimotor gating and have similar ability to filter out unnecessary sensory information when compared to WT mice.

POS-TUE-100

p53 AND NDFIP1 REGULATE STRESS INDUCED EXOSOME RELEASE

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The ability to remove unwanted proteins involves ubiquitylation followed by degradation of the marked protein in the proteasome. An alternative mechanism for protein removal and trafficking is provided by exosomes, which are small vesicles (50–90-nm diameter) originating from late endosomes and multivesicular bodies (MVBs). Cells undergoing stress conditions show increased exosome release. p53 KO cells (n = 6) show a decrease in stress induced exosome release and a role for p53 target genes in exosome release was postulated. The activation of target genes of p53 is regulated by at least two members of the Nedd4 family of E3 ubiquitin ligases (NEDL1 and WWP1). Ndfip1 (Nedd4 family-interacting protein 1), an adapter protein for the Nedd4 family, is also involved in exosomal release and further plays a role in the export of specific proteins in exosomes. Overexpression of Ndfip1 in different cell lines (n = 10) enhances exosome release and stressed cells show an increase in exosomal release of Ndfip1. Ndfip1 KO cells show a similar phenotype to p53 KO cells regarding stress-induced exosome release: After the induction of stress, Ndfip1 KO cells are also unable to increase exosome release, like p53 KO cells (n = 10). Therefore p53 and Ndfip1 may share a common pathway leading to increased exosome release after stress. Given the positive roles of Ndfip1/Nedd4 in improving neuronal survival during brain injury, it is possible that increased exosome secretion provides a novel route for rapid sequestration and removal of proteins during stress.

POS-TUE-101

EXPLORING SOLUBLE ANTAGONISTS OF EPHA4 AS A POTENTIAL THERAPEUTIC FOR SPINAL CORD INJURY

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The Ephs and the ephrins comprise a receptor:ligand system capable of bidirectional signalling with important roles in cell migration and segregation during development. EphA4, a unique Eph receptor capable of interacting with both classes of ephrins, is an important regulator of CNS development and function. Mice lacking EphA4 receptors show a decrease in the gliotic response and remarkable functional recovery following a lateral hemisection injury of the spinal cord. Exploiting the ability of Fc fusion proteins to block Eph:ephrin signalling, we have now generated soluble antagonists of EphA4 by fusing the extracellular domain of EphA4 or its high-affinity ligand, ephrin A5, to the human IgG1 Fc domain. Previous experiments of hemisectional injury in wild type mice with a two-week treatment permits significant functional improvement and substantial axon regeneration in 6 weeks. Here we present data from two additional cohorts. Extending the duration of treatment from two weeks (n=11) to four weeks (n=10) does not substantially improve functional outcome. Furthermore, mice recovering for periods of 8 weeks (n=41), 12 weeks (n=27) and 6 months (n=13) display maximal recovery within 8-12 weeks of injury and no functional deterioration at 6 months. These results demonstrate that a two-week treatment and an 8-week recovery period are optimal for assessing the therapeutic benefit of EphA4-Fc in spinal cord injuries. EphA4-Fc is continually demonstrating beneficial outcomes for treating spinal cord injuries and proving to be an important therapeutic opportunity for many CNS injuries and diseases.

POS-TUE-102

THE ROLE OF SOCS2 IN NEURITE MORPHOLOGY OF CULTURED DORSAL ROOT GANGLIA NEURONS

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Overexpression of Suppressor of Cytokine Signalling-2 (SOCS2) promotes increases in neurite length and neurite number in PC12 cells and cortical neurons. The mechanisms by which SOCS2 regulates the signals that control neurite outgrowth and neuronal differentiation are unresolved but appear to involve Trk receptors. To examine potential effects of SOCS2 and Trk interactions in primary neurons, morphology of TrkA expressing Dorsal Root Ganglion (DRG) neurons from SOCS2 overexpressing SOCS2 transgenic (SOCS2-Tg) mice were compared to wildtype neurons. DRGs were obtained from 1 day old post-natal wildtype (WT) (n=3) or SOCS2-Tg mice (n=4) pups and dissociated neurons were cultured for 4 hours with 50 ng/mL NGF. DRG neurons were fixed and immunostained for the neuronal marker beta III tubulin. Neurons were classified based on neurite morphology and the relative proportions of DRG neurons in each subpopulation were compared between WT and SOCS2-Tg genotypes. DRG neurons from SOCS2-Tg mice demonstrated an increased proportion of neurons with complex neurite morphology. Conversely, a decrease in the proportion of neurons with immature or absent neurites in the SOCS2-Tg cultures was observed. No change was observed in the proportion of neurons bearing simple neurite morphology between genotypes. Overexpression of SOCS2 promotes the culture of a neuronal subpopulation from Dorsal Root Ganglia with increased neurite complexity and a reduction in the early prevalence of neurons without neurites and those bearing immature neurites. This finding may reflect enhanced neurite outgrowth or branching in response to treatment with nerve growth factor *in vitro* or changes in the relative abundance of DRG neuronal subtypes in the developing SOCS2 transgenic mouse due to survival or differentiation effects.

POS-TUE-103

ESTABLISHING AN *IN VITRO* MODEL TO INVESTIGATE MICROGLIA INFLUENCE ON HIPPOCAMPAL NEURAL PRECURSORS

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Microglia are capable of secreting factors that can either stimulate or inhibit proliferation and differentiation of neural precursor cells. The activation status and secretory profile of microglia thus partly shape the molecular microenvironment of the neurogenic niche, which in turn can influence neurogenesis under both normal and pathological conditions. To date, however, detailed understanding of the influence of microglia on neural precursors has been hampered by the absence of an assay to study microglia-neural precursor interactions under defined conditions. The main aim of this study was therefore to establish a quantitative *in vitro* model of hippocampal microglia and neural precursor cell interactions. In this study, we took advantage of $CX3CR1^{+/GFP}$ mice which express green fluorescent protein (GFP) in all cells of monocytic lineage, including brain microglia. Neurosphere frequency of wild-type and $CX3CR1^{GFP/GFP}$ (i.e. CX3CR1-deficient) mice were ascertained to determine whether targeted deletion of one CX3CR1 allele interfered with neural precursor proliferation. No difference in neurosphere-forming frequency was observed between wild-type and CX3CR1-deficient mice (n=3) even in assays which activated latent precursor with high KCl (Walker et al. 2008). Interestingly, however, KCl depolarisation increased total microglia numbers in neurosphere cultures by 27%. GFP^+ microglia were sorted from $CX3CR1^{+/GFP}$ hippocampi using FACS with an average 2218 ± 820 live microglia isolated per hippocampus (n=3). Isolated cells displayed the well-defined microglia phenotype as determined by expression of the markers $CD11b^+$ and $CD45^{dim}$. Ongoing co-culturing experiments indicate a dose-dependent effect of microglia on the neurosphere-forming frequency from neural progenitors. Walker TL, White A, Black DM, Wallace RH, Sah P, Bartlett PF (2008) J Neurosci 28:5240 .

POS-TUE-104

METALLOTHIONEINS INDUCE GROWTH CONE CHEMOTAXIS

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Disruption of neuronal networks can occur in brain injury or as a consequence of neurodegenerative disease. The metallothionein (MT) family of proteins are candidates for enhancing neuronal repair, and are known to be crucial in functional recovery after CNS injury. MTIII has been shown to inhibit neuronal outgrowth. MTI and MTII (MTI/II) exhibit neuroprotective, anti-apoptotic and growth-promoting effects. The neuronal response to MTI/II is characterised by significant increase in neuronal outgrowth and sprouting of neurites. In this study, using the well established growth cone turning assay, the acute response of actively navigating embryonic (E15-18) rat sensory neurons to a gradient of MTI/II and MTIII was measured in vitro. Neurites responded to MTI/II by chemoattraction, and MTIII by chemorepulsion (8.7° ± 1.3, n=12 and -13.8° ± 1.9, n=14 respectively, compared to PBS control, -1.1° ± 1.8; $P<0.02$). It was found that LRP-receptor inhibitor, RAP, abrogated the chemotactic effect of MTs, suggesting that MT chemotaxis occurs via binding LRP receptors, such as megalin. Immunocytostaining of growth cones established that megalin is distributed appropriately in growth cones for chemical sensing. MT chemotaxis is dependent on the presence of extracellular calcium: in low calcium media, a molecular switch turns chemoattraction into chemorepulsion, and vice versa, whereas bathing neurons in calcium free media abolishes MT-mediated chemotaxis altogether. Understanding the mechanisms by which MTs elicit this response in neuronal growth cones has important implications in future therapeutic developments. The ability of MTs to induce chemotaxis, coupled with their neuroprotective and neuroregenerative properties, may render them an effective modality in rescuing damaged neurons and assisting re-innervation and repair at the site of injury.

POS-TUE-105

FUNCTIONAL DOPAMINERGIC DIFFERENTIATION OF PURIFIED MICE MüLLER CELLS IN CULTURE

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Müller cells constitute the main glial cell type in the retina and span the tissue from the inner to the outer limiting membranes. Several functions have been attributed to these cells including structural and nutritional roles as well removal of ions and neurotransmitters from the extracellular space. The potential of Müller cells to actively participate in cellular communication within the nervous system has been recently uncovered. Moreover, it has been suggested that this type of cell can generate neurons under appropriate conditions. Knowledge of the mechanisms controlling this particular differentiation allow production of cells that could be used in potential therapy against some neurodegenerative disorders. In the present work, we evaluated the capability of Müller cells to be used as a source of dopaminergic cells and the factors that influence this process. We have shown that Müller cells in culture (n=3) not only express dopaminergic markers proteins such as the dopamine transporter (DAT), tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and the transcription factor for dopaminergic differentiation Nurr1, but they are also able to produce dopamine *in vitro* (n=5), reaching values of 50 ± 10nmoles or 65 ± 8nmoles, when treated either with forskolin 10µM or PACAP38 10nM, respectively. A mouse model of Parkinson's Disease is obtained with stereotactic injection of 6-OH-DA in the striatum. In these mice, injection of functional dopaminergic differentiated Müller cells clearly reduced the typical rotational behavior presented by the animals (from 15±2 rpm to 2±1 rpm) (n=7).

POS-TUE-106

LOW AND HIGH AFFINITY CATECHOLAMINE BINDING SITES IN TYROSINE HYDROXYLASE SHOW STRUCTURAL SIMILARITY

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PURPOSE: Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of the catecholamines dopamine, noradrenaline and adrenaline and controls the rate of production of catecholamines in cells. Short-term control of TH activity is achieved through a combination of feedback inhibition by the catecholamines and reactivation by phosphorylation. Catecholamines bind TH irreversibly and with high affinity to inhibit the enzyme. Phosphorylation of TH allows dissociation of bound catecholamine, thereby increasing enzyme activity. We have identified a second catecholamine inhibitory site which is readily reversible and functions independently of the phosphorylation state of the enzyme, thus controlling the level of cytosolic catecholamines under both basal and stimulated conditions. In this study TH mutants were generated to determine the position of the novel low affinity catecholamine binding site in TH. METHODS: The crystal structure of the TH active site was used to identify residues responsible for low affinity site. The dopamine dependent inhibition of TH activity in wild-type and active site TH mutants was measured. RESULTS: The IC50s for dopamine inhibition through the low affinity site in TH mutants Tyr371Phe and Glu332Asp were 70-fold and 10-fold higher than wild-type respectively ($p<0.005$, n=3). Catecholamine bound to the high affinity site produced a 10-fold increase in the Km for the cofactor in wild-type TH. In Tyr371Phe, Glu332Asp and Ala297Leu this inhibitory effect was absent. CONCLUSIONS: The results from this work indicate that the low affinity catecholamine binding site is localised to the active site of TH and is likely to be structurally similar to the high affinity site.

POS-TUE-107

MECHANISMS OF PHOSPHORYLATION-SENSITIVE CAMKII TARGETING

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Calcium/calmodulin stimulated protein kinase II (CaMKII) is an important regulator of neuronal function. The biological properties of CaMKII are regulated by multi-site autophosphorylation and targeting to cellular microdomains through interactions with specific proteins. The role of autophosphorylation at Thr286 has been well characterised and shown to regulate CaMKII function by altering CaMKII activity and CaMKII targeting. We have identified a new autophosphorylation site at Thr253, which regulates CaMKII function exclusively through targeting. To identify which regions of CaMKII are responsible for binding to interacting proteins, short peptides corresponding to different regions of CaMKII (α-CaMKII 310-320, 249-258, 282-291) were examined for their ability to inhibit CaMKII binding to a panel of known binding partners in a semi-quantitative overlay binding assay (n=3-6). We observed highly selective inhibition profiles indicating that: 1. One region of CaMKII can interact with more than one binding partner; 2. Autophosphorylation induced changes in CaMKII binding can change the region of CaMKII involved in the interaction and, by implication, also the region of the binding protein involved in this interaction. We have also shown that non-phosphorylated CaMKII and CaMKII phosphorylated at Thr253, but not Thr286, binds neurogranin. However, phosphorylation of neurogranin by PKC completely abrogates binding (neurogranin is not a CaMKII substrate). These results imply that phosphorylation-induced alterations in the targeting of CaMKII can mediate crosstalk between different signalling pathways leading to changes in the signalling pathways into which CaMKII is linked, thereby altering functional outcomes.

POS-TUE-108

REGULATION OF PROLIFERATION OF NEUROBLASTOMA CELLS BY CAMKII

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CaMKII is an important regulator of a variety of cellular functions including cell growth and proliferation. The biological properties of CaMKII are regulated by phosphorylation and targeting to cellular domains via interactions with proteins. The roles of phosphorylation at T286 have been well characterised. We have identified a new phosphorylation site at T253 whose phosphorylation in vivo is dynamically regulated independently of T286 phosphorylation. We have demonstrated that, following transfection of the SHSY5Y neuroblastoma cell line with wild type CaMKII (WT), T286D-CaMKII (mimicking phosphorylation at T286), or T253D-CaMKII (mimicking phosphorylation at T253), the morphology and growth rate of these cells is differentially altered (n=3). Transfection with WT approximately doubled the growth rate without any alteration in cell morphology. The effect of transfection with T286D-CaMKII was identical to that produced by WT showing that phosphorylation at T286 had no effect on growth rate or 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 at various stages of the cell cycle (n=3). We found that while there is no change in total CaMKII expression, there appears to be a marked decrease in T253 phosphorylation at the G2/M border. These results strongly suggest that phosphorylation of CaMKII at T253 is involved in regulating neuronal cell growth and morphology independently of phosphorylation at T286, and clearly identifies functional consequences following T253 phosphorylation.

POS-TUE-109

CHEMOKINES AND INFLAMMATORY MEDIATORS REGULATE NEURAL PROGENITOR CELL DIFFERENTIATION

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Adult neural progenitor cells (NPCs) respond to injury or disease of the CNS by migrating to the site of neural damage and/or differentiating locally to replace lost neurons, astrocytes and oligodendrocytes. Factors that mediate this injury induced NPC response include pro-inflammatory cytokines, such as tumour necrosis factor-α (TNFα) and interferon-γ (IFNγ), which we have shown previously promotes neuronal differentiation, as well as chemokines. RT-PCR was used to compare expression of CXC, CC, C and CX3C family chemokines and their receptors in normal adult mouse brain, and in cultured NPCs in response to IFNγ and TNFα. Basal expression of several chemokines and their receptors was found, predominantly in neurogenic regions, with OB>SVZ>hippocampus and little or no expression in non-neurogenic regions, such as cortex. Treatment of SVZ-derived NPCs with IFNγ and TNFα (alone and in combination) resulted in significant up- or down-regulation of expression of specific chemokines, with CXCL1, CXCL9 and CCL2 most highly upregulated and CCL19 downregulated. Unlike IFNγ, chemokine treatment of NPCs in vitro had little or no effect on migration, survival or proliferation. Neuronal differentiation was promoted by CXCL9 or CCL21, while glial differentiation was modestly promoted by CCL19 and CCL21 and blocked by IFNγ. IFNγ (+/- chemokines) promoted oligodendrocyte maturation but had little effect on overall oligodendrocyte differentiation. Therefore, not only do NPCs express chemokine receptors, they also produce several chemokines, particularly in response to inflammatory mediators. This suggests that autocrine or paracrine production of specific chemokines by NPCs in response to inflammatory mediators may regulate differentiation into mature neural cell types and may alter responsiveness to CNS injury or disease. Keywords: neural progenitor cells, inflammatory mediators, chemokines

POS-TUE-110

GALANIN POTENTIATES AMYLASE SECRETION BY MOUSE PANCREATIC LOBULES BUT NOT BY ISOLATED ACINAR CELLS

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Galanin is implicated in the pathogenesis of acute pancreatitis. Many rodent models of acute pancreatitis use supramaximal concentrations of caerulein to induce the disease. The effect of galanin on amylase secretion under these conditions is unclear. We hypothesised that galanin modulates pancreatic amylase secretion evoked by a supramaximal concentration of caerulein (10^{-7}M) by a direct action on the acinar cells (AC). We compared the effect of exogenous galanin on amylase secretion from pancreatic lobule and AC preparations evoked by 10^{-7}M caerulein. Lobules and AC were prepared from mouse pancreata by standard collagenase digestion techniques. Lobules or AC (n=5-7 preparations) were incubated with galanin (10^{-13}–10^{-7}M), caerulein (10^{-12}–10^{-7}M), alone or in combinations for 60 min at 37°C. Control lobules or AC were incubated in medium alone. Amylase activity into the incubation medium was measured and expressed as % of total amylase (medium plus lobules/AC). Caerulein stimulated amylase secretion from lobules and AC in a dose-dependent manner ($P<0.05$). The peak secretion from lobules and AC was 170% and 330%, respectively, of control and evoked by 10^{-10}M caerulein in both preparations. Secretion then declined with increasing concentration in both preparations. Galanin alone did not influence basal amylase secretion from lobules and AC. Caerulein (10^{-7}M) alone stimulated amylase secretion from lobules to 124% of control, whereas co-incubation with galanin (10^{-12}M-10^{-7}M) potentiated caerulein-stimulated amylase secretion up to 160% of control ($P<0.05$). In contrast, galanin had no effect on the caerulein-stimulated amylase secretion from AC. We conclude that galanin does not act directly on AC to regulate pancreatic amylase secretion.

POS-TUE-111

CALCITONIN GENE-RELATED PEPTIDE (CGRP) ANTAGONISTS DECREASE THE EXCITATORY INPUT OF PRIMARY AFFERENT INPUT ONTO TRIGEMINAL SUBNUCLEUS CAUDALIS NEURONS

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Calcitonin gene-related peptide (CGRP) is the primary neuropeptide released during migraine via activation of trigeminovascular neurons. Whilst much is known about the peripheral vasoactive role of CGRP, less is known about the effects CGRP may have on central nociceptive neurons in the trigeminal nucleus caudalis. Using whole-cell patch-clamp electrophysiological recordings in brainstem slices, we examined the effect of the nonpeptide CGRP receptor antagonist BIBN4096BS on evoked excitatory postsynaptic currents (eEPSC) in second order trigeminal neurons. In response to electrically stimulated trigeminal primary afferent fibres, bath application of BIBN4096BS decreased mean (± SEM) eEPSC amplitude by 22.6 ± 5.3% (n=12; p=0.012). The results of the current study suggest that a proportion of excitatory neurotransmission is mediated by CGRP receptors at the first synapse in the spinal trigeminal nucleus. These results suggest that at least part of the mechanism of BIBN4096BS in clinical trials occurs at the primary afferent-second order central synapse and strengthens the idea that central inhibition CGRP receptors may be effective in the treatment of migraine pain.

POS-TUE-112

GALANIN AND ITS RECEPTORS ARE EXPRESSED IN THE WHOLE MOUSE PANCREAS, ISOLATED ACINAR AND ISLET CELLS

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Galanin is a neurotransmitter/neuromodulator associated with the pancreatic vasculature in many species. Galanin also modulates pancreatic exocrine secretion. Galanin acts via 3 known receptors, galanin receptor 1(GalR1), GalR2 and GalR3. The galanin receptor expression in the pancreas however is not fully described. We aimed to establish if galanin and its 3 receptors are expressed in normal mouse pancreas, acinar and islet cells. Pancreata were rapidly harvested from mice. Acinar and islets cells were isolated from mouse pancreas by standard protocol specifically techniques. Extraction of total RNA used a Trizol designed for pancreatic tissue. The expression of galanin, GalR1, GalR2 and GalR3 mRNA was determined using real-time reverse transcription-polymerase chain reaction (RT-PCR) with primers designed specifically for these transcripts. 18S rRNA was used as a housekeeping gene for normalisation of expression data. In the whole pancreas (n=3), expression of galanin and its 3 receptors was detected. GalR3 showed the highest expression followed by GalR1 then GalR2. In islet cells (n=2 preparations) GalR3 was highly expressed whereas GalR1, GalR2 and galanin appear to be poorly expressed. By comparison with islet expression, the acinar cell (n=3 preparations) expression of the 3 galanin receptors was very low, but surprisingly, galanin was well expressed. We conclude that the 3 galanin receptors are present in the mouse pancreas, but their relative expression varies with the different cell types studied. The poor expression of galanin receptors on acinar cells is consistent with our finding that galanin does not modulate amylase secretion by directly acting on acinar cells.

POS-TUE-113

ANATOMY AND PHYSIOLOGY OF GABA-ERGIC FEEDBACK EXCITATION IN PARVALBUMIN EXPRESSING INTERNEURONS OF THE BASOLATERAL AMYGDALA

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Parvalbumin expressing interneurons of the basolateral amygdala have previously been shown to form an excitatory feedback excitation loop involving at least one glutamatergic cell. This circuit can be identified in juvenile mice at post-natal day 21 and is preserved in mature adults. GABAergic interneurons with identified feedback excitation were filled with biocytin and developed for light and electron microscopy. Axonal length and arborization varied greatly from cell to cell, however in each case (n=32) the presynaptic interneuron was observed to make traditional axo-somatic basket synapses as well as axo-dendritic and axo-axonic synapses confirmed by co-labeling with the axon initial segment marker Ankyrin-G. Axo-axonic synapses also varied between the more classical “cartridge” type and single synaptic contacts. We tested the hypothesis that the Na+/K+/Cl- cotransporter (NKCC1), which maintains a high internal [Cl-] by pumping Cl- into the cell along the Na+ gradient, is required for the excitatory GABAergic feedback. Application of the antagonist bumetanide had no effect, suggesting that the feedback excitation is due to the lack of K+/Cl- cotransporter (KCC2), which sets a low internal [Cl-] by extruding Cl- along the K+ gradient. This was confirmed by immunohistochemical labeling of KCC2, which appeared to be present in dendrites but not in initial segments.

POS-TUE-114

PROPERTIES OF THE INTERCALATED NEURONS IN THE RODENT AMYGDALA

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The intercalated cell masses (ITCs) of the amygdala are a cluster of interneurons located between the basolateral complex and central nucleus, the main input and output stations of the amygdala. These neurons play an important role during extinction of conditioned fear and act as feed forward interneurons for cells in the central amygdala. However, their physiological properties are little understood. Methods: Using immunohistochemistry and whole-cell recordings in acute slices we have characterised the ITCs in GAD67-EGFP transgenic mice. Results: Immunohistochemical staining for calretinin, calbindin, parvalbumin, somatostatin and cholecystokinin showed that there was no expression for calretinin, calbindin, parvalbumin and somatostatin. Some cells expressed cholecystokinin in cell bodies and nerve terminals. Two types of neurons (n=50) were found on electrophysiological recordings: 34 % (17/50) of ITCs showed clear adaptation during the course of the 700 ms current injection and 66 % (33/50) of neurons fired repetitively. Synaptic stimulation evoked large polysynaptic IPSCs in most neurons. However, paired recordings revealed a connection rate of only 13.4 % (4/30 pairs). This result shows that ITCs have a different phenotype from most interneurons, lacking the typical calcium binding proteins. In further studies we will investigate whether synapses onto the ITCs are plastic and if these cells could not only be a site for the expression of extinction but also a site for the storage of extinction memory.

POS-TUE-115

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE MEDIAL AMYGDALA IN MICE

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BACKGROUND: The medial nucleus of the amygdala (MeA) is a subcortical structure that processes olfactory signals and plays a key role in regulating social, defensive and reproductive behaviours. However, the cellular and synaptic properties of MeA neurons have not been well characterized. **METHODS:** We have characterized neurons in the MeA of adult male GAD67-eGFP knock-in mice using immunohistochemistry and whole cell recordings in acute brain slices. Synaptic responses in these neurons were examined by stimulation of putative afferent olfactory inputs within the MeA external layer. **RESULTS:** Immunohistochemistry showed the presence of calbindin and calretinin in the MeA. However, there was no immunoreactivity for parvalbumin and somatostain. The majority of calbindin positive cells were GFP+, whereas calretinin positive cells appeared to be largely GFP-. Both GFP+ (n=36) and GFP- (n=57) cells could be divided into two types based on their responses to depolarising current injections: Repetitive firers (42% GFP+, 30% GFP-) and accommodating neurons (58% GFP+, 70% GFP-). There was no significant difference in the amplitude of afterhypepolarizations between the two firing subtypes in either the GFP+ or GFP- groups. Most cells possessed I_h (80% GFP+, 50% GFP-) and I_T (75% GFP+, 54% GFP-) which frequently gave rise to rebound spikes. Synaptic stimulation largely evoked monosynaptic glutamatergic responses that were mediated by both AMPA and NMDA receptors. A polysynaptic GABAergic input was often present in both GFP+ and GFP- neurons. These results represent the first step towards understanding the internal circuitry of the MeA and its role in olfactory processing and social recognition.

POS-TUE-116

EFFECTS OF SENSORY STIMULI ON AMYGDALA NEURON ACTIVITY IN VIVO

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Pavlovian fear conditioning involves the pairing of a non-aversive stimulus (conditioned stimulus, CS) with an aversive stimulus (unconditioned stimulus, US). The basolateral amygdala (BLA) is critical for fear conditioning, and convergence of CS and US-related inputs onto single BLA neurons is thought to lead to an enhanced synaptic response to CS presentations. Central to this hypothesis is that CS- and US-related inputs converge onto single BLA neurons. The strongest evidence for the convergence of CS and US-related information comes from single unit in vivo recordings obtained from the BLA. However, there are a number of limitations to single unit recordings. To address these issues, we employed whole-cell patch-clamp recordings from single BLA neurons in vivo and examined the convergence of auditory- and footshock-related inputs onto single BLA neurons. A glass electrode containing a standard potassium internal-solution was lowered into the BLA of Wistar rats (P18-21) and a whole-cell recording configuration obtained. Using urethane-anesthetized animals we show that a single footshock (1ms, 5mA) can recapitulate UP-states while white noise (0.5-1s, 80 dB) only generates excitatory post-synaptic potentials (latency 100±11.2ms; duration 432±75 ms; amplitude: 10.43mV±1.70). Under isoflurane, the majority of BLA principal neurons displayed a depolarizing response to both white noise and footshock. The response-latencies were: white noise, 119.1 ± 6.3 ms; and footshock, 88.4 ± 3.6ms. These results demonstrate for the first time that single BLA projection neurons receive synaptic inputs related to both auditory and footshock stimulation.

POS-TUE-117

MAPPING THE PRIMATE ZONA INCERTA BY COMPARISON WITH THE PATTERN OF IMMUNOMARKERS IN THE RAT

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The zona incerta (ZI) has recently proved to be a more effective target than the subthalamic nucleus for deep brain stimulation (DBS) in patients with Parkinson's disease. The caudal ZI seems to be the most important area for DBS in humans, but the anatomy of the human ZI has not been mapped in detail. We have therefore attempted to identify the subnuclei of the primate ZI by comparison with the rat, in anticipation of extending the primate studies to the human brain in the future. We have examined serial brain sections stained with a number of markers (calbindin, parvalbumin, calretinin, SMI32, NADPH diaphorase, acetlylcholinesterase, tyrosine hydroxylase, and Nissl) in the rat (n=1)(Paxinos et al 2009) and in the pygmy marmoset (n=1) (Tokuno et al, 2009). We have identified dorsal, ventral, rostral, and caudal regions of the ZI in the marmoset, which we believe to be homologous with similarly named areas in the rat. Parvalbumin, SMI32, and calretinin have proved the most useful markers in delineating sub-regions in the primate ZI. In the next phase of our study, we will extend the marmoset data to similarly stained sections of human ZI. Our long term aim is to correlate the area of optimal DBS in humans with an area with a characteristic pattern of histological markers.

POS-TUE-118

NEUROMODULATION OF STOCHASTIC RESONANCE IN CORTICAL NEURONS

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We investigated the properties of mouse cortical layer II/III pyramidal neurons to show stochastic resonance (SR), the property of signal transmission systems that allows them to function optimally under non-zero noise. Stochastic resonance was shown (N=10 cells) as the increase in signal to noise ratio with addition of noise to sub-threshold EPSPs injected via dynamic clamp. Depending on the intrinsic neuronal properties the optimal noise levels as well as the optimal timing of the inputs was found to differ. Even further, application of neuromodulatory agents such as charbachol modified the SR responses of neurons. As the ability of individual cell to respond and synchronize to subthreshold inputs greatly influences the large-scale behavior of the network, these results provide insights into the neuromodulation of synchronous activity of the cerebral cortex.

POS-TUE-119

NETWORK ACTIVATION STATE MODULATES GAIN IN THE MOUSE LATERAL GENICULATE NUCLEUS

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Purpose. Neurons in the visual thalamus receive synaptic inputs from a number of sources including the cerebral cortex, retina and brainstem nuclei. These inputs reflect the activation state of the network and interact with intrinsic membrane properties to determine individual neuronal output. We asked how the network activation state altered the output properties of neurons in the mouse (C57BL/6) lateral geniculate nucleus (LGN). **Methods.** Whole-cell current-clamp recordings were made from coronal brain slices (250 µm) containing the LGN. Intrinsic membrane properties of visualised LGN neurons were characterised using a set of stimuli that included incremental steps of depolarising current. Changes in network activation state were simulated by convolving a suite of white noise stimuli drawn from a Gaussian distribution (standard deviations; 6, 12, 25 or 50) onto the depolarising current stimuli. **Results.** LGN neurons showed a range of sensitivities to depolarising steps (firing response gain; 0.1-0.7 Hz/pA; n = 18). Increased noise altered firing response gain such that the distribution of gains in the LGN neuron population was narrowed. In 8/13 cells tested in this way, increased noise increased gain ($p = 0.005$), while in 5/13 neurons it decreased gain ($p = 0.02$). In both cases, changes were toward the population mean (0.32 ± 0.05 Hz/pA). However, pharmacologically altering the state of the corticothalamic network using the specific mGlurR1a agonist 1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD) always increased gain when compared to control ($p = 0.016$; n = 3). **Conclusion.** These results suggest the output of LGN neurons is stabilised around a mean level in a state-dependent manner, but presynaptic mGlurR1a receptors on corticothalamic projecting neurons are not sufficient for this stabilisation.

POS-TUE-120

ALCOHOL AND PROTEIN EXPRESSION: PERSPECTIVES ON ALCOHOL-INDUCED BRAIN DAMAGE

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Background: Alcohol can be harmful to the physical health, social life and brain of the individual. Alcohol-induced brain damage appears to be region-specific with major microstructural dysmorphology observed in the prefrontal cortex, the striatum, the cerebellum, the hippocampus and the white matter including the corpus callosum (CC). The molecular mechanisms underlying these microstructural changes in the human brain are largely unknown, particularly at the level of proteomics. Methods: Human postmortem brain samples were collected from NSW Tissue Resource Centre. Proteins were extracted from different brain regions (10 control; 7 uncomplicated alcoholic and 6 alcoholic complicated with hepatic cirrhosis) and separated by 2-D gel electrophoresis. Digitized gel images were analysed and identified protein through MALDI-TOF and the MASCOT search engine techniques. Metabolic pathway analysis of the identified proteins was performed using the Ingenuity database. Results: Four separate experiments were conducted using tissue from the hippocampus, and the genu, body and splenium of the corpus callosum. Differential expression of 21, 50, 46 and 43 proteins was found relative to controls in the alcoholic hippocampus, genu, body and splenium respectively. Pathway analysis suggested that differentially expressed proteins in these regions related to abnormal carbohydrate metabolism, lipid peroxidation, oxidative stress, signaling and apoptosis pathways. Comparison within alcoholic groups revealed that at least 40% of these proteins had differential expression in complicated (impaired liver function) compared to uncomplicated alcoholism. Conclusion: Sub-regional expression profiles indicate that alcohol-induced changes in protein expression are region specific. Liver complications have a synergistic effect on changes in brain protein expression. Thiamine-related cascades do not appear to be the major pathways for brain damage. Rather deleterious oxidative stress, methyl glyoxalation, lipid peroxidation, deacetylation and apoptosis pathways are dominant. Alcohol-induced microstructural damage detected histologically may therefore reflect cascades of these multiple biochemical mechanisms.

POS-TUE-121

LONG-TERM MODERATE BEER CONSUMPTION CAUSES MAJOR CHANGES IN THE STRIATAL PROTEOME OF THE RAT

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Background: Dopamine systems centred on the ventral and dorsal striatum have been heavily implicated in the mechanisms underlying drug and alcohol abuse and addiction. Neuroimaging and other studies suggest that drug and/or alcohol addiction alters the neuronal plasticity in striatal regions through modification of neuronal structure and synaptic architecture. The biochemistry underlying those micro-morphological modifications induced by alcohol remains largely unknown. Methods: Group housed male Wistar rats given ad libitum home cage access to beer (Toohey's New) consumed alcohol at an average of 3.2 g/kg/d ethanol for 8 months. This represents a moderate level of alcohol consumption that is not associated with physical dependence. At the end of this drinking period, protein expression was studied in striatal tissue from these animals using 2-DE gel based proteomics. Results: Fourty-four protein spots, recognised as 28 unique proteins, were differentially altered in the beer group relative to the controls ($P<0.05$). Functional analysis of the identified proteins indicated that they belonged to the general classes of metabolic (40%) followed by signal transduction (25%), oxidative stress (18%), cytoskeletal (10%) and Ca+2 regulation (7%). Interestingly several dopamine (DA) regulating proteins such as tyrosine hydroxylase (enzyme of DA biosynthesis), pyridoxal phosphate phosphatase (coenzyme providing enzyme for DA biosynthesis), dopamine-and cAMP regulating phosphoprotein (DARPP-32) (protein of dopamine receptor and transporter regulator) and protein tyrosine phosphatase (DA signaling protein) and nitric oxide synthase (DA uptake modulating enzyme) were differentially expressed in the striatum. Conclusion: Prolonged moderate intake of alcohol can cause major changes in protein expression in the striatum of rats. The cascades of above identified proteins can be linked to altered synaptic plasticity and habitual alcohol seeking behaviour.

POS-TUE-122

IONIC HOMEOSTASIS IN RESPONSES OF NEURAL CELLS TO STRESS

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It is well established that transport of ions is of paramount importance to neural cells and is believed to be among the first responses to chemical or physical injury. In our studies we assessed kinetics of net K^+, Ca^{2+} and H^+ fluxes in primary cortical neurons in response to a neurotransmitter glutamate thus simulating neural injury using a non-invasive microelectrode ion flux measuring technique (MIFE). All the chemical and physical injuries used led to a transient K^+ efflux and Ca^{2+} uptake. Inhibitors of specific K^+ and Ca^{2+} transport systems were used to assess their contribution to the observed ionic fluxes in response to stimuli thus revealing roles of relevant transporters in stress perception and signalling. Application of various concentrations of glutamate (between 1 to 100 µM) led to a transient K^+ efflux and Ca^{2+} and H^+ uptake with the magnitude of response being concentration dependent. Peak of K^+ efflux increased from 184.63 nmol m^{-2} s^{-1} to 1814.42 nmol m^{-2} s^{-1}, respectively, when glutamate concentration was raised from 1 to 100 µM (n=3-4). The magnitudes of fluxes were age specific with 3-fold increase in Ca^{2+} uptake in 14 DIV as compared to 7 DIV neurons, while K^+ efflux measured from the same cells was decreased in mature cultures. Inhibitors of ionotropic glutamate receptors MK-801 and CNQX significantly reduced net Ca^{2+} influx suggesting their involvement in the observed flux changes. TEA and 4-AP reduced K^+ efflux by 50% and 30%, respectively (n=3-5), suggesting involvement of more routes for K^+ efflux. Overall, our results demonstrate that studies of kinetics of ion transport across cellular membranes can help to understand better mechanisms underlying those processes.

POS-TUE-123

ACUTE, REVERSIBLE AXONAL ENERGY FAILURE DURING A STROKE-LIKE EPISODE IN MELAS

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Introduction:The pathophysiology of stroke-like episodes in MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) remains unresolved. In-vitro studies of mitochondrial cytopathies have demonstrated reduced capacity to produce ATP during states of energy demand and membrane depolarization, resulting from Na^+/K^+ pump inhibition. To date, in-vivo studies have largely focused on cerebral neuroimaging. Methods:Serial recordings of peripheral axonal excitability were undertaken at baseline and at 2, 4, 6, 24 and 48 hours from the onset of clinical symptoms during a stroke-like episode in a ten-year-old with MELAS. Serum electrolytes, lactate and pH were collected with each recording. Results:There were marked and progressive changes in multiple axonal excitability parameters during the first six hours of the stroke-like episode. The stimulus response curve shifted to the left, strength-duration time constant increased, threshold electrotonus 'fanned in', refractoriness increased and superexcitability reduced. These changes were consistent with axonal depolarization, similar to acute ischemia. There was a subsequent reversal of excitability parameters, with a return towards baseline by 24 hours. The excitability parameters correlated with the clinical assessment of CNS dysfunction and degree of lactic acidosis($R=0.97$). Conclusions:There is dynamic and reversible depolarization in the peripheral axon due to disruption of energy dependent processes during a stroke-like episode in a 10-year-old with MELAS. Significance:Our results suggest that these pathophysiological processes may not be confined to the cortex, but rather may be deleterious to tissues with high energy demand, resulting in simultaneous energy insufficiency throughout the neural axis. As such, axonal excitability techniques may be useful as a surrogate marker of the central pathophysiological events that develop during a stroke-like episode in MELAS.

POS-TUE-124

PATHOPHYSIOLOGY OF PACLITAXEL-INDUCED NEUROTOXICITY

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Purpose Sensory neurotoxicity is a prominent side-effect of the chemotherapy paclitaxel, commonly utilised in early-stage breast cancer. Although *in-vitro* studies suggest that disruption of axoplasmic transport may underlie neuropathy development, pathophysiology remains largely undefined. The present study aims to investigate the development of paclitaxel-induced neurotoxicity *in-vivo* to explore pathophysiological mechanisms. **Method** Sensory axonal excitability studies, quantitative sensory testing and clinical neurotoxicity grading scales were undertaken in 15 paclitaxel-treated patients (126 studies), across assessed prospectively at baseline and every month during treatment. A cohort of 17 oxaliplatin-treated patients (590 studies) was included as a disease control. **Results** Following 930±64.3 mg/m^2 paclitaxel over 11.5 weeks, 60% of patients reported neuropathic symptoms, which developed at a median of 6 weeks of treatment. Stimulus threshold significantly increased ($P<0.05$) after 4 weeks, while by mid-treatment, maximal sensory amplitude was significantly reduced from 50.1±4.7 µV to 41.2±4.0 µV ($P<.005$). By final treatment, sensory amplitude was reduced to 36.5±3.3 µV ($P<.005$), revealing development of sensory nerve damage. However, excitability measures of membrane potential and ion channel function remained within normal limits. In contrast, 11 weeks of oxaliplatin treatment produced no reduction in maximal sensory amplitude but significant excitability changes revealing membrane hyperpolarization ($P<.005$). **Conclusion** Chemotherapy-induced neurotoxicity is associated with a diverse range of excitability profiles reflecting differing underlying pathophysiologies. Specifically, paclitaxel treatment produces progressive changes in sensory nerve function, in the absence of alterations in membrane potential or ion channel function. In contrast, oxaliplatin causes significant changes in excitability suggestive of membrane hyperpolarization that precedes axonal degeneration. These findings suggest that excitability studies may provide novel biomarkers of axonal function in chemotherapy-induced neurotoxicity.

POS-TUE-125

HCN CHANNELS IN INFLAMMATION OF THE RAT TEMPOROMANDIBULAR JOINT

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Introduction: Hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels are active at resting membrane potentials and thus contribute to neuronal excitability. Four HCN subunits have been cloned (HCN1-4) and have been shown to contribute to nociception following nerve injury. However, little is known of the contribution of HCN channels to nociception following inflammation. Methods: In one series of experiments, an animal model of sensitivity to temporomandibular joint (TMJ) inflammation was used to determine whether peripheral HCN channels contribute to inflammatory pain. Sensitivity was tested with calibrated von Frey filaments, applied over the TMJ, 1 day pre- and 1 day post-injection of 4µl of complete Freund's Adjuvant (CFA; to induce inflammation; n=7 animals), saline (control; n=7 animals) or CFA+ZD7288 (inflammatory agent+HCN antagonist; n=8 animals). Withdrawal thresholds at day 1 post-injection were determined and expressed as a percentage of the pre-injection value for each animal. In another series of experiments (n=3 animals), retrograde tracing was used to identify trigeminal sensory neurons that innervate the TMJ, and immunohistochemistry was used to examine HCN1-4 subunit immunoreactivity in these neurons. Results: There was a significant increase in mechanical sensitivity (decrease in withdrawal threshold) in animals following CFA injection, but not following saline injection, and ZD7288 blocked this increase in sensitivity (ANOVA on ranks; $P<0.05$). Of the neurons retrogradely labelled with Fast Blue, 13±1.7% (mean±SEM), 13±4.6%, 1.4±0.8% and 0% were immunoreactive for the HCN1, HCN2, HCN3 and HCN4 subunits respectively. Conclusion: These data suggest that HCN channels on trigeminal primary afferent neurons contribute to nociception induced by inflammation.

POS-TUE-126

DISTINCT MEMBRANE AND SYNAPTIC PROPERTIES OF CALRETININ EXPRESSING NEURONS IN THE SUPERFICIAL DORSAL HORN OF THE MOUSE SPINAL CORD

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Neurons in the superficial dorsal horn (SDH) receive and process noxious and innocuous peripheral inputs. One barrier to understanding how SDH neurons process such inputs has been the regions neuronal heterogeneity. **Purpose**: We used transgenic mice, expressing enhanced green fluorescent protein (eGFP) in calretinin-positive neurons, to record selectively from presumptive excitatory interneurons. **Methods**: Mice (2-3 months) were anaesthetised (Ketamine 100 mg/kg i.p.), decapitated, and transverse slices were prepared from lumbar spinal cord. Targeted patch-clamp recordings were made from eGFP-positive neurons and compared to those from randomly sampled neurons in littermate controls. **Results**: eGFP neurons (n = 30) had similar input resistances (345 ± 27 MOhm vs. 364 ± 43 MOhm) and RMPs (-63 ± 1 mV vs. -63 ± 2.5 mV) to randomly sampled neurons (n = 18), however membrane capacitance was increased (25 ± 1 pF vs. 20 ± 2 pF, $p<0.05$). Spontaneous excitatory postsynaptic currents (sEPSC) in eGFP neurons (n = 20) had similar amplitudes (-32.2 ± 12.1 pA vs. -28.5 ± 1.8 pA), rise times (0.55 ± 0.02 ms vs. 0.60 ± 0.03 ms) and half widths (1.7 ± 0.1 ms vs. 1.9 ± 0.1 ms) to randomly sampled neurons (n = 18), however sEPSC frequency was higher (29.0 ± 1.0 Hz vs. 16.6 ± 1.9 Hz, $p<0.05$). The rapidly activating and inactivating potassium current (IAr) was observed in all eGFP neurons (18/18) but only 11/17 of randomly sampled recordings. **Conclusions**: The distinct properties of calretinin expressing eGFP-positive neurons suggest a specific role for these presumptive excitatory interneurons in the spinal sensory processing.

POS-TUE-127

ELECTROPHYSIOLOGICAL PROPERTIES OF SUPERFICIAL DORSAL HORN NEURONS DIFFER IN UPPER-CERVICAL SPINAL SEGMENTS IN NEONATE AND ADULT MICE

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Injury to deep structures in the neck produces significant disability, including ongoing pain. Such injuries often occur in neonates because of torsional forces associated with forceps delivery, and in adults following whiplash. Surprisingly, little is known about how sensory information from deep neck structures is processed in upper-cervical spinal segments in neonates or adults. **Purpose:** To investigate excitability and sensory processing in upper-cervical (C2-4) superficial dorsal horn (SDH) neurons in neonate (P0-5) and adult (≥ P24) mice. **Methods:** Mice (C57Bl/6) were anaesthetized (Ketamine 100 mg/kg i.p.) and decapitated. Transverse slices were prepared from C2-4 spinal segments and whole cell recordings (at 32°C) were obtained from SDH neurons (KCH_3SO_4 internal). **Results:** Passive membrane properties differed significantly between neonatal (n=101) and adult (n=99) SDH neurons. Input resistance was higher in neonates (711 ± 41 MΩ vs. 428 ± 21 MΩ) and resting membrane potential was more depolarised (-58.4 ± 1.0 mV vs. -65.8 ± 1.0 mV). Action potential (AP) and after-hyperpolarization amplitude were lower in neonates (29.5 ± 1.4 mV vs. 48.9 ± 1.3 mV; -11.5 ± 1.0 mV vs. -36.9 ± 0.7 mV), whereas AP half-width was greater in neonates (2.29 ± 0.1 ms vs. 0.72 ± 0.02 ms). Neonate SDH neurons also differed in their response to step current injection. The prevalence of tonic firing and initial bursting responses was greater in adult neurons. Following dorsal root stimulation (C2 nerve) both Aδ and C-fibre inputs were observed in adult SDH neurons. **Conclusion:** Our data suggest nociceptive processing in the upper-cervical SDH differs in neonates and adults.

POS-TUE-128

COMPARISON BETWEEN T-LYMPHOCYTES IN DORSAL ROOT GANGLIA OF WISTAR AND LEWIS RATS AFTER SCIATIC NERVE TRANSECTION

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T-lymphocyte invasion of dorsal root ganglia (DRGs) projecting in damaged peripheral nerves may arise from an auto-immune response [1] and be involved in retrograde death of axotomized neurones. Here we compared T-cell responses to nerve transection between Wistar and Lewis rats. The latter (with few T-cells and heightened susceptibility to autoimmune disease) might respond more than Wistar rats. The left sciatic nerve was transected in groups of 6 female rats (7-9 weeks old) under anaesthesia with ketamine (60 mg/kg) and xylazine (10 mg/kg) i.p. After one or 10 weeks, the rats were anaesthetized with pentobarbitone (100 mg/kg i.p.) and perfused with Zamboni's fixative. Bilateral L5 DRGs were processed for double labelling immunohistochemistry, using combinations of antibodies to α/β T-Cell Receptor, CD8, CD3, CD68 and MHC II to define the subtypes of T-cells and macrophages. After one week, T-cell density was higher in Wistar than Lewis rats and, after 10 weeks, was markedly increased in both strains. However the increase relative to that after one week was similar between strains. The data suggested that CD4+ T-cells are more prevalent after injury in Lewis than Wistar rats. Increased MHC II+ macrophage density paralleled that of T-cells in both strains. As T-cells can be beneficial or detrimental for survival of axotomized adult retinal ganglion cells [2], the degeneration of cutaneous nociceptive neurones after peripheral nerve injury [3] needs evaluation in both strains. 1 Olsson, T. et al (1992) Autoimmunity 13:117-126. 2. Luo, J-M. et al (2007) Eur. J. Neurosci. 26:3475-3485. 3 Hu, P. & McLachlan, E.M. (2003) J. Neurosci.23:10559-10567.

POS-TUE-129

LONG-TERM FACILIATION OF SYMPATHETIC NERVE ACTIVITY DOES NOT REQUIRE INCREASED PHRENIC NERVE ACTIVITY FOLLOWING ACUTE INTERMITTENT

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Intermittent hypoxia (IH) elicits a long-lasting augmentation of phrenic nerve activity known as long-term facilitation (LTF), a type of plasticity of respiratory motor neural activity, even after the stimuli have ceased and blood gases have normalized. It is still unclear whether the sympathetic system similarly expresses the IH-induced plasticity, even though the respiratory and sympathetic control systems are coupled with each other. The aim of this study was to investigate the relationship between the sympathetic and phrenic LTF after IH. We recorded splanchnic (sSNA) and phrenic nerve activities (PNA) in urethane-anaesthetized (1.2 g/kg, i.p.), vagotomized and mechanically ventilated Sprague-Dawley rats (n=16). Animals (n=11) were exposed to 10 45s episodes of 10% O_2-90% N_2, separated by 5 min interval of 100% O_2, and the recordings were continued for 60 min following the last hypoxic exposure. The other five animals were prepared in the same manner but not exposed to hypoxia (time-control). Cycle-triggered averages of integrated PNA and sSNA from periods preceding, and 15, 30, 45 and 60 min following the hypoxic stimuli were compared. We found that (1) all animals manifested the sustained increase of sSNA (P<0.001) after AIH, but only five of them also expressed phrenic LTF compared with the time control group; (2) both the inspiratory, and post-inspiratory, peaks of SNA increased regardless of phrenic LTF; (3) the baroreflex was enhanced after the sympathetic LTF was established ($Gain_{max}$ from 2.15±0.37 to 3.62±0.98 %/mmHg, P=0.008). These findings indicate that the respiratory-sympathetic coupling does contribute to sympathetic LTF, but that an additional effect on sympathetic tone is also present.

POS-TUE-130

NERVE EXCITABILITY AND MECHANISMS OF IMMUNOTHERAPY IN PATIENTS WITH CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY

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Purpose Chronic inflammatory demyelinating polyneuropathy (CIDP) is a disorder of the peripheral nervous system treated using immunotherapy intravenous immunoglobulin (IVIg). However, although IVIg is the first-line treatment for CIDP, the underlying mechanism of IVIg remains unclear. To investigate the pathophysiology of immunotherapy, nerve excitability studies were undertaken to monitor changes reflected in axonal membrane potential during treatment. Method Motor nerve excitability studies were undertaken in 20 patients undergoing immunotherapy (IVIg), pre-and-post monthly infusion. In addition, CIDP patients were assessed longitudinally across treatments. The median nerve was stimulated at the wrist, recording compound motor action potentials (CMAP) from abductor pollicis brevis muscle. Multiple excitability parameters were recorded including stimulus-response curves (SR), threshold electrotonus (TE), recovery cycle of excitability (RC) and current-voltage relationship (I/V). Results Patients with CIDP demonstrated significant differences (paired t-test) in multiple excitability parameters pre and post infusion. Post-infusion, the stimulus response curve shifted to the left (decreased threshold) with increased peak response (p<0.05). SDTC was significantly reduced (p<0.02) accompanied with fanning-in appearance of TE (TEh(90-100)ms, p<0.01) and increase refractoriness and decreased superexcitability in the RC. Longitudinal changes in these excitability parameters, specifically superexcitability demonstrated correlation with the clinical recovery of patients. Conclusion This is the first study using novel threshold tracking techniques to investigate the mechanisms of action of immunotherapy in CIDP patients. Significant changes in excitability properties illustrated a subtle depolarizing effect post-immunotherapy. These results may imply the role of immunotherapy is to stabilize the axonal membrane potential to thereby prevent further progression due to the underlying disease state.

POS-TUE-131

DEVELOPMENTAL CHANGES IN SUBTHRESHOLD POTASSIUM CURRENTS CONTRIBUTE TO INCREASED EXCITABILITY OF THE NEONATAL SPINAL CORD

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There is now increasing evidence that innocuous and noxious peripheral stimuli are processed differently in the spinal cords of neonates and adults. Spinal superficial dorsal horn (SDH) neurons receive and process these stimuli, and their excitability plays an important role in determining SDH output to higher centres. Subthreshold potassium (K^+) currents are important in setting excitability and discharge in a variety of CNS neurons. It is not known how these K^+ currents differ in neonate and adult SDH neurons. **Purpose:** To compare the properties of subthreshold K^+ currents in neonatal (P0-5) and adult (≥ P21) SDH neurons. **Methods:** Mice were anaesthetized (Ketamine 100 mg/kg i.p.), decapitated and transverse slices (300 µm, L3-5 segments) were prepared. Whole cell recordings were obtained (at 32°C) from SDH neurons using a KCH_3SO_4-based internal. **Results:** Two distinct outward currents were observed in adult neurons, one exhibiting rapid activation/inactivation (rapid A; I_{Ar}) and a second with slower kinetics (slow A; I_{As}). Only I_{Ar} was observed in neonatal SDH neurons, so further comparisons were restricted to I_{Ar}. The peak amplitude (158.97 ± 13.97 pA vs. 346.28 ± 38.99 pA; neonates (n = 80) vs. adults (n = 59)) and decay time constant (26.3 ± 26 ms vs. 38.8 ± 3.5 ms) of I_{Ar} was lower in neonates. Further analysis of I_{Ar} (n=10; both ages) revealed neonatal I_{Ar} activated at more depolarised (~ 5 mV) potentials. Inactivation kinetics did not differ in neonates and adults. **Conclusion:** The lower expression, faster kinetics and more depolarised activation threshold of I_{Ar} are consistent with the increased excitability of the neonatal spinal cord.

POS-TUE-132

SODIUM CURRENT PROPERTIES DIFFER IN NEONATE AND ADULT MOUSE SUPERFICIAL DORSAL HORN NEURONS

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Superficial dorsal horn (SDH: laminae I-II) neurons are important for spinal processing of nociceptive information and their excitability is determined, in part, by the properties of voltage-gated sodium channels (I_{Na}). Recently, we have shown excitability and action potential (AP) properties in SDH neurons are altered during development (Walsh et al., 2009 J Neurophysiol 101: 1800). **Purpose:** To compare I_{Na} currents in neonate (P0-5) and adult (≥ P21) SDH neurons. **Methods:** Mice were anaesthetized (Ketamine 100 mg/kg i.p.) and decapitated. Transverse slices were prepared from L3-5 spinal segments and whole-cell recordings were made (at 32°C) from visualized SDH neurons using a CsF-based internal. **Results:** A fast activating and inactivating inward current was evoked by depolarising neurons from - 60 to - 20 mV. The peak amplitude of this current increased during development (1.34 ± 0.35 nA vs. 6.58 ± 0.68 nA; neonates (n = 10) vs. adults (n = 12)), and was completely abolished by 1 µM TTX in both neonates (n = 2) and adults (n = 3), thus confirming the current was mediated by I_{Na}. Time to peak and half width was slower in neonates (0.82 ± 0.08 ms vs. 0.46 ± 0.06 ms; 1.40 ± 0.25 ms vs. 0.43 ± 0.05 ms). I_{Na} activation voltage and peak current voltage also differed in neonatal and adult neurons (- 50 mV vs. – 60 mV; - 15 mV vs. – 25 mV). **Conclusion:** These data show I_{Na} expression and kinetics differ in neonate and adult SDH neurons and provide an underlying mechanism for the differences observed previously in AP properties of developing SDH neurons.

POS-TUE-133

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION CHANGES AN ABNORMAL - BUT NOT NORMAL - NEURAL PROJECTION

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Purpose: Non-invasive and painless stimulation of brain tissue by administration of repetitive transcranial magnetic stimulation (rTMS) benefits a wide range of neurological and psychiatric disorders. However, investigations have been limited to synaptic changes in healthy animals, short-term effects which are unlikely to explain long-lasting behavioural improvements in neurological conditions. Here, we investigate the potential for rTMS to alter connectivity in a representative neural projection, the mouse retinocollicular projection. In addition, we compared the effect of rTMS on a normal (wildtype) and abnormal (ephrin-A2/A5-/- mice) projection. **Methods:** After 14 days (10min/day) of high frequency (>75Hz) stimulation to the superior colliculus, or sham treatment, the retinocollicular projection was assessed anatomically (anterograde tracing) and functionally (electrophysiological recording, visuomotor behaviour) in WT (n=40) and ephrin-A2/A5-/- (n=19) mice. Data were analysed using ANOVA and Scheffe post-hoc tests. **Results:** rTMS altered functional properties of the retinocollicular projection in ephrin-A2/A5-/- but not WT mice. Sham-treated ephrin-A2/A5-/- mice showed a longer latency of response to off-light stimulation, and this was reduced by rTMS treatment to the same as WT ($p<0.05$). In addition, rTMS increased the size of the retinocollicular receptive fields ($p<0.0001$ vs sham). No anatomical or behavioural changes were detected following rTMS. **Conclusion:** Chronic, high frequency rTMS can induce subtle functional changes in a mature neural projection. However, these changes were only observed in a neural system with abnormal connectivity (ephrin-A2/A5-/- mice), suggesting homeostatic mechanisms may prevent such changes in normal mice. Our results highlight the importance of using relevant animal models of neurological disorders in investigating the impact of rTMS.

POS-TUE-134

LONG-TERM DEPRESSION CAUSED BY LOW FREQUENCY STIMULATION IN PAIRED-PYRAMIDAL CELLS OF LAYER II/III OF RAT BARREL CORTEX

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Multiple forms of long-term depression (LTD) have been reported at neocortical synapses, implying their varied roles in different forms of behaviour. Using low frequency presynaptic stimulation (<0.3 Hz) in paired-recordings, we observed LTD with unique properties. **Purpose:** Identifying the induction site and exploring underlying molecular mechanism(s) of this LTD. **Methods**: Paired whole-cell recording from layer II/III pyramidal cells, verified by histology, were obtained in 300 µm thick slices of barrel cortex (P15-19) at 36±1°C. Pre- and postsynaptic cells were current- and voltage-clamped, respectively. 300 presynaptic stimuli were evoked at 0.2 Hz using short current pulses (~1 nA, 3 ms) in the presence of gabazine (3 µM). **Results**: Repeated stimulation caused LTD of 69% after 25 min, lasting >2.5 h. Rate of depression was well fit by a single exponential (tau=25.5±3.9 min at 0.25 Hz, n=9) and still observed at 0.03 Hz (tau=121±29 min, n=5). tau was shorter for paired versus single stimuli at 0.1 Hz (25±7 vs 109±28 min, n=3). Paired-pulse interval did not affect tau. Chelation of postsynaptic Ca^{2+} with intracellular BAPTA (20 mM), did not prevent LTD, indicating a presynaptic mechanism. Blockade of presynaptic adenosine-1, NMDA and P2X/Y receptors by DPCPX (200 nM), APV (20 µM) and suramin (20 µM), respectively, had no effect. **Conclusions:** A presynaptic form of LTD is described, lasting > 2.5 h in layer II/III pyramids of barrel cortex, which is independent of presynaptic A_1, NMDA or P2X/Y receptor activation. CB1R and mGluR are currently being explored.

POS-TUE-135

DOPAMINE D2 RECEPTOR EXPRESSING STRIATAL PROJECTION NEURONS DISPLAY LONG TERM POTENTIATION AFTER HIGH FREQUENCY STIMULATION OF CORTICAL AFFERENTS

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Activity-dependent synaptic plasticity in the neostriatum has been proposed to play an important role in the integration of cortical information into specific reward related actions. Experimental analysis of plasticity in this system is complicated by the existence of two major subtypes of striatal projection neurons, which predominantly express either dopamine D1 receptors (D1 cells) or D2 receptors (D2 cells). Here, we study synaptic plasticity in corticostriatal slices from transgenic mice that have cell specific markers to allow definitive identification of D1 and D2 cells. After high frequency cortical stimulation (100 Hz), D1 and D2 cells displayed cell specific responses. The D2 cells potentiated to a significantly higher degree than the D1 cells, suggesting that afferent information integration and processing is different in the two cell types. The potentiation observed in the D2 cells was sensitive to the specific D2 receptor antagonist sulpiride (10µM) and the adenosine A2A receptor antagonist ZM241385 (1µM). Analysis of the cells' electrophysiological properties revealed D2 cells were more excitable than D1 cells. Application of the A2A antagonist modulated the pattern of firing of the D2 cells so that they resembled those seen in the D1 cells. These data suggest that there are specific differences in synaptic plasticity between D1 and D2 cells in response to specific patterns of excitation and that there is a significant role for both the dopamine D2 receptor and adenosine A2A receptor in modulating the plasticity in D2 cells.

POS-TUE-136

PROTEIN SYNTHESIS-DEPENDENT ENHANCEMENT OF TRANSMITTER RELEASE IN PERSISTENT FORMS OF HIPPOCAMPAL LTP

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Long-term potentiation (LTP) of synaptic transmission is an important process underlying learning and memory in the brain. At CA3-CA1 synapses in the hippocampus, three discrete forms of LTP (LTP1, 2 and 3) can be differentiated on the basis of maintenance and induction mechanisms. However, the relative roles of pre- and postsynaptic expression mechanisms in LTP1, 2 and 3 are unknown. In this study, the potential role of enhanced neurotransmitter release in the expression of LTP1, 2 and 3 was investigated by measuring electrically-evoked destaining of the styryl dye FM1-43 from potentiated CA3 terminals in 400µm brain slices taken from male Wistar rats (7-8 weeks). No difference in vesicle turnover rate was observed for LTP1 at 60 min or 120 min following induction by 1 train of theta-burst stimulation (1TBS). A significant increase in release was found for LTP2 only at 120 min after induction by 4TBS ($n=6$; $p<0.05$), and for LTP3 at both time points after induction by 8TBS (60 min, $n=14$; 120 min, $n=10$; $p<0.05$). Inhibition of protein synthesis with anisomycin blocked both LTP2 maintenance ($n=4$, $p<0.05$) and the associated enhanced exocytosis ($n=3$, $p<0.05$), whereas the transcription inhibitor Actinomycin-D had no effect. LTP3 was found to be dependent on both protein synthesis ($n=4$, $p<0.05$) and transcription ($n=6$, $p<0.05$), however the associated enhanced release was dependent only on protein synthesis ($n=4$, $p<0.05$). This study shows that more durable forms of LTP involve an enhancement of transmitter release, which is dependent on *de novo* protein synthesis, but not gene transcription, confirming the existence of mechanistically discrete forms of LTP in CA1.

POS-TUE-137

CHEMOKINE-MEDIATED GUIDANCE OF THE NEUROINFLAMMATORY RESPONSE BY MULLER CELLS FOLLOWING LIGHT-INDUCED RETINAL DEGENERATION

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AIM: To investigate the role of the chemokine CCL-2 – a monocyte chemoattractant protein – in shaping the retinal inflammatory response following photoreceptor degeneration induced through exposure to excessive light. **METHODS:** SD rats were exposed to 1000lx of light for up to 24hrs, after which some animals were kept in dim light (5 lux) to recover. At specific time points during (1, 3, 6, 12, 17, and 24hrs) and following exposure (3 and 7 days), animals were euthanized and retinas processed. CCL-2 expression was assessed by qPCR (n=4), immunohistochemistry (n=3), and *in situ* hybridization (n=3) at each time point. In conjunction, counts were made of monocytes on retinal cryo-sections immunolabeled with ED1 (n=4), while photoreceptor cell apoptosis was assessed using TUNEL labeling (n=5). Statistical significance was determined using the Students t-test. **RESULTS:** Up-regulation of CCL-2 gene expression was evident in retinal tissue after 12hrs exposure, which correlated with the significant increase ($p<0.05$) in photoreceptor cell death. CCL-2 expression peaked by 24hrs exposure, coinciding with the peak in cell death. Immunohistochemistry and *in situ* hybridization on retinal cryo-sections revealed that CCL-2 is expressed by Müller cells from 12hrs exposure onward, predominately in regions of heavy photoreceptor degeneration. From 24hrs exposure, a significant ($p<0.05$) recruitment of monocytes to the choroidal and retinal vascular supplies was observed. CCL-2 immunoreactivity was also observed in many of these infiltrating monocytes at 24hrs exposure. **CONCLUSION:** Our data indicate that photoreceptor death promotes CCL-2 expression by Müller cells, which facilitates the targeting of monocytes to sites of injury, thereby contributing to the guidance of the neuroinflammatory response following retinal injury.

POS-TUE-138

ROLE OF THE COMPLEMENT SYSTEM IN ACUTE AND CHRONIC MODELS OF RETINAL DEGENERATION

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AIM: To investigate the role of the complement system in the pathogenesis of retinal disease by assessing the expression profile of key complement components in two distinct rodent models: acute degeneration induced through exposure to excessive light, and chronically using a degenerative rodent strain (P23H). **METHODS:** In the acute model, SD rats were exposed to 1000lx of light for up to 24hrs, after which some animals were kept in dim-light (5 lux) to recover. At specific time points during and following exposure, animals were euthanized and retinas processed. In the chronic model, degenerative P23H rats and non-degenerative SD rats of similar ages were euthanized and retinas processed for comparative analysis. The expression of complement component genes (C1s, C3, and C5), complement receptor genes (C1qR, C3aR, C5aR), and a retinal stress gene (GFAP) were assessed by qPCR (n=3). Photoreceptor cell apoptosis was determined at each time point (n=5) using TUNEL labeling. Statistical significance was assessed using the One-way ANOVA. **RESULTS:** A significant up-regulation ($p<0.0001$) of C3, C1s, C3aR, C1qR, and C5aR was observed during and following the course of light exposure, correlating with significant increases in photoreceptor apoptosis ($p<0.001$) and GFAP up-regulation ($p<0.0001$). Comparison between SD and P23H rats showed modest increases in C3 and C1s expression in the P23H strain, consistent with the slow, persistent degeneration characterized in this model. **CONCLUSION:** While the degenerative stimuli in both models differ, the increased expression of key complement components – which fuel the complement cascade – in conjunction with increasing photoreceptor death provides evidence for a common pathway in retinal degeneration involving the activation of complement.

POS-TUE-139

ANTI-INFLAMMATORY EFFECT OF 670NM LIGHT IN WHITE LIGHT-INDUCED PHOTORECEPTOR DEGENERATION

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Purpose: The aim of this study is to assess the long term effect of 670nm red light in modulating inflammatory response in retinas damaged by exposure to white light. **Methods:** Young SD rats were exposed to bright continuous light (BCL) for 24 hours. Animals were divided into 3 groups (n=8 per group). The first group was treated with 670nm red light (NIR) at 10J/cm^2 using an LED array 1x daily for 5 days prior to light exposure (pre-treatment). The second group was treated immediately after the cessation of BCL for 5 days (post-treatment). The third group was treated 1 day prior to BCL, then 2x daily during and immediately after BCL (mid-treatment). Retinal function was evaluated 1week and 1month after light exposure using ERG. Cell damage was assessed using classical histology and TUNEL. Immunohistochemistry, Western Blot and qPCR were performed to assess localisation and regulation of activated microglia and complement components. **Results:** Population of photoreceptors was maintained in all NIR treated groups compared to the non-treated animals ($p<0.01$). The photoreceptor function was only reduced by 10-30% in pre- and mid-treatments groups compared to the 80-90% loss in non-treated retinas ($p<0.01$). The post treatment group showed functional damage at 1week but recovered to 80% of baseline by 1month ($p<0.01$). The invasion of activated microglia/macrophages was prominent in non-treated but was not observed in the NIR treated retinas. Complement activation was also reduced in treated animals compared to non-treated groups. **Conclusions:** Present results suggest that NIR treatment may ameliorate the effect of damaging light thereby providing protection and long term stability of the retina against light-induced degeneration.

POS-TUE-140

RATIONALE FOR NON-INVASIVE TREATMENT OF ROP: DARK REARING MINIMISES VASO-OBLITERATION DURING HYPEROXIA AND MIMICS PHYSIOLOGICAL VASCULARATION

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Preventing hyperoxia-induced regression of retinal vasculature via down regulation of VEGF could preclude the onset of ROP. We tested our hypothesis that dark rearing minimises vaso-obliteration of retinal vessels during hyperoxia as it causes a metabolic sump via depolarising photoreceptors. 10 litters of SD pups were raised in the dark and normal light under hyperoxic conditions (60% and 75% oxygen) from P0-P4 and placed in room air from P4-P8. Blood vessel density and vessel stability were assessed using anti-SMA, NG2, CD39, S100 and GS lectin,. Quantitative PCR was used to evaluate levels of VEGF expression. Retina of dark reared rats raised in room air had significantly greater blood vessel density compared to age matched controls (39.4±4.2 vs. 50.8±2.4, $p<0.05$). When combined with 60% oxygen, vascular density showed no statistical difference compared to pups raised in normal light and room air (39.4±4.2 vs. 38.0±1.2, $p>0.05$). When dark rearing was combined with 75% oxygen, oxygen flux from the arterial oxygen tension exceeded the increased metabolic demands from photoreceptor depolarisation, resulting in significant vaso-obliteration (33.8±1.6, $p<0.05$). When neonates returned to room air, dark reared and 60% hyperoxia retina showed near normal mural cell ensheathment whereas at 75% oxygen, abnormal preretinal neovascular formations were seen. Quantitative PCR suggests that dark-rearing modifies levels of VEGF expression. When dark rearing was combined with hyperoxia, physiological growth was mimicked, in terms of vessel density and mural cell ensheathment. Given the non-invasive nature of our treatment, successful application of dark rearing could have an enormous benefit on the visual outcome of premature infants.

POS-TUE-141

THE MAMMALIAN EYE AVERAGES COMPETING DEFOCUS

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AIMS. Myopia (short-sightedness) can be induced or retarded in animals with spectacle lenses. Minus lenses cause the eye to accelerate its growth and become myopic, while plus lenses have the opposite effect. Using a concentric Fresnel lens with simultaneous positive and negative defocus, we studied whether the mammalian eye can integrate opposite signs of defocus. **METHODS.** 65 guinea pigs raised in a 12/12hr light-dark cycle wore a lens on the right eye from 4-15 days of age. In different groups, the power of the lens either varied in consecutive concentric rings (fresnel dual-power: +5/-5D, 0/-5D, or 0/+5D) or was the same power throughout (single vision: +5D and -5D). Control animals wore only the lens spacer (SP) without any lens. Refractive errors and axial dimensions of the eyes were measured respectively using retinoscopy and ultrasound A-scan after 5 and 11 days of lens-wear. **RESULTS.** Animals wearing -5D lenses became myopic and elongated, while those wearing +5D lenses became hyperopic and relatively shorter. The difference in refractive error between the lens-wearing and fellow-eye for the groups +5D, 0/+5D, +5/-5D, SP, 0/-5D and -5D were 2.73D, 0.48D, -0.72D, -0.09D, -1.58D and -5.68D respectively. Their mean interocular ocular lengths were -0.054 mm, -0.018 mm, 0.041 mm, -0.020 mm, 0.031 mm and 0.073mm respectively. Thus, the dual-power lenses induced less refractive error compensation compared to the corresponding single vision lenses, and competing plus and minus defocus was averaged by the eye. **CONCLUSIONS.** The mammalian eye can integrate opposite optical signals to modulate its growth and refractive error. It implies that optimally designed dual-power lenses will be able to inhibit myopia progression in humans while still providing clearly corrected vision.

POS-TUE-142

ONE EXPOSURE PER DAY TO HYPEROPIC BLUR CAUSES MYOPIA

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AIMS Myopia occurs when the eyeball is too long for the power of its optics, so that images are short-focussed. It can be induced if a young growing eye wears a negative spectacle lens which creates hyperopic defocus. The eye compensates by elongating excessively to cancel the defocus. When the lens is removed, the eye is myopic. Using this paradigm, we determined the amount of hyperopic defocus required to induce myopia in the mammalian eye. **METHODS** 136 guinea pigs wore a -4D lens worn on one eye for 12 days. Lenses were worn continuously (n=9), or with an intermittent light cycle designed to vary the exposure (15 min and 1 hr) and signal decay periods (0.25, 1, 2, 4, 6, 23, 35, or 47 hrs of darkness between episodes). Refractive error and ocular dimensions were compared between the two eyes at the end of the lens-wearing period. **RESULTS** Eyes wearing a -4D lens continuously, elongated by 50 µm and developed -4.3D of relative myopia. Significantly more growth (p= 0.02) was induced with one hr exposures every 4 hrs (120 µm growth, -5.1D). Eyes no longer became myopic when the periods between defocus exposures were greater than 42 hrs (18 µm growth, -0.5D). Just 15 minutes or one hr of defocus per day was enough to cause the eye to become myopic (50 µm or 100 µm of growth and -2.9D or -2.7D respectively). **CONCLUSION** The mammalian eye only needs one exposure period per day of hyperopic defocus to induce myopia. This implies that the retinal signal underlying myopia is sustained for long periods, and suggests that if the human eye has brief regular exposures to hyperopic defocus it may induce myopia.

POS-TUE-143

REGIONAL VARIATION IN SENSITIVITY TO FORM DEPRIVATION MYOPIA IN THE GUINEA PIG

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Purpose: Myopia (short-sightedness) can be induced by depriving a growing eye of patterned vision (form deprivation, FD). We have developed a guinea pig mammalian model of FD, and determined whether all parts of the visual field were equally susceptible to FD. Methods: Guinea pigs (n=26) were either raised normally or with a diffuser worn over one eye from 7-14 days of age to induce FD. At 14 days of age, refractive error was mapped in the center of the pupil, and off-axis in the superior (S), inferior (I), temporal (T) and nasal (N) visual fields (VF) under cycloplegia. Eye shape was analysed from digital images of frozen sections in both horizontal and vertical planes. Results: Untreated animals had more myopia in the I-VF and were hyperopic in the S-VF (I,0.7±0.5D; S,6.5±0.3D; N,2.3±0.3D; T,3.0±0.3D; C,2.7±0.4). FD eyes developed central myopia (-7.6±0.7D) and axial elongation (142±29um) relative to their fellow eye. Peripheral regions varied in their sensitivity to FD, with less myopia and elongation in the N- and T-VF, high sensitivity in the I-VF while the S-VF was resistant to change (N,-3.5±0.7D; T,-3.7±0.6D; S,-0.5±0.7D; I,-6.3±0.9D). This variation was correlated with the difference in the vitreous chamber depth from frozen sections (central; 142±29um Vs. N, 12±19um; T, 60±12um; I, 112±19um; S, 48±17um). The sclera perimeter also grew more than twice as much in the retinal area corresponding to the I- than the S-VF (180 Vs 80 um). Conclusion: Peripherial retinal regions vary considerably in their sensitivity to FD. We speculate that the resistance of the superior VF to FD may be related to either the hyperopic set-point and/or the preponderance of blue cones in this retinal region.

POS-TUE-144

FORM DEPRIVATION INDUCES MYOPIA AFTER OPTIC NERVE SECTION IN THE MAMMALIAN EYE

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Purpose: Myopia develops when a growing eye is deprived of patterned vision (form deprivation, FD). We have developed a mammalian model of myopia using the guinea pig, and ask: Is FD myopia possible when the eye is disconnected from the brain by optic nerve section (ONS)? **Methods:** 15 guinea pigs underwent ONS or Sham surgery at 4-5 days of age. 3 days later, a diffuser was worn on the right eye for 2 weeks. Nine additional animals underwent ONS but did not wear a diffuser. Refractive error and ocular parameters were measured before, during and after FD (at 8, 15 and 22 days of age), and 18 and 33 days after diffuser removal. Retinas were processed for retinal cell degeneration at the completion of the experiment. **Results:** Sham eyes responded like normal eyes to FD (1 week: -4.9D; 2 weeks -5.5D). FD ONS eyes developed excessive myopia (1 week: -7.5D: 2 weeks: -8.5D), some of which could be accounted for by the surgery since ONS alone induced a small amount of myopia (-1.3 to -2.9D). All eyes recovered from their myopia when the diffusers were removed, losing 6.0D (Sham) and 4.9D (ONS) after 18 days with almost complete recovery by 33 days. Severe degeneration of the retinal ganglion cells was found 60 days after ONS. **Conclusions:** Mammalian eyes can respond to FD without the optic nerve, suggesting that acceleration of eye growth from abnormal visual input depends on visual activity intrinsic to the retina. Recovery from FD myopia is also independent of the brain and the retinal mechanisms do not require functional retinal ganglion cells.

POS-TUE-145

PERMANENT FUNCTIONAL REORGANIZATION OF RETINAL CIRCUITS INDUCED BY EARLY LONG-TERM VISUAL DEPRIVATION

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Purpose: Light deprivation during developmental critical period modifies the response to light stimuli in the retina but is reported to be mostly reversible if visual experience is restored before the end of critical period. To determine if dark-rearing induces permanent rewiring of retinal networks, we characterized its effects on the receptive field (RF) organization and response strength in retinal ganglion cells (RGCs) in controls and in rats born and raised in complete darkness during the critical period and then returned to normal environment (DR/R). **Methods:** Rats were born and raised in complete darkness for 2-4 months and returned to normal circadian rhythm for at least 2-8 months. Rats of matching age were used as controls. Retinas were dissected under infrared light and maintained in oxygenated Ames medium. Light-evoked responses elicited with spots of different diameters were recorded from RGCs in whole-mount retinas either in voltage-clamp or in current-clamp configuration. **Results:** The analysis of area-response function in controls (n=14 cells) and in DR/R animals (n=10 cells) shows a reduction in both the response strength and their RF size ($p<0.01$). The ratio of inhibition-to-excitation for control (n=8 cells) and DR/R animals (n=13 cells) was significantly different ($p<0.05$). The spatial distribution of excitatory inputs in controls is similar to the one of DR/R animals, while inhibitory inputs from DR/R are spatially disorganised compared to control animals. **Conclusions:** These results show that early visual experience is critical for the refinement of retinal circuits, and suggest that abnormal visual experience during the critical period impacts on retinal network and consequently on vision.

POS-TUE-146

FEATURES OF THE HUMAN ROD BIPOLAR CELL ERG RESPONSE DURING FUSION OF SCOTOPIC FLICKER

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The ability of the eye to distinguish between intermittently presented flash stimuli is a measure of the temporal sensitivity of vision. The aim of this study was to examine the relationship between summation of the human rod bipolar cell response (as measured from the scotopic ERG *b*-wave) and the psychophysically measured critical fusion frequency (CFF). Stimuli consisted of dim (~0.35 Rh* per rod), blue flashes presented either singly, or as flash pairs (at a number time separations, betweewn 5 - 200 ms). Single flashes of double intensity (~0.70 Rh* per rod) were also presented as a reference. Visual responses to flash pairs were measured via (1) recording of the ERG *b*-wave, and (2) threshold determinations of the CFF using a two-alternative forced-choice method (flicker vs. steady illumination). Participants were healthy adults, with normal or corrected-to-normal visual acuity, from whom informed written consent was obtained. The results of this experiment suggest that ERG *b*-wave responses to flash pairs separated by < 100 ms undergo response summation, consistent with the threshold for the CFF; while those of shorter duration (< 50 ms) may be electrophysiologically similar to presenting a single flash of double the intensity. In conclusion, the visual system's ability to discriminate between scotopic stimuli may be determined by the response characteristics of the rod bipolar cell, or before, by the rod photoreceptor itself.

POS-TUE-147

SPATIAL AND TEMPORAL STIMULUS VARIANTS OF MULTIFOCAL PUPILLOGRAPHIC PERIMETRY

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Purpose: To investigate pupillary responses of dichoptic multifocal pupillography to spatial and temporal stimulus variants in normal subjects. **Methods:** Peak pupillary constriction amplitudes, time to peak and width of contractions were analysed for 29 normal (mean age 70.9 ±6.0) subjects with 4 different stimulus protocols. Stimuli were presented dichoptically and pupil responses were measured concurrently. All protocols presented multifocal stimuli with a dartboard layout having 24 or 44 independent test regions/eye with a mean presentation interval of 1 or 4 s/region and a presentation duration of 33 ms, subtending ±15° of visual field. Luminance of the stimulus regions was 250 cd/m^2 and background 10 cd/m^2. **Results:** Stimuli presented in a 24 region layout with a 4 s/region presentation rate achieved the largest mean amplitude, shortest time to peak and response width. These measures were compared with a reference stimulus which consisted of a 44 region array at 1 s/region presentation rate. Relative to this reference, the 24 region 4 s/region presentation rate increased amplitudes by 3.5x (b = 5.56 dB); and decreased latencies by 76.0x (b = 18.81 dB); and width of responses decreased by 24.5x (b = 13.89 dB), all with $p<0.00001$. Median signal to noise ratios expressed as Z-scores of a normal distribution per protocol ranged from 1.73 to 3.15 with 4 s/region presentation rates achieving the highest Z-scores. **Conclusion:** Long duration stimulus presentation rates with low resolution layouts produce the largest effect on amplitudes, time to peak, and response widths.

POS-TUE-148

PUPILLARY RESPONSES TO BLUE STIMULATION USING MULTIFOCAL PUPILLOGRAPHIC OBJECTIVE PERIMETRY

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Purpose: To assess the effect of stimulus luminance and duration on pupillary responses of normal subjects using blue multifocal pupillographic objective perimetry (mfPOP) stimuli. Slow blue stimuli are of interest given the possible contribution of melanopsin containing retinal ganglion cells to mfPOP responses. **Methods:** Five normal subjects were tested in two experiments (n=1, n=5) comprising 13 stimulus protocols and two different stimulus layouts (24 regions, 44 regions). The characteristics of pupillary response waveforms to various combinations of stimulus durations (750-2667ms) and luminances (40–80cd/m^2) were investigated. Effects were quantified using a multivariate linear model. **Results:** Longer stimulus durations resulted in significantly longer times to peak of the contraction (n=1: 1.34ms delay per 100ms increased duration, $t(838) = 5.73$, $p<.0001$). Longer stimulus durations also resulted in small but significant reductions in pupil contraction amplitudes (n=1: -0.24µm per 100ms increased duration, $t(838) = -2.68$, $p<.01$). Increased luminance produced significantly larger amplitudes (n=1: 2.55µm per 10cd/m^2, $t(838)=5.73$, $p<.0001$; n=5: 4.74µm per 10cd/m^2, $t(3475) = 9.45$, $p<.0001$) as well as significantly shorter times to peak (n=5: -8.3ms per 10cd/m^2, $t(3475) = -14.79$, $p<.0001$). Response waveforms at longer pulse durations displayed a sustained component which ended with a small contraction. The period between response onset and the offset contraction corresponded to the stimulus duration in each case, indicating that the integration time of the pupillary system had been exceeded. Contraction amplitudes were much smaller or not detectable to stimulation of the parafoveal retina. **Conclusions:** Pupillary responses to blue multifocal stimuli vary with changes in stimulus luminance and duration. The largest, fastest contractions were obtained to brighter, shorter stimuli.

POS-TUE-149

ALTERED IPSILATERAL TOPOGRAPHY, INDUCED BY THE DELETION OF TEN-M3, LEADS TO THE EMERGENCE OF NOVEL OCULAR DOMINANCE DOMAINS IN MICE

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The visual system is characterised by a precise, topographically appropriate representation of the visual field. We recently showed that deletion of Ten-m3 causes mismapping of the ipsilateral terminals in the thalamus, and is associated with deficits in visual behaviour. Notably, monocular-inactivation rescues these deficits. Transneuronal tracing showed that ipsilateral inputs map aberrantly to the medial, typically monocular, region of primary visual cortex (V1) in Ten-m3 knockout (KO) mice. Terminals were grouped in clusters that spanned V1 (n=4), different to WT (n=4) where a single patch of terminals was always seen in lateral V1 ($p<0.01$; Multivariate ANOVA). Immunohistochemistry for c-fos in monocularly-inactivated mice revealed discrete clusters of ipsilaterally-driven cells in medial V1 of KOs (n=6) but not in WTs (n=6, $p<0.01$, Multivariate ANOVA). Clusters of low reactivity were seen contralaterally in KOs. In vivo single-unit recordings revealed that single V1 neurons receive disparate inputs via each eye in KOs; the mean separation of receptive fields (25.9° ± 3.7°, median 18.0°; 32 cells) was significantly increased compared to WT (9.5° ± 2.2°, median 0°; 25 cells; $p<0.01$; Mann-Whitney U-test). A significant shift in the monocularity index ($p<0.01$, Kolmogorov-Smirov test) was also observed. Intrinsic optical imaging revealed complementary regions of strongly ipsilateral or contralateral drive in V1, consistent with an increase in monocularity. We propose that the subcortical ipsilateral mismapping drives the emergence of an ocular dominance structure in Ten-m3 KOs.

POS-TUE-150

ANATOMICAL AND PHYSIOLOGICAL CHANGES IN THE VISUAL SYSTEM FOLLOWING DAMAGE TO THE PRIMARY VISUAL CORTEX (V1) EARLY IN LIFE

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Injury to the primary visual area (V1) is common following hypoxia, traumatic brain injury and stroke in humans. Although some sparing of visual functions occurs in adults, a more robust recovery occurs in neonatal humans and nonhuman primates, which suggests that the brain loses its plastic capabilities with age. To assess this, we identified anatomical, cellular and functional alterations in extrastriate areas of the visual cortex following neonatal (PD10, n=2) and adult (4 yrs, n=2) V1 unilateral lesions in the marmoset monkey (*Callithrix jacchus*). Using immunohistochemistry, we were able to identify changes in a subset of excitatory neurones by their expression of nonphosphorylated neurofilament, and two subsets of inhibitory interneurones that express either calbindin or parvalbumin. Functional visual activity was determined by detecting the expression of the Fos protein, potently activated by neural stimulation. Following V1 lesion, changes in both excitatory and inhibitory neurones in the contralateral V1, second visual area (V2) and middle temporal area (MT) were detected, suggesting a role for transcallosal projections. The ipsilateral hemisphere of the neonatal animal closely resembles the control, demonstrating a high capacity for plasticity. More importantly, we detected alterations in the adult lesioned animal, revealing that the adult still retains some plastic capabilities. Using the Fos protein, we have been the first to show changes in the activity of excitatory and inhibitory interneurones following a V1 lesion in MT of nonhuman primates. These findings highlight the role of both excitatory and inhibitory neurones in plasticity following a cortical injury and suggest that alterations in their regulation have the potential to re-induce developmental plasticity in the adult brain.

POS-TUE-151

A MODEL FOR SIGNAL PROCESSING IN PRIMARY VISUAL CORTEX

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Aim. Existing models of primary visual cortex describe orientation selectivity, direction selectivity, and complex responses, in individual cortical cells. The models tell us less, however, about population properties. Our aim is to produce a mathematical model for primary visual cortex that not only yields cortical cells with these fundamental properties, but also explains the diversity of behaviour across the population. **Methods.** The model consists of four sub-cortical stages - photoreceptors, bipolar cells, ganglion cells, and lateral geniculate nucleus - and three cortical stages. There are two sub-cortical channels, one relaying off-centre signals and the other on-centre signals. Each cortical stage comprises a rectangular grid of neurons with spacing substantially smaller than the distance between the two geniculate inputs. The input to each stage is convergent, with a Gaussian spread of synaptic weights. Each neuron in the model is a low-pass temporal filter implemented by one differential equation; the output of cortical neurons is rectified. **Results.** The model simulates the direction selectivity by assuming that signal processing in one sub-cortical channel is slower than in the other. This also results in a range of selectivities across cells. The resulting cortical cells have elongated on- and off-subfields resulting in orientation selectivity. The orientation tuning curve also confirms this property. Responses in cortical stage 1 are simple-like, in that they are strongly modulated by a drifting grating, and the responses become progressively more complex-like in stages 2 and 3 because of signal rectification at each stage. **Conclusion.** This model, representing one cortical column, simulates three fundamental properties of primary visual cortex. It also shows the diversity of direction selectivity and the origin of complex-like behaviour.

POS-TUE-152

MULTIPLE LINEAR REGRESSION FOR ANALYSING CORTICAL RECEPTIVE FIELDS: BETTER THAN YOUR AVERAGE SPIKE-TRIGGERED AVERAGE

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Simple and complex cells are the two dominant classes of neuron found in the primary visual cortices of mammals. These cell types can be distinguished by the way they spatially and temporally integrate contrast stimuli that are both brighter (ON stimuli) and darker (OFF stimuli) than the mean background - their receptive field (RF) properties. For example, simple cells have spatially distinct ON and OFF zones whereas complex cells have ON and OFF zones that mostly overlap. Simple cells have linear RF properties that are analytically easier to evaluate than the nonlinear RF properties of complex cells. Here we demonstrate a novel white noise technique that enables previous linear analyses to be extended to evaluate the ON and OFF RFs of complex cells. We recorded extracellular responses from complex cells (n=8) in cat visual cortices (areas 17 and 18) to pseudorandom stimuli and estimated their RFs using the extended spike-triggered average (STA) and multiple linear regression (MLR) methods. We found that in all cells, the correlation coefficient between the measured response and the response predicted using the extended MLR method was higher than it was for the extended STA method. Furthermore, the correlation coefficients for the extended MLR method are comparable to more complicated techniques such as spike-triggered covariance (STC). We suggest that given the ON and OFF segregation in the visual pathways of mammals, the two linear RF subunits (ON and OFF) produced by our novel method are more biologically plausible than the multiple linear subunits produced using the STC method.

POS-TUE-153

RESOLUTION OF V1 AND V2 ACTIVITY IN HUMAN VISUAL CORTEX BY INTEGRATION OF MULTIFOCAL FMRI, EEG AND MEG NEUROIMAGING

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Distinguishing the contribution of cortical areas in evoked response studies in human neuroimaging is made difficult due to the close proximity of the areas involved, the complex folding of the cortical sheet, and the largely overlapping time-course of responses. We used multifocal methods we have now developed [1, 2] to image activity in visual cortex of six subjects separately in three modalities, each with an identical spatial layout stimulating 60 regions of the visual field. Regions were concurrently stimulated, in a block design for FMRI [2] and in a pattern-pulse design for EEG and MEG recording, similar to [1]. Coregistration of FMRI activation volumes [2] with high resolution anatomical scans gave the mapping of areas V1 and V2 on the cortical sheet for each subject. EEG responses on 74 channels (BioSemi) and MEG on 306 channels (Neuromag) were decomposed to estimate elementary responses for each region using multiple linear regression [1]. Source currents within V1 and V2 were estimated using a novel equivalent normal vector method integrating over curved patches of cortical sheet, to give waveforms of current dipole density per unit area. V1 downward current peaked at 80-95ms, while V2 current had a prominent upward peak earlier, at 70-80ms. Peak dipole current density estimated from the independent methods of EEG and MEG corresponded remarkably closely, at 0.2-0.3 nAm/sqmm. 1. James AC, 2003. The pattern-pulse multifocal visual evoked potential. IOVS, 44(2), 879-890. 2. Vanni S, Henriksson L and James AC, 2005. Multifocal FMRI mapping of visual cortical areas. Neuroimage. 27(1):95-105.

POS-TUE-154

THE FOVEAL CONFLUENCE IN PRIMATE, INVESTIGATING, MODELING, EXPLAINING

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Background: A basic organizational principle of the primate visual system is that it maps the visual environment repeatedly and retinotopically onto cortex. Simple algebraic models can be used to describe the projection from visual space to cortical space not only for V1, but also for the complex of areas V1, V2 and V3. Typically a conformal (angle-preserving) projection ensuring local isotropy is regarded as ideal and primate visual cortex is often regarded as an approximation of this ideal. Using high resolution fMRI (1.2x1.2x1.2mm) we demonstrated systematic deviations from this ideal that are especially relevant in the foveal projection (*Schira et al. J. Neurosci. 2009*). Here we present and investigate a simple algebraic model that accurately predicts the observed data. **Methods/ Findings:** The retino-cortical map can be optimized towards a space-conserving homogenous representation or a quasi-conformal mapping. The latter would require a significantly enlarged representation of specific parts of the cortical maps, which is not supported by empirical data. Further, the recently published principal layout of the foveal singularity cannot be explained by existing models. We suggest a new model that accurately describes foveal data, minimizing cortical surface area in the periphery but suggesting that local isotropy dominate the most foveal part of the projection at the expense of additional cortical surface. **Significance:** The foveal confluence is an important example of the detailed trade-offs between the compromises required for the mapping of environmental space to a complex of neighboring cortical areas. Our models demonstrate that the organization follows clear principles that are essential for our understanding of foveal vision in daily life.

POS-TUE-155

DEVELOPMENT OF THE SPECIALISATION OF CENTRAL PRIMATE RETINA

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Purpose: Three overlapping phases of development characterize the morphological specialization of the macula: (1) ganglion cell (GC) axon pathfinding in the retina; (2) definition of the foveal avascular area, and (3) retinotopic mapping onto visual targets. We aimed to identify candidate genes with roles in these different phases. **Methods:** We carried out a microarray analysis, using human fetal RNA at 19-20 weeks' gestation (n=4), to identify genes differentially expressed in the macula and confirmed expression by quantitative RT-PCR (QPCR) and by *in situ* hybridisation, using macaque retinas aged between fetal day 55 and adulthood. **Results:** Gradients of mRNA expression in the GC layer were observed for the axon guidance genes EphA6, unc5h4 and netrin G1, which changed over time. EphA6 was highly expressed in the macula during fetal life and levels of expression in the macula increased postnatally. Netrin G1 was highly expressed early in fetal life, but decreased postnatally. Un5h4 was highly expressed in the macula during formation of the avascular area, but was low in early development and postnatally. The anti-angiogenic factors pigment epithelium-derived factor (PEDF) and brain natriuretic protein (BNP) were highly expressed in the macula during development and postnatally. **Conclusion:** Changing levels of expression of these genes in the macula during pre- and postnatal life suggests they have sequential roles in the three phases of development. The data suggest that EphA6 regulates vascular patterning early in development and characterises the projection from foveal GC in the postnatal phase. The findings give insight into how the characteristics of the macula may have evolved.

POS-TUE-156

FACILITATION IN HYPERACUITY OF DRAGONFLY HYPERCOMPLEX NEURONS

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We recently identified similarities in processing of target motion by STMD (small target motion detector) neurons in the insect lobula complex (3rd optic ganglion) and hypercomplex cells in the mammalian cortex [1]. One surprising feature is that such neurons achieve selectivity for small features at the limits for eye resolution. We recently argued that this is achieved through powerful mechanisms of local inhibition [2,3]. However, 'target hyperacuity' (the response to 'sub-pixel' features) requires neural mechanisms to amplify very low contrasts in the image (<1%), i.e. extremely high contrast gain. How this is achieved whilst maintaining lack of spontaneous activity remains poorly studied, but our modelling suggests that such a mechanism is a pre-requisite for reliable detection once receptor noise is accounted for. One possibility is that gain is facilitated by higher order interactions between local motion detecting elements. To test this, we used recordings from dragonfly STMD neurons and single target stimuli that either drifted through the receptive field, or which began at discrete locations within it. By normalizing responses for the receptive field shape (determined with the drifting stimulus) we show that responses are facilitated by very slow mechanisms. Best fits to data for one identified neuron (CSTMD1) suggest time constants for this facilitation on the order of 300 ms (T_{50}=217ms, N=4) - several times slower than the neural delay intrinsic to local motion detection. [1] Nordström & O'Carroll (2009) Trends in Neurosciences 32: 383-391 [2] Wiederman et al. (2008) PLoS ONE, 3, 7, e2784 [3] Bolzon et al. (2009) Journal of Neuroscience (in press).

POS-TUE-157

RECLASSIFICATION OF SIMPLE AND COMPLEX CELLS IN THE PRIMARY VISUAL CORTEX OF THE CAT

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Ever since Hubel and Wiesel noted that neurons in the primary visual cortex fit into two distinct cells types (simple and complex) it has been standard for authors to divide their cell populations into those two groupings. Neurons are most often divided into simple and complex cells based on their responses to drifting gratings. Current quantitative techniques decompose the response into its mean (F0) and the first Fourier component (F1), which oscillates at the temporal frequency of the grating. The cell is then classified as simple if F1/F0>1 or complex if F1/F0<1. We show that in an ideal model of a complex cell both the mean and variance of the F1/F0 ratio increase as the spike count decreases. We show analytically that the expected F1/F0 ratio for a cell that spikes n times in a grating cycle is 2/sqrt(n). A new quantitative classification technique is presented in which cells are classified as simple based on their relationship between spiking and F1/F0. We test this new definition with a large sample (n=468) of neurons in the primary visual cortex of the cat, demonstrating that there is a clear relationship between cell classification and the neuron's laminar location that is more clearly revealed using this new classification scheme. Specifically, simple cells are predominantly found within deeper laminar when examined using the new definition, and relatively evenly distributed across layers when the spiking of the cell is not used in the classification of the cell. As our technique only requires extracellular recording and uses moving sine wave gratings, to which cortical cells respond very strongly, we believe that it should be considered for adoption as the standard classification technique in cortex.

POS-TUE-158

CONTRAST RESPONSE FUNCTIONS FOR GRATINGS AND PLAIDS IN HUMAN VISUAL CORTEX

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How do visual systems code the contrast of different patterns? A recent intrinsic signal optical imaging study in tree shrew showed surprisingly that the population response of V1 to plaid patterns comprising orthogonal grating components of equal contrast is predicted by the average of the responses to the individual components (MacEvoy *et al.*, 2009). This prompted us to compare responses to plaids and gratings in human visual cortex as a function of contrast. We used fMRI at 3T to measure the BOLD response of retinotopically-defined regions in 8 subjects. We found that the responses of areas V1-V3 to a plaid comprising superposed orthogonal grating components of equal contrast were on average 15-30% higher than the responses to a single grating of the same contrast as the components. However, the response to a plaid was predicted to within 4% by the response to a grating of twice the contrast of the plaid components. These data show that in humans the fMRI BOLD response of early visual cortex to plaid patterns is not the average of the response to the components. Instead, the population response to a given pattern appears to depend on the contrast energy within that pattern regardless of whether that energy is distributed over one or more orientations. Reference: MacEvoy SP, Tucker TR, Fitzpatrick D. (2009) A precise form of divisive suppression supports population coding in the primary visual cortex. *Nat Neurosci.* **12**:637-645.

POS-TUE-159

STRUCTURE OF EXTRA-CLASSICAL RECEPTIVE FIELDS OF NEURONES IN CAT'S AREA 18

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The 'hypercomplex' cells of the mammalian visual cortex were originally described as complex cells with silent, suppressive regions on one or both ends of the discharge (spike-generating) field, along the axis of a cell's optimal orientation[1]. **Purpose:** To examine the spatial structure of silent, extra-classical receptive fields (ECRFs) and its relation to the 'hypercomplexity' of single neurones in the parastriate part (area 18, V2) of cat primary visual cortex. **Methods:** Single neurones, recorded from area 18 of anaesthetized and immobilized adult cats, were identified on the basis of the ratio of the phase-variant (F1) component to the mean firing rate (F0) of their spike-responses to patches of optimised, achromatic sine-wave gratings drifting through their receptive fields (simple: F1/F0>1; complex F1/F0<1). **Results:** The majority of cells tested (26/34;18 of them, simple[2]) could be identified as 'end-stopped' since presentation of patches of gratings restricted to the silent subregion(s) along the axis of the optimal orientation, reduced substantially (>30%) the magnitude of responses to the gratings restricted to the spike-generating regions. However, in the great majority of these (22/26), suppressive subregions were not confined to the regions along the axis of optimal orientation (cf. 'higher-order' hypercomplex cells[1]). **Conclusions:** The higher-order hypercomplexity of area V2 cells does not necessarily imply the higher-order status of the area[1]. Indeed, the ECRFs of most area 18 neurones, like the ECRFs of their afferent neurones in the dorsal lateral geniculate nucleus, appear to completely surround the discharge regions.
[1]Hubel DH and Wiesel TN (1965) J. Neurophysiol., 28, 229-289. [2] Dreher B (1972) Invest. Ophthalmol., 11, 355-356.

POS-TUE-160

DISTINCT MECHANISMS UNDERLYING THE EFFECT OF VISUAL MOTION ON PERCEIVED POSITION

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As the visual system suffers from substantial neural latencies (~100 ms), by the time a moving object is processed, its position will have changed significantly. To explain various illusions, a popular theory is that the brain shifts perceived position of moving objects in the direction of its motion to overcome neural latencies. For latency compensation, shifts should increase with speed. The flash-lag illusion – where a flash is perceived to lag a moving object despite being aligned – does increase with speed. However, this is also consistent with an attentional shift unrelated to compensation. We investigated the speed dependence of a related illusion (flash-drag), where nearby irrelevant motion shifts the perceived position of flashed objects. The flash-drag cannot be explained by an attentional shift, so its speed dependence is a critical test of compensation theories. EXPERIMENTS. Dots above fixation moved to the right while dots below fixation moved to the left. Two flashed bars were presented near each dot field. Consistent with the flash-drag, participants (n=5) perceived the flashes biased in the direction of the nearby motion. Unlike the flash-lag illusion, which increases rapidly over a wide speed range, the flash-drag saturated at a slow 5º/sec. Furthermore, by using a fast speed (9º/sec) and alternating the motion direction of the dots between right and left at slower and slower rates, we found that the flash-drag did not saturate until 1.5 Hz, which suggests that a longer interval of motion (300ms) is used than in the flash-lag effect (80 ms). CONCLUSIONS. As the flash-drag saturates at slow speeds and integrates motion for an extended interval after the flashes, it might not reflect compensation for neural latencies.

POS-TUE-161

DOES THE PULVINAR NUCLEUS CONTRIBUTE TO THE EARLY MATURATION OF THE DORSAL STREAM VISUAL CORTICAL AREAS?

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In the present study, we demonstrate the perinatal development of the retinopulvinar pathway to the middle temporal visual cortical area (MT) and morphological development of the pulvinar nucleus. Marmoset monkeys (*Callithrix jacchus*) aged between embryonic day 130 and 9 months (n=9) were injected with fluorescently conjugated CTb and fast blue in the eyes and left hemisphere area MT, respectively. Following a one week survival period, animals were perfused, their brains frozen and coronal sections processed immunohistochemically and histologically to assist in the demarcation of thalamic nuclei boundaries. Colocalisation of fluorescently labelled contralateral retinal ganglion terminals and area MT relay cells was confirmed using synaptophysin and statistical analyses of resultant confocal images. In the medial nucleus of the pulvinar (PIm) we observed dense labelling of relay cells to ipsilateral MT area. In the adult a small number of these cells were recipient of retinal projections predominately from the contralateral eye whereas younger animals received increased labelling of binocular input to the PIm as well as to neighbouring subnuclei of the pulvinar nucleus. Furthermore, it was confirmed that contralateral retinal afferents formed synaptic connections with labelled area MT relay cells in the PIm. These results provide evidence of the early development of a direct extrageniculate pathway from the retina to area MT early in life, prior to the maturation of all visual cortical areas. Furthermore, these data demonstrate an alternate pathway that may be responsible for the early maturation of the dorsal stream cortical areas.

POS-TUE-162

THE RESPONSES OF MT COMPONENT AND PATTERN CELLS TO TRANSPARENTLY MOVING DOTS

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MT Neurons can be categorized into two types on the basis of their response to drifting plaid patterns; those which respond to the direction of the individual grating components (component cells) and those which respond to the average motion (pattern cells). Here, we investigated how these categories of neurons respond to moving transparent dot-fields. Standard preparation techniques were used (Camp et al, J. Neurosci. 2009, 29(15):5009-5021) for electrophysiological recording from MT in 4 marmosets. Neuronal signals were isolated and their optimal spatio-temporal parameters were quantified. On the basis of their direction tuning to single gratings and to plaids, neurons were categorized into component and pattern cells. Finally the responses of the neurons to 2 transparent fields of moving dots (120 degrees apart), at a range of speeds were measured. The component cells tended to respond optimally to the average direction of the two planes of dot-fields and pattern cells generally responded optimally to the individual dot-fields. Remarkably, this pattern of responding is the reverse of the grating/plaid case. If, however, the speed of the stimulus was far from the optimal speed of the pattern cell, then its direction tuning often resembled that of a component cell. These responses, both to grating/plaid and transparent dot-fields, can be accounted for by the model of Simoncelli and Heeger (Vis. Res. 1998, 38(5):743-761), both at optimal and non-optimal speeds.

POS-TUE-163

PARALLEL SUB-PATHWAYS OF S-CONE SIGNALS TO THE MACAQUE'S MIDDLE TEMPORAL AREA

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We have recently demonstrated (Roy et al., Eur. J. Neurosci., 30, 1517-1526, 2009) that in the macaque, the short-wavelength sensitive cone (S-cone) signals from the retina are sent largely to the koniocellular regions of the dorsal lateral geniculate nucleus (LGN). In the present study, we investigated whether the S-cone signals to the cortical middle temporal area (MT) are relayed via the primary visual cortex (area V1) or reach MT bypassing V1, such as through the direct projections to MT from the koniocellular layers of the LGN (Sincich et al., Nat. Neurosci., 7, 1123-1128, 2004). Twenty-one cells in our sample of MT cells recorded in the anaesthetized and immobilized macaques showed significant responses to S-cone isolating stimuli. We studied their responses before and during reversible inactivation (by cooling) of V1. Twelve of these S-cone input cells showed a significant reduction in the magnitude of response to the S-cone isolating stimuli during cooling. The rest of the cells showed no significant change in response to S-cone stimuli during cooling. The former group, which presumably receive their S-cone signals via area V1 had longer response latencies (81.5 ± 38.3ms) compared to the latter group whose S-cone signals bypass V1 (57.8 ± 29.5ms). This difference in latencies, however, failed to reach statistical significance (Student t test; p=0.12). Our results indicate that the S-cone signals might reach MT via at least two distinct pathways, one through V1 and the other bypassing V1. The alternative route(s) may be via the LGN and/or the pulvinar.

POS-TUE-164

HOW MOTION INTEGRATION BY NEURONS IN PRIMATE AREA MT DEPENDS ON TEMPORAL FREQUENCY

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Some cells in the middle temporal area (MT) of macaque are selective for the overall motion of a visual pattern, while others respond to the motion of the components of that pattern. Yet while MT cells have broad temporal frequency (TF) tuning, it is not known how the motion integration depends on TF. We addressed this by making extracellular recordings from 100 cells in the middle-temporal region of four opiate-anaesthetised adult male marmosets (Callithrix jacchus); direction tuning curves were measured for brief presentations of 50% contrast sinusoidal gratings drifting at each of four TF (3, 6, 12, & 25 Hz), and plaids made of two gratings that drifted at the same TF in directions 120 degrees apart. From these responses we calculated a pattern-selectivity index, and determined how this index evolved over the first 300 ms of response [Smith MA, Majaj NJ, Movshon JA (2005) Dynamics of motion signaling by neurons in macaque area MT. Nat Neurosci, 8:220-8]. We first established that the pattern/component distinction holds in the marmoset, a diurnal New World primate: of cells that could be classified, 37 were component-selective, 17 were pattern-selective, and 5 responded to plaids but not the component gratings. Among individual cells motion integration was generally stable across the TF to which the cell responded – for those that responded robustly to both, the index at 3 Hz predicted that obtained at 25 Hz ($r = 0.84$, $n = 33$) – but motion integration was always more pronounced at low TF. The response of component cells was transient, and component cells could be reliably classified from the very earliest part of their response. The response of pattern cells was more sustained, and it took longer for their response to clearly signal motion integration; this lag was most pronounced at low temporal frequencies.

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POS-TUE-165

ADAPTATION TO SURFACE MOTION PERCEIVED THROUGH TOUCH

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Sustained observation of a moving surface causes adaptation in the human visual system, such that subsequently observed motion appears to be slower. Our goal was to explore the possibility of the same phenomenon of speed adaptation in the tactile domain. To create tactile motion, we used ridged, rotating drums on which participants rested their fingers. After adapting to motion applied to one hand, participants (n = 6) judged the speed of a test stimulus that was a) the same direction as the previously exposed adapting stimulus, and b) the opposite direction to the adapting stimulus. Perceived speed was equally reduced following adaptation in both conditions. That is, the surface appeared to be slower regardless of the direction of the adapting motion. This result contrasts with those from visual experiments, where the direction of the adapting stimulus affects the perceived speed of the test stimulus. The lack of direction selectivity in our results suggests that tactile motion is processed quite differently than is visual motion. The reduction in perceived speed that we observed might be due to adaptation in temporal frequency channels.

POS-TUE-166

AUDITORY EVOKED POTENTIALS IN THE RAT AND THE "TWO-HIT" HYPOTHESIS OF SCHIZOPHRENIA

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Reduced mismatch negativity (MMN) is a robust finding in schizophrenia. It is correlated with other cognitive impairments in patients and with grey matter loss in frontotemporal regions. The goal of the present project is to establish a rodent model based on the "two-hit" hypothesis of schizophrenia and assess the success of the model by the extent to which it produces a reduction in MMN. However, the first step is to assess whether MMN-like activity in the rat meets criteria for identification as MMN, namely, sensitivity to probability effects. Event related potentials to 3 kHz tones were measured over auditory cortex in a freely moving animal (n = 1). The stimulus paradigms allow (1) control for stimulus attributes and extraction of long (100 ms) and short (50 ms) duration MMN, (2) the effects of deviant probability on long duration MMN and (3) investigation of other auditory evoked potentials (AEPs). Preliminary data indicate a negativity around 50 ms after the offset of the longer duration deviant (1600 standard and 400 deviant trials). In addition, we observed four AEPs in non-deviant ERPs from 4800 trials: P10 (latency = 8 ms), N17 (22 ms), P23 (29 ms) and N38 (37 ms). While the preliminary data are encouraging, data need to be collected from more animals.

POS-TUE-167

THE EFFECT OF CONTOUR LENGTH ON THE PERCEPTION OF LOCAL PERTURBATIONS

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It is thought the visual system evolved to optimally process contours (Geisler, Perry, Super & Gallogly, 2001) as well as informative contour irregularities (e.g. junctions and changes in orientation) which are frequently present in natural scenes, (Elder & Goldberg, 2002). However, relatively little is known about how context affects the perception of these irregularities. Here we investigate the effects of contour length on the perception of local contour irregularities. Experiment 1: 12 participants matched the amplitude of a sinusoidal line perturbation of an isolated test-segment to the perturbation amplitude of a match-segment embedded in contours of different predetermined lengths. Embedding the match segment in extended contours was found to cause systematic overestimation of perceived perturbation amplitude (p <.05). Experiment 2: In a 2-alternative-forced-choice task, 15 participants judged whether very small segment perturbations of different amplitudes bowed leftward or rightward of absolute vertical. The task was performed for isolated segments and segments embedded in an extended contour. Surprisingly, detection of the perturbation direction was found to be better for isolated segments than embedded segments (p <.05). The results from Experiment 1 can be explained with a gain control mechanism model similar to that which has been previously proposed to account for the tilt illusion (Schwartz & Simoncelli, 2001). However, Experiment 2 cannot be easily reconciled with this explanation. We discuss the results of Experiment 2 in terms of local versus global processing and relate both experiments to optimal processing.

POS-TUE-168

A COMPARISON OF NEURONAL AND BEHAVIOURAL DETECTION THRESHOLDS IN RAT WHISKER-BARREL SYSTEM

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A fundamental goal of systems neuroscience is to identify the link between neuronal activity and behaviour. The present study employed an animal model the rat whisker-barrel system – to compare the perceptual detection thresholds with the neuronal detection thresholds for a set of vibro-tactile stimuli. The whisker-region of rat somatosensory cortex (barrel cortex) is well-suited for examining the brain's encoding and decoding mechanisms due to its functional efficiency and its well-established anatomic and physiological organization. Here, we delivered sinusoidal whisker vibrations (frequencies of 30 to 100Hz with increment steps of 10Hz; amplitudes of 7.6, 15.2, and 30.4μm) to anaesthetised rats (n=6) while recording extracellularly from barrel cortex neurons. A second group of rats (n=4) were trained in a bahvioural licking paradigm where a plate generated whisker vibrations at varying intervals and water became available at a spout 400ms after the onset of each vibration. The licking-rate during this stimulus presentation phase, prior to reward delivery, was taken as a response in anticipation of reward. As rats learned to detect vibrations, the amplitude and frequency was reduced to measure the behavioural detection thresholds. Our results confirmed that cortical activity encoded the product of amplitude and frequency which is proportional to the mean vibration speed. Neuronal response (spike rate) and behavioural response (licking rate) were both well-fitted by sigmoid curves (cumulative Gaussian). However, neuronal response profiles typically indicated a higher sensitivity compared to the behavioural detection thresholds. Although the lowest intensity stimulus was detected by most of the recorded neurons, none of the rats showed an enhanced licking response to this stimulus. These results suggest a superiority of performance for single cortical neurons over behaviour.

POS-TUE-169

DIFFERENTIAL CORTICAL REPRESENTATION OF INPUTS ARISING FROM THE UPPER ARM

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Purpose: Peripherally-induced pain has a range of qualities that depend on the tissue stimulated. Cutaneous pain is often described as a sharp and/ or burning, well-localised sensation, whereas muscle pain has a dull-aching quality that is poorly localised. Intriguingly, bone pain shares qualities of cutaneous and muscle pain. **Methods:** Three 21-element platinum electrode arrays (provided by Cochlear Ltd.) were used to record the responses of the primary somatosensory cortex to electrical stimulation of the median nerve, the nerve innervating the triceps and/ or biceps muscles and the bone nerve entering the nutrient foramen of the humerus in anaesthetised rabbits (70mg.kg^{-1} α-chloralose, n=5). Electrical stimuli (1mA, 2ms) were applied to each nerve at an inter stimulus interval of ~2sec. **Results:** Cortical representation of bone and muscle inputs was contained within an area identified by stimulation of the median nerve. The positive-going cortical potentials tended to cluster on electrodes around distinct loci. The amplitude and extent of cortical activation varied between inputs from bone and muscle nerves relative to the median nerve (latency 13ms, amplitude 750μV). Bone-evoked cortical responses were observed in the majority of active recording electrodes identified by stimulating the median nerve and were of comparable amplitude but of longer latency (14ms). In contrast, stimulation of the muscle nerve either failed to evoke a cortical response or evoked responses but on fewer electrodes. Furthermore, the responses were of smaller amplitude and longer latency (17ms). **Conclusion:** Consistent with perceptual differences experienced during muscle and bone pain, a more limited pattern of cortical activation was observed following muscle stimulation.

POS-TUE-170

RESPONSES IN PRIMARY AND SECONDARY SOMATOSENSORY CORTICAL REGIONS IN THE CAT TO DUAL FREQUENCY VIBROTACTILE STIMULI APPLIED TO THE GLABROUS SKIN OF THE FOREPAW

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The relative roles of the primary (SI) and secondary (SII) somatosensory regions of cortex in processing vibrotactile stimuli of high and low frequency remain unclear. To investigate this issue further we recorded the spatio-temporal patterns of activation of neurons in the cat SI and SII using multichannel electrodes and data acquisition. Sinusoidal vibrotactile stimuli of 20Hz, 200Hz or combined 20/200Hz were presented at various amplitude combinations to the glabrous skin of the forepaw of anaesthetized cats (n=4). Multi-unit spike activity was recorded from penetrating multi-electrode arrays (100-channel Utah or 64-channel NeuroNexus arrays) inserted into contralateral SI and SII, in each case in the region receiving input from the glabrous skin of the forepaw. The responses recorded in many cases were tuned specifically to either 20 or 200 Hz. However, in ~25% of the responsive electrode sites there was an inhibition of the response to the combined sinusoidal stimulation. In these cases, spiking activity was principally driven by either the 20 or 200 Hz stimulus, and as the amplitude of the other stimulus frequency was increased, the spike count reduced. In ~10% of responsive sites, the dual frequency stimulus produced spike counts greater than the linear summation of spiking activity to the individual frequencies. Inhibitory responses were more common in SI than SII, which is consistent with previous reports that SII provides inhibitory feedback to SI.

POS-TUE-171

A HIGH THROUGHPUT ASSAY TO MEASURE SENSORIMOTOR GATING (PPI) IN DROSOPHILA

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Introduction In every day life, sensory systems are exposed to an incredible amount of information. To protect the brain from being overwhelmed, this information is filtered so only relevant information gets processed and transformed into action. Sensorimotor gating is one such filter mechanism. It refers to the state-dependent regulation of transmission, or gating, of sensory information to motor systems. Impairments in sensorimotor gating, such as pre-pulse inhibition (PPI), have been described in several psychiatric disorders like schizophrenia, autism and ADHD. **Methods** We study sensorimotor gating in *Drosophila* with a high-throughput behavioral paradigm that allows us to measure motor responses to moving visual stimuli in walking fruit flies. Groups of 25-30 flies are loaded into a maze placed over a screen showing moving visual stimuli. Flies move through the maze and pass eight successive left/right choice points after which they emerge in one of nine end tubes where they are counted. The average response is the Optomotor Index (OI). We measured the effect of briefly flashed visual stimuli on OI in two wildtype Drosophila strains: *Canton-S* and *Berlin*. **Results** Flash duration varied from 13-106 ms (n=240 per condition). In both strains, only 40 ms black flashes against a green background significantly reduced OI. Additionally, fly behavior was recorded using custom-made fly-tracking software. During 40 ms black flashes, behavior changed considerably. Flies spend longer in the maze and backtracked more. **Conclusions** We are currently investigating whether this behavioral change can be suppressed with a low intensity visual pre-pulse, in order to create a model of PPI in the fly. The development of such a model could be of enormous use given PPI deficits are considered a viable endophenotype of schizophrenia.

POS-TUE-172

PHYSILOGICAL NOISE IN THE FIRING OF HUMAN MUSCLE SPINDLE AFFERENTS

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Depending on their proximity to blood vessels some muscle spindle afferents may be driven by arterial pulsations. We explored whether a subtle cardiac modulation could be discerned in the background discharge of spontaneously active muscle spindles, which could contribute to their overall discharge variability and hence their capacity to encode length changes. Recordings were made from 11 primary and 6 secondary muscle spindle afferents innervating the pretibial flexors via microelectrodes inserted into the common peroneal nerve of awake human subjects. Pulse-related modulation of discharge was observed in 7 primary and 3 secondary afferents. On average, arterial pulsations could explain 17% (range 4-47%) of the total variance in muscle spindle discharge. The largest modulation within the cardiac circle was observed ~300 ms after the R-wave. The size of the pulse wave modulation correlated neither with mean discharge rate, overall discharge variance nor pulse pressure ($p>0.05$; Spearman's rank correlation). To identify common features of modulation we used principal component analyses. Most of the modulatory pattern features were explained by the first principal component ($p<0.05$): 53% of the modulatory effect was accounted for by a pattern that shared common features between different afferents. The second principal component explained 21% of the variance; this was a modulation of the first mode that captured latency differences across afferents. The eigenvector coefficients indicated that the arterial pulse wave could show inhibitory as well as excitatory effects on discharge variability. We conclude that the spontaneous discharge of human muscle spindle afferents contains significant physiological noise that is determined primarily by mechanical disturbances by local blood vessels.

POS-TUE-173

REACTION TIMES IN AN ATTENTION TASK IS SHORTENED BY MICROSTIMULATION IN POSTERIOR PARIETAL CORTEX

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Electrophysiological studies in macaques have shown attention related responses in a part of the posterior parietal cortex (lateral intraparietal area or LIP), but it is not known whether such neuronal activity affects decision making by the monkey. To demonstrate any causal relationship that may exist between LIP neurones and spatial attention, we applied brief pulses of a biphasic current to a small region of the LIP during a specific period of an attention-demanding delayed match to sample memory task. The task involved the monkey covertly attending to two grating patches presented sequentially for 100 msec each with a delay of 600 msec between them and matching the visual field location and orientation of the two gratings. We reported earlier that LIP neurones show increased coherence and response around the time of presentation of the second grating when the two gratings have the preferred orientation and location (Saalmann et al., Science, 316, 1612-1615, 2007). In the present study, we applied in one monkey microstimulation (20-50 microamp 200 microsecond pulses at 200 Hz) for 200 msec, starting 100 msec before the second grating. In match trials, when both stimuli were presented at the visual field location represented by the site of the stimulating electrode, reaction times were significantly shorter with microstimulation (n= 7 sessions; Paired t test, p=0.007). In trials with stimuli at visual field locations away from the stimulating electrode, there was no difference between unstimulated and stimulated trials (n=7; p=0.90). These results support the idea that activity in LIP may be causally related to focal spatial attention.

POS-TUE-174

STEADY-STATE VISUALLY EVOKED POTENTIALS (SSVEPS) REVEAL ATTENTION-LIKE BEHAVIOUR IN THE FRUIT FLY *DROSOPHILA MELANOGASTER*

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Introduction: Correlates of visual selective attention can be measured in human EEG when subjects are presented with competing visual stimuli tagged by distinct flickering frequencies (“frequency tags”). Typically, transitions in attention are associated with changes in amplitude and coherence in the induced waveforms. How such changes relate to endogenous attentional mechanisms remains unclear. In order to investigate visual attention in a reductionist model, we have developed an SSVEP paradigm in the fruit fly, Drosophila melanogaster. Methods: Local Field Potentials (LFPs) were recorded from the brains of tethered flies presented with competing flickering images on a surrounding LED arena (n > 30 flies). Spectral analyses of fly brain activity were contrasted for frequency-tagged visuals. Results: We found central brain responses to different objects flickering simultaneously at distinct frequencies. These responses displayed temporal dynamics characteristic of attention-like states, notably when salience (novelty or heat) was associated with one or the other flickering object. In general, the amplitude of the more salient (i.e., attended) frequency was increased, and coherence between the attended frequency and endogenous 20-30 Hz oscillations was transiently increased as well. Data from training experiments (n = 10 flies) revealed that operant learning could be restricted to fly brain activity in response to competing visual objects. Conclusion: Our Drosophila SSVEP model offers a powerful approach to dissect fundamental mechanisms of visual selective attention. To more thoroughly explore visual selection and suppression effects in this small brain, we have developed a method of recording from multiple sites throughout successive layers of visual processing in the fly. Together with genetic tools, this strategy should help uncover the neuroanatomy responsible for attention-like processes in the fly brain.

POS-TUE-175

GENETIC CONTRIBUTION TO INDIVIDUAL VARIATION IN BINOCULAR RIVALRY RATE

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Binocular rivalry (BR) occurs when conflicting images are presented in corresponding locations of the two eyes. Perception alternates between the images at a rate that is relatively stable within individuals but that varies widely between individuals. The determinants of this variation are unknown. In addition, slow BR has been demonstrated in bipolar disorder, a psychiatric condition with high heritability (Pettigrew & Miller, 1998, Proc Roy Soc 265:2141-2148; Miller et al., 2003, Psychol Med 33:683-692; Nagamine et al., 2009, Bipolar Disord 11:539-546). The present study therefore examined whether there is a genetic contribution to individual variation in BR rate. We employed the twin method and studied both monozygotic twins (MZ; genetically identical; N=128 pairs) and dizygotic twins (DZ; who share roughly half their genes; N=220 pairs). Twin correlations for BR rate were 0.51 and 0.19, respectively. Genetic modeling showed 52% of the variance in BR rate was accounted for by additive genetic factors. In contrast, non-shared environment accounted for only 18%, with the remaining variance due to measurement error. This is the first study to report such a finding for BR and is also, to our knowledge, the first large study to show a substantial genetic contribution to individual variation in any post-retinal visual processing phenomenon (Wilmer, 2008, Spat Vis 21:561-579). The results suggest vigorous pursuit of genetic and molecular studies of BR, and further characterisation of slow BR as an endophenotype for bipolar disorder.

POS-TUE-176

THE ROLE OF NEURAL SYNCHRONIZATION IN HIERARCHICAL MOTOR CONTROL

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Neural dynamics display oscillatory activity at multiple spatial and temporal scales. Oscillations at large scales are due to synchronized activity in and between smaller subsystems and reflect a reciprocal relationship between such interactive parts and the collective pattern. These interactions can extend over several scales, giving rise to so-called nested oscillations such as phase-amplitude coupling between theta and gamma oscillations. In the present study, we sought to characterize nested oscillations during coordinated, rhythmic motor behavior in two experimental conditions. The first experiment comprised a bimanual tapping task in which participants (n=9) learned to perform a 5:3 polyrhythm during the acquisition of MEG and EMG data. Time-frequency analyses revealed distinct patterns of corticocortical and corticospinal synchronization. Specifically, neural activity between bilateral motor cortices was synchronized at approximately 30 Hz, whereas corticospinal activity was synchronized at 20-25 Hz. Both synchronization patterns exhibited event-related modulations such that the envelope was coupled to the force output of the contra-lateral finger. In the second experiments, EMG data were acquired whilst participants (n=10) tracked a sinusoidal target signal with their centre of gravity. Bilateral EMG coherence between homologous leg muscles was found to occur in two distinct frequency bands. Again, synchronization was not constant but modulated within a movement cycle and followed the time course of the activation patterns of the muscles. These nested synchronization patterns provide a framework for hierarchical motor control in which large-scale oscillations impose coordinated top-down influences that modulate neural processing and organize motor commands.

POS-TUE-177

DISCRIMINATION OF COMPLEX FORM BY SIMPLE OSCILLATOR NETWORKS

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Purpose: Natural images are rich in higher order spatial correlations. Brain scanning, psychophysics and electrophysiology indicate that humans are sensitive to these image properties. Isotrigon textures are useful for studying this sense. Like natural images these textures have low dimensionality (entropy) relative to random images, but like random images contain no average structure in their first to third order correlation functions. Thus, the structured appearance of these textures, and our ability to discriminate them from random textures, results from higher order spatial correlations. Recursive nonlinear processing can generate the higher order products of higher order correlations. We therefore examined whether small recursive oscillator networks could produce isotrigon texture discrimination performance that matches that of humans. **Methods:** The 23 subjects discriminated random textures from 53 isotrigon texture types. A range of network types were examined, all of which had a pooling readout oscillator. Differences in readout oscillator activity for isotrigon and random textures were measured. The inputs to the networks were small spatial receptive fields (RFs). We have shown that the entropy of textures sampled by these RF shapes also predicts human discrimination performance [Taylor et al. 2008. J Vision 8: 1-13]. The input oscillators are of a novel cubic form. **Results:** The two best network types found contained as few as 4 oscillators. Network activity matched human performance reasonably well, r^2 up to 0.81 +/- 0.13 SE, even when the network parameters were fixed for all 53 texture types. The best RFs matched those of our entropy study. The networks resemble published models of illusory contour tours in V1 and V2, where isotrigon discrimination occurs. **Conclusions:** Overall it appears that relatively simple, short range, and biologically plausible, recursive processing could provide the basis for discrimination of complex form.

POS-TUE-178

LEARNING AND MEMORY PATHWAYS IN THE *DROSOPHILA* MUSHROOM BODIES AFFECT MOTION RESPONSE TO VISUAL STIMULI

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Introduction: Attention-like processes modulate behavioural responses to visual stimulation. Here, we use the *Drosophila* model to characterize visual behaviour and dissect its neural correlates. **Methods**: We developed a high-throughput paradigm that scores responsiveness of fruit flies to a variety of visual stimuli. Flies were exposed to visual stimuli while progressing through mazes overlaid on top of CRT monitors. Upon completion of the maze, flies emerged at the exit points and were automatically counted, after which an average response, the optomotor index, was calculated for each experiment. **Results**: Wild-type flies and *dunce*^{1}, a learning and memory mutant, were used to evaluate this automated paradigm. *dunce*^{1} mutants responded more strongly than wild type flies to moving stimuli across a wide range of luminosity, contrast, spatial, and temporal frequency values ($p<0.05$, $n=192$). To further dissect visual motion responses in *dunce*^{1} mutants, we introduced conflicting visual stimuli. *dunce*^{1} mutants retained a higher response than wild-type flies even when competing stimuli were occluding most of the field of view ($p<0.05$, $n=202$). Since dunce is strongly expressed in the mushroom bodies, the learning and memory brain structures, we hypothesized that they are involved in producing these optomotor responses. Expressing wild-type *dunce* cDNA in the mushroom bodies rescued the visual response to wild-type levels. Conversely, silencing the synaptic output from the mushroom bodies produced *dunce*-like visual responses. **Conclusion**: We have developed an efficient new method of screening visual phenotypes in *Drosophila*, and our results suggest that the mushroom bodies, structures previously associated with olfactory learning memory, are also involved in modulating visually driven behaviour in flies.

POS-TUE-179

THE CORRIDOR TASK AS A SENSORIMOTOR TEST FOR 6-OHDA LESIONED MICE

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The midbrain dopamine cell loss associated with Parkinson's disease is frequently modelled by unilateral 6-hydroxydopamine (6OHDA) injections into the nigrostriatal pathway resulting in side-biased motor impairments including akinesia, sensorimotor neglect and rotational behaviour. While behavioural tests for these impairments have been well established for rats, many are not well translated using mice. The corridor task is a drug free behavioural test that has been shown to detect lateralised neglect in 6OHDA lesioned rats. In the present study adult female Swiss mice (n=51) with large 6OHDA unilateral SNpc lesions were subjected to the corridor task to determine whether it could also be a suitable sensorimotor test in mice. The corridor task involved placement of food rewards at regular intervals along the left and right sides of a long narrow corridor. The number of retrievals made from the left and right side within 5 minutes were counted. The cylinder test and amphetamine induced rotational behaviour was also conducted to confirm and compare the validity of the corridor test with established behavioural tests. The corridor test revealed contralateral sensorimotor neglect in 98% of mice whilst only 82% mice responded to amphetamine induced rotations. The cylinder test did not show a significant impairment in the contralateral forelimb. Histological examination revealed that performance in the corridor test correlated with the loss of DA neuron - a discrepancy that commonly exists when comparing with rotational behaviour. Our findings indicate that the corridor task may be a sensitive test of lateralised sensorimotor response selection in mice and possibly a more reliable test to assess loss of midbrain dopamine neurons compared to amphetamine induced rotations and the cylinder test.

POS-TUE-180

STRUCTURE AND PUTATIVE FUNCTION OF ANTENNAL SENSILLA OF WORKERS OF *COPTOTERMES FORMOSANUS* SHIRAKI

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The difficulty in controlling termites by fungi as a biological agency is often dependent on the ambiguousness of their habitat and behavior. Therefore fundamental studies are needed to elucidate the nature of termites. A better understanding of the antennal sensory system, which is considered important in communication among termite individuals, will be helpful in developing fungal control of termites. Although early studies reported the external and ultrastructures of sensilla on the termite antennae, the number and distribution of sensilla on a whole antenna were not detailed yet. We categorized the antennal sensilla of *Coptotermes formosanus* Shiraki into nine types on the basis of the external structures of the cuticular apparatus of sensilla, and precisely examined their distribution by scanning electron microscopy. The classified antennal sensilla were chaeticum I, II and III, s. trichodeum I and II, s. basiconicum, s. capitulum, s. campaniformium and marginal sensillum. The morphological features and functions of these sensilla were also discussed in consideration of their structural and distributional characteristics.

POS-TUE-181

RE-EXAMINING PREVIOUSLY 'ESTABLISHED' NEURAL PATHWAYS USING CLASSICAL AND NOVEL METHODS

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Intracellular recordings from regularly discharging motoneurons in brain slices recently showed that the classical methods (SEMG, PSTH) contain inherent errors (Türker and Powers, Trends Neurosci 2005). These errors are minimized using peristimulus frequencygram (PSF). We now aim to re-examine the synaptic connections in several human reflex pathways using PSF and to compare the results with classical methods in order to source possible errors. After obtaining informed consent from volunteers (n=47), wire electrodes were inserted into one of the following muscles: tibialis anterior, soleus, gastrocnemius, masseter and first dorsal interosseous. Subjects maintained constant discharge rates of selected motor unit while sensory afferents (spindle, tendon organ, mechanoreceptors) around these muscles were stimulated. The evoked responses were represented by the SEMG, PSTH and PSF. We found that the onset of inhibitory events was reliably determined from the reduction in spike counts in PSTH. However, the endpoints of the inhibitory periods were more optimally shown by the PSF compared with the other two methods ($p<0.001$). Similarly, the duration of excitation is better established using the PSF, as the falling phase of excitation appeared as an inhibition in the SEMG and PSTH (lower activity / spike counts). The PSF on the other hand showed this event as a continuation of excitation as the discharge rates remained above prestimulus levels. This study confirms the predictions from the slice work that the timing of synaptic events on motoneurons, is more optimally reconstructed by a combination of classical and PSF methods.

POS-TUE-182

AN ASSOCIATION BETWEEN INEQUITY-AVERSE MORAL PREFERENCE AND RISK AVERSION IN DECISION-MAKING

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Neuroimaging studies of decision-making (DM) in moral and non-moral contexts have shown activation of insular and prefrontal cortex regions, implicating processes in common to both forms of DM. However, the relationship between an individual's moral and non-moral DM preferences has yet to be investigated. This study therefore examined whether moral preferences in a distributive justice task (DJT) are associated with preferences in financial risk-taking tasks. Twenty young adult participants completed a computerised DJT to assess their individual preferences for equity versus total material benefit when distributing meals amongst a group of disadvantaged children. Participants also completed three computerised financial gambling tasks (Iowa Gambling Task, Cambridge Risk Task, Balloon Analogue Risk Task) and a trait questionnaire (Dahlbaeck Risk-Taking Propensity Scale) to assess their risky DM preferences. DJT inequity aversion was found to correlate significantly with risk aversion (r_s =.60, p =.003) when risk aversion was assessed by the Iowa Gambling Task, which contained uncertain potential outcomes, but not when risk aversion was assessed by the other measures. Inequity-averse distributive justice DM preferences therefore appear to be associated with risk-averse DM preferences when the potential outcomes of risky choices are uncertain. This finding suggests that factors contributing to individual differences in DM may generalise across moral and non-moral contexts, and is consistent with previous neuroimaging studies of insular and prefrontal cortex function during both types of DM.

POS-TUE-183

FUNCTION OF EGF RECEPTOR SIGNALING IN THE NEONATAL HIPPOCAMPAL LESION MODEL OF SCHIZOPHRENIA

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There are EGF and EGF receptor (ErbB1) abnormalities in brain tissues and blood of schizophrenic patients. Here we examined pathological roles of ErbB1 signaling in the schizophrenia model rats that are established with bilateral microinjections of ibotenic acid to the ventral hippocampus as neonates. Treatment with ibotenic acid altered HB-EGF and TGF alpha protein levels in the forebrain regions in parallel with persistent activation of ErbB1 receptors. On postnatal week 8, rats were tested for behavioral tasks. The hippocampus-lesion significantly impaired the behavioral scores of PPI and latent learning in adults. In context fear conditioned task, memory retentions were not affected although their latent learning was significantly disrupted. We determined the antipsychotic effects of several ErbB1 tyrosine kinase receptor inhibitors in this model. The abnormalities in PPI and latent inhibition were ameliorated by icv infusion of all the ErbB1 inhibitors from an osmotic pump. These results indicate that blockade of ErbB1 signals may be a novel antipsychotic target for schizophrenia and its related disorders.

POS-TUE-184

SPATIAL LEARNING-INDUCED INCREASE IN AGMATINE LEVELS AT HIPPOCAMPAL CA1 SYNAPSES

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Agmatine, a metabolite of L-arginine, is considered a novel putative neurotransmitter. Agmatine-like immunoreactivity has been detected in axon terminals that synapse with pyramidal cells of the hippocampus. However, the role of endogenous agmatine in learning and memory is poorly understood at present. Recent research has demonstrated water maze training-induced increase in the tissue concentration of agmatine in the CA1 sub-region of the hippocampus, a brain area that is critically involved in spatial reference memory. This finding suggests that endogenous agmatine may directly participate in learning and memory processes as a neurotransmitter. The aim of the present study was to further address this issue by investigating whether levels of agmatine are increased at synaptic terminals in the CA1 region following water maze training. Quantitative immunogold-labelling and electron-microscopical techniques were used to analyse agmatine levels in CA1 Schaffer collateral (SC) terminals (n=600) of rats that had been trained to find a hidden escape platform in the water maze task (WM, n=3), or forced to swim in the pool with no platform presented (SW, n=3). All experimental procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Agmatine levels were significantly increased in the synaptic terminals of SCs of trained WM group compared to the SW control group (all $p<0.001$). These results provide further evidence of the participation of endogenous agmatine in learning and memory processing.

POS-TUE-185

CAN RATS BE TRAINED TO DISCRIMINATE BETWEEN PRE- AND POST-SYNAPTIC SEROTONIN 1A RECEPTORS? INVESTIGATING THE EFFECTS OF NOVEL ANXIOLYTIC AND ANTIDEPRESSANT COMPOUNDS USING DRUG DISCRIMINATION

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As a pharmacological target, the 5-HT_{1A} receptor has shown considerable efficacy in clinical studies for the treatment of anxiety and depressive disorders. One explanation that has been proposed to account for the 2-4 week delay in the onset of therapeutic effects of antidepressants is that this is the time required for desensitization of pre-synaptic 5-HT_{1A} receptors (autoreceptors). If true, it is likely that combining an autoreceptor-selective agonist with antidepressant treatment may lead to a more rapid onset of antidepressant or anxiolytic action. In this study we examined whether rats could be trained to report differences in the effects of a post-synaptic 5-HT_{1A} selective agonist (F15599; 0.05-0.5mg/kg i.p.; 20 min ptt) and a pre-synaptic 5-HT_{1A} selective agonist (F13714; 0.005-0.05mg/kg i.p.; 20 min ptt). Twenty-one (12 M, 9 F) Sprague-Dawley rats were trained to discriminate between the selective 5-HT_{1A} agonist 8-OH-DPAT (0.1mg/kg i.p.; 20 min ptt) and its vehicle, saline. Both F15599 (n=8) and F13714 (n=8) fully substituted for the training drug. 8-OH-DPAT-appropriate responding for all three drugs was blocked by the selective $5HT_{1a}$ receptor antagonist, WAY-100,635(n=4). There was a 20-fold difference in the doses at which F15599 (0.5mg/kg) and F13714 (0.025mg/kg) generalized to the 8-OH-DPAT training cue, supporting the conclusion that interoceptive cues produced by 8-OH-DPAT (0.1mg/kg) are mediated primarily through pre-synaptic 1A receptors. Preclinical evaluation of their antidepressant and anxiolytic utility will be the next important step in determining whether differences in their efficacy can be ascribed to actions at pre-synaptic, post-synaptic or the combination of these receptors.

POS-TUE-186

BEHAVIOURAL CORRELATES OF GESTATIONAL LOW DOSE ETHANOL EXPOSURE IN AGED OFFSPRING

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Purpose: Excessive alcohol consumption during pregnancy can lead to a wide spectrum of disorders and defects in offspring, which are collectively referred to as Foetal Alcohol Syndrome. However, little is known about the long term effects of mild alcohol consumption during pregnancy. The aim of this study was to examine the effect of exposure to a low dose ethanol diet during gestation on behavioural changes in aged adult offspring. **Methods:** Female Sprague Dawley rats were fed a liquid diet containing a low dose of ethanol (6% vol/vol, Ethanol) or a calorie matched control diet for the duration of pregnancy (Control). Male (n=14, Control; n=8, Ethanol) and female (n=10, Control; n=9, Ethanol) offspring were tested at 15-18 months of age to assess a number of behavioural domains including anxiety, exploration, sensorimotor gating and spatial memory, as well as ethanol preference. **Results:** Prenatal exposure to a low ethanol diet resulted in a subtle, however not significant ($p=0.08$), behavioural phenotype affecting aspects of anxiety and neophobia. However, there was no effect of prenatal treatment on locomotion, sensorimotor gating or spatial memory ($p>0.05$). Finally, the Control rats tended to have a greater preference for ethanol than did rats in the Ethanol group in a two bottle choice paradigm ($p=0.06$). **Conclusions:** Exposure to low dose ethanol during early neural development did not lead to long lasting behavioural changes in aged adult offspring. This indicates that a small amount of alcohol during pregnancy does not result in significant changes in foetal nervous system development.

POS-TUE-187

ALCOHOL REINSTATEMENT INDUCED NEURONAL ACTIVATION FOLLOWING PROTRACTED ABSTINENCE COMPARED TO REINSTATEMENT IMMEDIATELY FOLLOWING EXTINCTION

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The enduring propensity for alcoholics to relapse even following years of abstinence presents a major hurdle for treatment. The neural mechanisms underlying continued susceptibility to relapse are largely unknown due to a lack of appropriate animal models to investigate relapse following extended abstinence. Here we report such a model and utilize it to investigate the pattern of neuronal activation during cue-induced reinstatement and following administration of the orexin-1 antagonist SB-334867 (SB). Rats were trained to self administer alcohol under an operant cue-conditioned paradigm then divided into two groups; early (reinstated immediately following extinction), and late (extinguished and then housed for five months before reinstatement). During reinstatement animals were treated with vehicle (early n=15, late n=9) or SB (20mg/kg ip; early n=12, late n=8). Fos expression was compared between each group and to animals who underwent extinction only (n=3). SB significantly attenuated reinstatement in both early and late groups. Early reinstatement increased Fos expression in the nucleus accumbens, the lateral and dorsomedial hypothalamus and bed nucleus, while late reinstatement further increased Fos expression in the accumbens shell, lateral hypothalamus, ventral bed nucleus and nucleus incertus. Activity in the basolateral and central amygdala was also increased late when compared to extinction. SB decreased Fos expression in the accumbens core in the early, but not late group. Cue-induced alcohol seeking can be triggered following protracted abstinence and involves a differential pattern of activation when compared to reinstatement immediately following extinction. SB was able to inhibit both early and late reinstatement and also demonstrated differential activation between the two paradigms.

POS-TUE-188

THE IMPACT OF STRESS ON A HETEROZYGOUS NEUREGULIN 1 MUTANT MOUSE MODEL

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Purpose: A mouse model for the schizophrenia candidate gene neuregulin 1 (*NRG1*) [i.e. transmembrane domain *Nrg1* mutant mice (*Nrg1* HET)] exhibits a schizophrenia-related behavioural phenotype. *Nrg1* HETs are more susceptible to environmental factors such as cannabis (i.e. acute and chronic Δ-9-tetrahydrocannabinol exposure) or altered housing conditions (i.e. environmental enrichment). During animal transfers between laboratories/institutes, *Nrg1* mutant mice appear to be differentially affected by stress compared to their non-mutant littermates. **Methods:** We investigated the endocrine and behavioural response of male and female heterozygous *Nrg1* HET and their wild type-like (WT) littermates ($n \geq 10$) after acute exposure to restraint or swim stress. Different sets of mice were tested in behavioural paradigms for locomotion, exploration and anxiety (e.g. open field, light dark, zero maze). Blood samples – collected at baseline and after stress exposure - were analysed for plasma levels of the stress hormone corticosterone. **Results:** WT and *Nrg1* HETs were susceptible to the locomotion-inhibiting and anxiety-elevating effects of restraint and swim stress. All test mice exhibited an endocrine stress response with corticosterone plasma levels being increased after stress induction. However, it was dependent on the stressor and the behavioural test paradigm used, whether *Nrg1* mutant mice showed an altered sensitivity to the behavioural effects of stress. The endocrine stress response of WT and *Nrg1* HET mice was identical. **Conclusion:** These data suggest that a variation in the Nrg1 gene alters the sensitivity specifically to some but not all environmental manipulations/factors. In future, the *Nrg1* mouse model will be tested regarding its response to social stress.

POS-TUE-189

EXERCISE OR COMFORT FOOD REDUCES STRESS RESPONSE FOLLOWING PROLONGED MATERNAL SEPARATION IN RATS

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Prolonged maternal separation (MS) causes HPA axis dysregulation with elevated plasma corticosterone (CORT), decreased glucocorticoid receptor (GR) mRNA expression and behavioural outcomes such as anxiety and depression-like behaviour. We investigated the effects of voluntary exercise and/or palatable high fat diet (HFD) following MS. Sprague-Dawley litters were assigned to brief (15 min, S15) or prolonged (180 min, S180) separation from postnatal day (PND) 2-14. At PND 21, males from each litter were assigned to either laboratory chow (11% fat) or HFD (32 % fat). Half the rats had continuous access to running wheels (voluntary exercise). Stress reactivity was measured at PND 60, using 15 min immobilization and sampling at 0 15, 30, 45, 60 min for plasma CORT. Hippocampus was collected at 14 weeks for GR mRNA expression. A significant interaction between treatment and diet was observed for CORT ($F=3.77$, $p=0.02$). S180 rats on chow had increased basal plasma CORT and CORT response to stress (area under CORT curve 1.4 times higher than S15 rats, ANOVA, $p<0.05$) and this was reduced by HFD consumption ($t=3.37$, $p<0.01$) or exercise alone ($t=2.28$, $p<0.05$). Combined exercise and HFD appeared to reduce plasma CORT however this was not significant ($p=0.06$). Hippocampal GR mRNA was significantly reduced in S180 versus S15 rats ($t=2.60$, $p<0.05$). S180 rats subjected to exercise and HFD alone had increased GR mRNA compared to S180 chow ($p<0.05$). Combining HFD and exercise did not alter expression of GR in S180 compared to chow fed rats. HFD or exercise blunted stress responsivity in S180 offspring and combining HFD and exercise had no added benefit. These effects appear to be mediated by increased hippocampal GR mRNA.

POS-TUE-190

BEHAVIOURAL EFFECTS OF MATERNAL OBESITY: REDUCED ANXIETY IN FEMALE OFFSPRING OF OBESE MOTHERS

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The obesity epidemic represents not only an immediate public health burden, but threatens future generations, as maternal obesity increases the risk of offspring obesity. We have shown that high-fat-diet consumption (HFD) after weaning exacerbates offspring metabolic risk. Obesity has been shown to influence behaviors such as learning, but data are conflicting. We tested the effects of maternal obesity on offspring anxiety levels, learning and memory. Female Sprague Dawley rats were fed chow (C) or HFD for 6 weeks before mating, throughout gestation and lactation. At 20 days, female pups were weaned onto either C or HFD, yielding CC, CH, HC and HH groups. Pups (12 per group) underwent anxiety testing (elevated plus maze, EPM), Y maze (spatial memory) and forced swim test (FST) from 9-19 weeks. Offspring of obese mothers (HC) were significantly heavier at weaning with increased adiposity and this was amplified by HFD (HH). At 11 weeks, HC and HH offspring spent more time, and made more entries, in the open arm of the EPM, with more exploratory behaviour assessed as head dips, compared to CC and CH offspring ($P<0.05$). Postweaning diet had no significant impact. No difference was observed in time spent in the novel arm of the Y maze. While some effects of maternal diet were observed on FST, with increased immobility in offspring of obese mothers, this appeared to be related to overall adiposity. At 21 weeks leptin was markedly increased in HH versus HC offspring. Therefore, in females, maternal obesity appears to reduce offspring anxiety-like behaviour; ongoing work is aimed at dissecting underlying mechanisms.

POS-TUE-191

ANXIETY AND CENTRAL RESPONSES TO RESTRAINT STRESS IN RATS MADE LEAN BY NEONATAL UNDERFEEDING

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Stress and the myriad diseases caused, triggered, or exacerbated by it costs countries millions of dollars annually and individuals their health and their lives. An excellent strategy to treat overactive and potentially dangerous responses to stress is to exploit the body's own inherent stress-inhibitory mechanisms. Stress responses can differ between individuals depending upon their level and distribution of adiposity, and we have recently shown that increased adiposity is associated with exacerbated responses to psychological stress. The converse may be true for lean animals. In this investigation we hypothesized that lean animals would have reduced anxiety and attenuated hypothalamic-pituitary-adrenal axis responses to psychological stress. Our findings show that rats made lean by being suckled in a large litter (n = 10 males, females) show reduced levels of anxiety compared with those from normal litters (n = 10 males, females) when tested in the elevated plus maze. These lean animals also have attenuated activation of the paraventricular nucleus of the hypothalamus in response to the psychological stress, restraint. Understanding the mechanisms by which these stress responses are attenuated in lean animals will be important for future strategies to treat overactive stress responses in humans.

POS-TUE-192

LYCIUM BARBARUM (WOLFBERRY) POLYSACCHARIDE FACILITATES EJACULATORY BEHAVIOR IN MALE RATS

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Objective: Lycium barbarum (wolfberry) is a traditional Chinese medicine, which has been considered to have therapeutic effect on male infertility. However, there is a lack of studies support the claims. We thus investigated the effect of Lycium barbarum polysaccharide (LBP), a major component of wolfberry, on male rat copulatory behavior. Method: Sprague-Dawley rats were divided into two groups (n=8 for each group). The first group received oral feeding of LBP at dosage of 1mg/kg daily. The control group received vehicle (0.01M phosphate-buffered saline, served as control) feeding daily for 21 days. Copulatory tests were conducted at 7, 14 and 21 days after initiation of treatment. Results: Compared to control animals, animals fed with 1mg/kg LBP showed improved copulatory behavior in terms of: 1. higher copulatory efficiency (i.e. higher frequency to show intromission rather than mounting during the test), 2. higher ejaculation frequency and 3. shorter ejaculation latency. The differences were found at all time points (Analyzed with two-tailed student's t-test, $p<0.05$). There is no significant difference found between the two groups in terms of mount/intromission latency, which indicates no difference in time required for initiation of sexual activity. Additionally, no difference in mount frequency and intromission frequency was found. Conclusion: The present study provides scientific evidence for the traditional use of Lycium barbarum on male sexual behavior. The result provides basis for further study of wolfberry on sexual functioning and its use as an alternative treatment in reproductive medicine.

POS-TUE-193

ASSESSING THE ROLES OF OXYTOCIN AND ARGININE VASOPRESSIN

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The neuropeptides oxytocin and arginine vasopressin are implicated in the regulation of depressive- and anxiety-like behaviours. Oxytocin is thought to promote anxiolytic and antidepressant-like effects while arginine vasopressin may increase anxious and aggressive behaviours. The present study examined the effects of the oxytocin agonist, carbetocin (2.5 and 5 mg/kg), and the vasopressin agonist, desmopressin (1 and 5 mg/kg) following intravenous administration, in a measure of depressive-like activity (forced swimming test; FST), and in two models measuring anxiety-like activity (elevated plus maze; EPM, and open field; OF). The oxytocin/AVP_{1A} receptor antagonist, atosiban (1 mg/kg), was also investigated, both in isolation and in combination with carbetocin and desmopressin. Young adult male Sprague-Dawley rats (n=110) were tested once in each behavioural paradigm following these drug treatments and their effects compared with those of imipramine (a tricyclic antidepressant; 10mg/kg) and midazolam (a benzodiazepine; 1mg/kg). The results supported the hypotheses that carbetocin would have antidepressant-like effects in the FST, and that desmopressin would have anxiogenic-like effects in the EPM. Surprisingly, the oxytocin/$vasopressin_{1A}$ antagonist atosiban produced anxiolytic effects in the EPM. As expected, the anxiogenic effects of desmopressin and anti-depressant effects of carbetocin were both attenuated by coadministration with atosiban. A lack of anxiolytic-like effects of carbetocin and depressant-like effects of desmopressin strengthens the conclusion that central AVP1A receptors are selectively implicated in the anxiety response, while antidepressant-like effects are the domain of central carbetocin receptors.

POS-TUE-194

ADVANCED PATERNAL AGE IS ASSOCIATED WITH SPONTANEOUS HYPERLOCOMOTION AND ALTERATIONS IN RESPONSE TO AMPHETAMINE IN C57BL/6J MICE

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Purpose: Advanced paternal age (APA) is associated with an increased risk of neurodevelopmental disorders in offspring, including schizophrenia and autism. We have developed a mouse model to examine behavioural effects in offspring from older fathers (advanced paternal age, APA) compared with young fathers (young paternal age, YPA). The aim of the present study was to investigate the effects of APA on locomotion in both novel and home-cage environments and in response to d-amphetamine (AMPH) challenge. **Methods:** The adult offspring (*N*=123) of young (3 month-old, YPA) and old (12-18 month-old, APA) C57Bl/6J sires underwent a behavioural test battery comprising tests for baseline locomotion, anxiety and exploration. Offspring were then treated with AMPH and their locomotor response recorded in either a novel environment (automated open field) or in a familiar home-cage (PhenoTyper chamber). **Results:** Male APA offspring travelled significantly further than YPA males in the holeboard (p=.03) and marble burying test (p=.01) as well as following AMPH exposure in a novel environment, though this failed to reach significance (p=.12). In the home-cage, male APA mice demonstrated decreased activity compared to controls, which was specific to the initiation of the dark phase of the cycle (7pm to midnight). There was no significant effect of paternal age on AMPH-induced locomotion in the home-cage. Behavioural changes in female APA offspring were restricted to their response to AMPH in the novel arena, in which APA females had transiently reduced locomotor scores compared with YPA females (p=.045). **Conclusion:** Male APA mice demonstrated spontaneous hyperlocomotion and we show a variable response to amphetamine, dependent on sex and arena. This may implicate abnormalities in dopamine signalling however given the sex- and arena-specific nature of these findings the role of sex hormones and other neurotransmitter systems cannot be excluded. These results provide clear evidence that APA is associated with long-term behavioural changes, particularly in male offspring.

POS-TUE-195

THE EFFECTS OF OREXIN-1 RECEPTOR ANTAGONISM ON ALCOHOL SELF-ADMINISTRATION AND RELAPSE BEHAVIOUR IN RATS

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The neuropeptide orexin (also known as hypocretin) has been implicated in arousal, feeding and more recently in drug-seeking. The current study aimed to (1) investigate whether the selective orexin-ı receptor antagonist SB-334867 could reduce the motivation to self-administer alcohol and (2) examine cue-induced alcohol-seeking following prolonged abstinence in alcohol-preferring (iP) rats. Intra-peritoneal injections of SB-334867 or vehicle were administered prior to operant sessions under fixed ratio (n = 20) and progressive ratio (n = 18) schedules. Rats were then withdrawn from alcohol for 7, 14, 28 or 56 days, following which they were returned to the operant chambers and tested under extinction conditions in the presence of alcohol-related cues. Results indicated that SB-334867 significantly attenuated volitional alcohol self-administration ($p<.05$) and also alcohol reward breakpoint under progressive ratio ($p<.05$). These data suggest orexins may be implicated in both consummatory and appetitive responding for alcohol. Furthermore, rats displayed robust cue-induced alcohol seeking following all periods of abstinence and SB-334867 attenuated this response in rats abstinent for 14 days ($p<.05$). These results further implicate orexin in the modulation of the rewarding effects of alcohol and the salience of alcohol-related cues.

POS-TUE-196

α4-S248F MICE SHOW INCREASED IV SELF-ADMINISTRATION OF LOW DOSE NICOTINE

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PURPOSE: A role α4β2 nicotinic acetylcholine receptors in mediating the reinforcing effects of nicotine has been asserted. Mice with a point mutation of the α4 nicotinic subunit that confers an increased sensitivity to low doses of nicotine (α4-S248F mice) were assessed in comparison to wildtype littermates for intravenous self-administration of nicotine. METHODS: Mice to undergo surgery for operant intravenous self-administration (IVSA) were first trained to respond for sucrose with two levers (reward-paired and inactive) on an FR1 schedule of responding, with olfactory and visual reward-paired cues. Following insertion of a catheter into the jugular vein, mice were then allowed to respond for nicotine (0.03, 0.05 and 0.07 mg/kg/infusion). Mice were also assessed for progressive ratio responding and cue-induced drug seeking following a period of withdrawal for all doses. RESULTS: Mice established stable responding for nicotine across all doses. At the doses of 0.05 and 0.07 mg/kg/inf, no difference was observed between the response rates of each genotype, for acquisition and maintenance of responding, motivation for drug as measured by progressive ratio responding nor for cue-induced drug-seeking. However, when the dose was reduced to 0.03 mg/kg/inf of nicotine, α4-S248F mice showed increased responding compared to WT littermates across all measures, with mice earning significantly greater rewards, and showing increased motivation for drug in progressive ratio responding and cue-induced drug seeking. CONCLUSION: The increased rewarding and motivational effects of nicotine in α4-S248F mice at the low dose of nicotine supports a role for α4* nicotinic receptors in mediating motivational and reinforcing properties of nicotine.

POS-TUE-197

MGLU5 RECEPTORS REGULATE OPIATE SELF ADMINISTRATION AND RELAPSE BEHAVIOUR IN MICE

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Opiates represent the single largest contribution to illicit drug-related mortality and morbidity worldwide and effective treatment remains a major clinical problem. The metabotropic glutamate 5 (mGlu5) receptor has been implicated in drug induced plasticity and is believed to play a role in mediating the reinforcing properties of drugs of abuse. Previously we showed that selective antagonism of mGlu5 receptors with 3-[(2-methyl-1,3-thiazol-4-yl) ethynyl]-pyridine (MTEP) decreases ethanol self-administration, however the effect of MTEP on opiate self-administration has not been determined. Therefore we hypothesized that mGlu5 receptors play a role in mediating the self administration of opiates and contribute to relapse to opiate-seeking elicited by drug-associated cues. CD1 outbred wildtype mice were implanted with indwelling intravenous catheters and trained to self administer morphine (0.1mg/kg/infusion, i.v.) on a fixed ratio (FR)-1 schedule. After self-administration had stabilized, mice were treated with vehicle or MTEP (20mg/kg, i.p.) prior to an operant session in a repeated measures design. Mice readily acquired self-administration of morphine preferentially responding on the morphine-paired lever ($p<0.05$). Pretreatment with MTEP selectively reduced responding on the morphine paired lever ($p<0.05$). This corresponded with a >50% reduction in total drug infusions ($p<0.05$). Responding on the morphine-paired lever returned to control levels the day after MTEP treatment. After 3 weeks withdrawal, mice were injected with vehicle (n=8) or MTEP (n=7) and returned to the chamber in the presence of drug-paired cues. MTEP also significantly reduced cue-induced opiate-seeking following abstinence ($p<0.05$). In conclusion we have shown that glutamatergic signaling via mGlu5 receptors plays role in regulating opiate self-administration and relapse to drug seeking behaviour.

POS-TUE-198

THE EFFECT OF OXYTOCIN AND VASOPRESSIN ON METHAMPHETAMINE AND MDMA ('ECSTASY')-INDUCED REWARD IN RATS

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The neuropeptide oxytocin is increasingly recognized as modulating the rewarding effects of psychostimulants. Oxytocin is known for mediating the social rewarding effects of 3,4-methylenedioxymethamphetamine (MDMA) and we have recently shown that systemic oxytocin pretreatment reduces the motivation for rats to self-administer intravenous infusions of methamphetamine (METH). However a role for oxytocin in MDMA or METH-induced contextual reward has not yet been demonstrated in rats. In addition, an effect of the neuropeptide vasopressin on psychostimulant reward has been less well described. This study investigated the effect of pretreatment with oxytocin (600 µg/kg ip) or [Arg^8]-vasopressin (3ng/kg ip) on MDMA (5mg/kg ip) or METH (1mg/kg ip) conditioned place preference (CPP) in male Sprague-Dawley rats. CPP was conducted using 6 daily conditioning sessions (30 min), where animals were paired to one context following drug administration for 3 sessions, alternated daily with 3 sessions of vehicle-paired context. Odour cues were paired to each context. In comparison to pre-conditioning, animals treated with METH spent significantly more time in the drug-paired context (n=10) indicating a CPP to METH. Pretreatment with oxytocin (n=10) or [Arg^8]-vasopressin (n=9) prior to drug conditioning sessions prevented METH-induced CPP. In comparison to pre-conditioning, animals treated with MDMA spent significantly more time in the drug-paired context (n=10) indicating a CPP to MDMA. Pretreatment with oxytocin (n=10) or [Arg^8]-vasopressin (n=10) did not prevent MDMA-induced CPP. These data show that both neuropeptides are involved in modulating the rewarding effects of METH but do not modulate MDMA-induced contextual reward.

POS-TUE-199

EVALUATING THE CONTRIBUTION OF SEROTONIN RECEPTOR SUBTYPES AND 'BINGE' MDMA EXPOSURE ON THE DISCRIMINATIVE STIMULUS EFFECTS OF MDMA IN RATS

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3,4-Methylenedioxymethamphetamine (MDMA; 'Ecstasy') shares psychoactive effects with both the stimulant, amphetamine (dopaminergic) and the hallucinogen, LSD (serotonergic). The majority of MDMA's reported effects and toxicity have been linked to its actions on serotonergic neurotransmission. One way to study MDMA's serotonergic effects is to train rats to distinguish between dopaminergic stimulant effects and mood and perception-altering serotonergic effects using a three-way drug discrimination paradigm. Once male and female Sprague Dawley rats (n=18) learned to reliably differentiate between amphetamine (0.75mg/kg), MDMA (1.5mg/kg) and saline, the contributions of serotonin$_{1A}$ (5-HT$_{1A}$) and 5-HT$_{2A/C}$ receptors to MDMA's interoceptive effects were evaluated. This was done both before and after the rats were exposed to an MDMA 'binge' (3 x 10mg/kg at two hourly intervals) to determine whether a reportedly neurotoxic dosing regimen would disrupt the interoceptive cues of MDMA. Blockade of 5-HT$_{1A}$ or 5-HT$_{2A/C}$ receptors, via administration of WAY 100,635 (1 mg/kg) or ritanserin (1.5 and 3 mg/kg), significantly disrupted MDMA-appropriate responding. Binge administration resulted in selective disruption to the MDMA training cue in the majority of rats during the two subsequent weeks. Once the discrimination had recovered, repeating the antagonist tests revealed that the contributions of the 5-HT$_{1A}$ and 5-HT$_{2A/C}$ receptors to the MDMA discriminative cues were not significantly different to what was measured prior to the 'binge'. This study provides support for the importance of 5-HT$_{1A, 2A/C}$ mediated cues in the discriminative, and by extension behavioural and neurotoxic effects of MDMA, and suggests that its discriminative stimulus effects are only temporarily disrupted following high-dose MDMA exposure.

POS-TUE-200

AGE-RELATED INCREASE IN MITOCHONDRIAL DNA DELETION MUTATIONS IN RAT BRAIN

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AIMS: Accumulation of mitochondrial DNA (mtDNA) mutations has been implicated in ageing. Consistent with this notion, studies in human brain show mtDNA deletions accumulate with age in a region specific pattern. The aims of the present study were to determine whether a similar phenomenon occurred in the brain of a commonly used animal model for ageing, the F344 rat. **METHODS:** DNA was extracted from cerebellum, midbrain, striatum and cerebral cortex of 6 young (4-6 months), 5 middle aged (12-15 months) and 6 old (24-27 months) F344 rats. Long PCR was carried out to amplify the major and minor arc regions of the mtDNA and reaction products separated by agarose gel electrophoresis. Quantification of mtDNA deletions was carried out using qPCR comparing the delta CT of a major arc gene, ND4, to that of a minor arc gene, 12S rRNA. **RESULTS:** Our preliminary long PCR results demonstrate a brain region-specific, age-related mtDNA deletion pattern, with the cerebellum being relatively spared and the striatum and cerebral cortex being most profoundly affected. The majority of deletions, in affected brain regions, were seen in the major arc of mtDNA with the minor arc being relatively free of deletions. These results are consistent with those previously reported for humans. The age-related mtDNA deletions apparent with long PCR, were confirmed by qPCR analysis which showed 28% and 25% increases in mtDNA deletion burden in the cerebral cortex and striatum, respectively, of old rats compared to young rats ($p<0.05$). **CONCLUSIONS:** These data indicate the rat is a suitable model for studying age-related mtDNA changes in the brain.

POS-TUE-201

CHARACTERISATION OF THE FOREBRAIN-NEURON SPECIFIC IRAP KNOCKOUT MOUSE

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IRAP (insulin-regulated aminopeptidase) is a membrane bound metalloprotease, which is colocalised with the insulin-regulated glucose transporter GLUT4 in hippocampal and cortical neurons. Substrates of IRAP include vasopressin and oxytocin, the actions of which are important in several memory domains. Central infusion of IRAP inhibitors attenuates memory deficits in several rodent models, and we propose that IRAP represents a novel target for the development of cognition-enhancing drugs. We sought to identify the role of IRAP in neurons of the forebrain using the IRAP lox x CamKIIα-Cre mouse. Animals carrying both the floxed IRAP gene and the CamKIIα directed Cre recombinase have a forebrain-neuron specific deletion of IRAP, as confirmed by western blot and autoradiography. Male forebrain-neuron IRAP KO mice and their WT littermates were subjected to behavioural assessment at 3, 9 and 15 months. There was a statistically significant interaction between arm preference and genotype for 3-month old animals in the Y-maze ($p=0.02$, RMANOVA, n= 19- 21). WT animals had intact spatial memory, spending 46.6% of the trial in the novel arm. However, KO mice did not perform above chance levels. For object recognition at the same age there was a statistically significant interaction between trial and genotype for the preference index ($p=0.049$, RMANOVA, n= 13- 16). WT animals indicated a preference for the novel object (19.2% more exploration) whilst KO mice had no preference. Western blot analysis of cortical and hippocampal membrane homogenates indicated altered expression of GLUT4. Since GLUT4 is required for facilitated glucose uptake during high metabolic demand, the altered GLUT4 expression may explain the poor learning demonstrated by the forebrain-neuron IRAP KO mice.

POS-TUE-202

INSULIN-LIKE PEPTIDE 3 (INSL3) IN MOUSE BRAIN: SOMATIC NEURONAL EXPRESSION AND EFFECTS OF CENTRAL ADMINISTRATION ON COMPLEX BEHAVIOUR

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Insulin-like peptide-3 (INSL3) is highly expressed by testicular Leydig cells and is present in the circulation of adult male rodents and humans, but neuronal expression has not been identified. Leucine-rich repeat containing G-protein coupled receptor 8 (LGR8), the native receptor for INSL3, is associated with excitatory pathways in mouse brain - the cortico-striatal and habenulo-interpeduncular systems, and in amygdala, midline thalamus, hypothalamus and brainstem, but its role is unknown. Therefore, these studies explored the possible presence of INSL3 expression in mouse brain and the effect of central LGR8 activation on mouse behaviour. Initial studies identified evidence of low levels of INSL3 mRNA in subcortical brain and the presence of Golgi-associated INSL3 immunoreactivity in soma of neurons in red nucleus and other areas. Groups of C57BL/6J mice (n = 8-13) received 4-µl icv infusions of aCSF or INSL3 (80-320 pmol) and were assessed in the automated locomotor cell, and the novel-object exploration (NOE), social interaction, and anxiety/stress tests. INSL3 (80-160 pmol) had no effect on locomotor activity over 60 min, but 320 pmol induced hypoactivity 45-60 min post-injection ($p < 0.05$). Exploration of inanimate objects in the NOE or other mice in a sociability/social novelty test) was not altered by INSL3, but inter-male aggression was elevated after 160 pmol INSL3 ($p <0.001$). Multiple tests revealed evidence for increased anxiety-like behaviour after INSL3, both in a social and environmental context - intruder-recognition test ($p < 0.05$), elevated plus maze ($p < 0.05$) and light/dark box ($p < 0.05$). The data suggest a role for INSL3/LGR8 signalling in sensorimotor and emotional behaviors in mice.

POS-TUE-203

CHARACTERIZATION OF A NOVEL COVALENTLY CROSS-LINKED Aβ PEPTIDE DIMER AND ITS ROLE IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of dementia and is characterized by progressive memory loss, confusion, and cognitive deficits. While the major cause in AD is unknown, it is generally accepted that the beta amyloid peptide (Aβ) is toxic and we are confident that the toxic species are a low molecular weight soluble oligomeric species of Aβ that is responsible for neuronal dysfunction and synaptic loss. Furthermore, we propose that the formation of the toxic oligomeric species occurs via dityrosine crosslinking of the Aβ peptide (dY-Aβ), the process which is brought about by an oxidative modification reaction. It is a fundamental question in understanding Aβ neurotoxicity to test a bona fida dimeric Aβ with no monomer present. The complexity of synthesizing dityrosine-containing dimeric Aβ peptides has hampered research to directly test its toxic effects. The task of synthesizing an 84 amino acid peptide is a very difficult process and requires novel chemistry paradigms and experience in handling the Aβ peptide. We have developed novel methods for successfully synthesising the dY-Aβ dimer peptides containing up to 84 residues with high yields of pure peptides. To date, we have successfully synthesized and purified a number of Aβ monomers and respective dY-Aβ dimers and control DAP-Aβ dimers (this is the control dimer peptide using the 2,6-diaminopimelic acid in place of dityrosine during the synthesis of the Aβ dimer). During the past five years, we have successfully investigated a number of mutant and modified Aβ peptides using a combination of neurotoxicity, synaptotoxicity, aggregation, biophysical, and biochemical assays in order to decipher the mechanisms of Aβ neurotoxicity. We now plan to subject the novel dYAβ peptides to a number of these well established assays in our laboratories and we will present some of the preliminary findings that we have collated so far.

POS-TUE-204

STUDIES ON THE MECHANISM OF HEPARIN-INDUCED STIMULATION OF PROBACE1

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The β-site APP cleaving enzyme (BACE1) is responsible for the first step in production of the β-amyloid protein (Aβ). BACE1 is synthesized as a partially active zymogen (proBACE1). We previously showed that heparin can increase the enzyme activity of proBACE1. In this study, the structural requirements and mechanism for the heparin-induced activation were examined. The effect of heparin on proBACE1 was influenced by the degree of sulfation ($p < 0.001$) and carboxylation ($p < 0.001$), as well as by the length of the sugar ($p < 0.005$). Although low molecular weight heparin fragments did not strongly stimulate proBACE1, they inhibited heparin-induced activation of the enzyme ($p < 0.001$). The zymogen structure was modeled using a known X-ray structure of the protease domain. The modeled structure suggested that a heparin-binding domain may reside near the prodomain, and that movement of loop region (residues 46 – 65) lying adjacent to the prodomain may be needed to accommodate heparin binding. This loop domain lies adjacent to the active site and may block access to the active site. The movement of this loop region upon heparin binding could expose the active site region to allow for increased substrate binding. The results suggest a model in which conformational changes close to the prodomain may be involved in the mechanism of heparin-induced activation.

POS-TUE-205

CELLULAR MODELS TO INVESTIGATE THE EFFECT OF OXIDATIVE STRESS ON BACE1

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Background and aim: Alzheimer's disease (AD) is associated with plaques consisting of Aβ peptides. BACE1 (β-site APP cleaving enzyme 1) initiates Aβ generation in neurons by cleaving the amyloid precursor protein (APP). BACE1 is elevated in AD brain cortex, and studies have shown that it is elevated in cells during oxidative stress (OS); however how endogenous BACE1 might be accumulated within cells remains unclear. The aim is to study BACE1 in cell culture in an OS condition. **Methods:** SH-SY5Y cells were cultured to 70% confluence and treated with 0, 100, 200 and 400μM H_2O_2 for 3h or with 1mM buthionine sulfoximine (BSO) for 24h. Protein carbonyl detection was used to measure OS in H_2O_2-treated cells, whereas OS by BSO treatment was measured using 2',7'-Dichlorofluorescein (DCFH). Cell lysate was separated by SDS-PAGE and analysed by Western blotting. Statistical analyses were performed with the SPSS software (significance when $p<0.05$). **Results:** H_2O_2 treatment resulted in a dose-dependent increase in protein carbonyl levels (n=6), however no statistical significance was obtained. Accordingly, the increase in BACE1 levels (n=12) were not significant. BSO was able to significantly induce OS (n=6), but with no change in BACE1 levels (n=6). **Conclusion:** Inducing OS in SH-SY5Y cells did not increase BACE1 levels. Studying of BACE1 in the context of OS may be better carried out in differentiated neurons. We are now investigating effect of OS on BACE1 in mouse primary cortical neurons.

POS-TUE-206

SCREENING OF SPHINGOLIPID BIOSYNTHESIS INHIBITORS AS AMYLOID-BETA MODULATORS

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Alzheimer's disease (AD) is characterized by cerebral amyloid deposits composed of the amyloid-beta peptide (Abeta) which is released by proteolytic processing of Abeta precursor protein (APP). Amyloidogenic processing of APP occurs within cell membranes in lipid raft microdomains that are enriched with glycosphingolipids (GSLs), sphingomyelin (SM) and cholesterol. It is established that inhibition of cholesterol biosynthesis suppresses Abeta production, whether modulation of cellular sphingolipids regulates Abeta production is not clear. CHO cells that constitutively express human wild type APP695 were employed to screen the impact of sphingolipid synthesis inhibitors on APP processing. Based on western blot analysis of secreted Abeta and soluble APP alpha (sAPPa), we demonstrate that a panel of inhibitors of glucosylceramide synthase: PDMP (D,L-threo-ethylenedioxy-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol), PPMP (D,L-threo-l-Phenyl-2-hexadecanoylamino-3-morpholino-propanol), EtDO-P4 (D,L-threo-ethylenedioxy-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol) and NB-DNJ (N-Butyl-deoxynojirimycin), reduced extracellular Abeta concentrations with approximate IC50 values of 0.020, 0.005, 0.001, 0.100 mM, respectively. In contrast to the well-characterized gamma-secretase inhibitor, DAPT (N-(3,5-Difluorophenyl)acetyl-L-alanyl-2-phenyl glycine-1,1-dimethylethyl ester, used at a concentration of 0.1 microM), GSL inhibitor compounds did not significantly increase extracellular levels of sAPPa or intracellular levels of the APP C-terminal beta (CTFbeta) fragment. We also found that there was a significant reduction of Abeta secretion after cells were treated with myriocin (2-amino-3,4-dihydroxy-2-(hydroxymethyl)-14-oxo-6-eicosenoic acid), a specific serine palmitoyl transferase inhibitor which reduces cellular GSLs and SM, or with D609 (tricyclodecan-9-yl-xanthogenate), or a SM synthase inhibitor (IC50 values of 0.100, 0.300 mM, respectively). These results indicate the potential to develop anti-amyloidogenic compounds via targeting sphingolipid biosynthesis.

POS-TUE-207

INVESTIGATION OF MATRIX METALLOPROTEINASE AS A BLOOD BIOMARKER FOR ALZHEIMER'S DISEASE

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Pathological changes in the Alzheimer's disease (AD) brain include the aggregation of A-beta and Tau proteins (in the form of plaques and tangles respectively), as well as neuronal death and synaptic loss. Matrix metalloproteinase -9 (MMP-9) and MMP-2 are able to degrade A-beta and their expression is increased in astrocytes near amyloid plaques. Similar changes may be reflected in plasma. We studied MMP-9 and MMP-2 activity in a well characterised plasma cohort consisting of healthy controls (HC, n=26), Mild cognitive impairment (MCI, n=26), AD (n=26), Dementia with Lewy Bodies (DLB, n=12) and Frontotemporal Lobar Degeneration (FTLD, n=9) groups. Using zymography, MMP-9 and MMP-2 activity were detectable in all of the plasma samples. A significant decrease in MMP-2 activity was found in the AD group compared to the HC ($p<0.05$), though no significance was found for MMP-9 activity between the groups. Thus, in AD patients, a decrease in MMP-2 activity in plasma might indicate an impairment in A-beta degradation, which could contribute to the pathogenesis of AD. This study shows promise for MMP-2 to be used as a biomarker in plasma, which could aid in the early diagnosis for AD.

POS-TUE-208

THE UNIQUE C-TERMINAL REGION OF SECRETED AMYLOID PRECURSOR PROTEIN ALPHA IS CRUCIAL FOR NEUROPROTECTION

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Alzheimer's disease (AD) is characterised by increased concentration and aggregation of the toxic *'sticky peptide'* amyloid beta (Aβ), and reduced concentrations of the neuroprotective protein secreted amyloid precursor protein alpha (sAPPα). In the healthy brain the pathways for Aβ and sAPPα production are balanced but in AD this equilibrium is disrupted. sAPPα differs from sAPPβ, produced in the Aβ pathway, by having its C-terminus longer by 16 amino acids, and yet it is up to 100-fold more potent than sAPPβ in conferring protective effects on neurons. The exclusive C-terminal sequence of sAPPα is strongly hydrophilic (11/16 residues) and has a number of sites that might be functionally important for neuroprotection, for example the consecutive histidines that may form a metal-binding site, a basic charged terminal amino acid, and a postulated heparin-binding domain (VHHQK). Stable cell lines that expressed substitution and deletion variants of sAPPα at potentially important sites were generated, and the recombinant proteins purified from the cultured cell media using a one-step purification. They were tested for their ability to protect human neuroblastoma cells (SH-SY5Y) against hypoglycaemic insult, compared with wild-type sAPPα. sAPPα was able to protect the cells from the adverse effects of hypoglycaemia in a number of protocols, while the variants had different activities ranging from 30% less potent to being significantly toxic to the cells ($n\geq6$, $p<0.05$). A peptide corresponding to the exclusive C-terminus of sAPPα mimicked the protection offered by sAPPα, but protection was independent of sequence order, highlighting the importance of the hydrophilic character of this region (n=3). This study confirms that the C-terminal region of sAPPα has a role in neuroprotection.

POS-TUE-209

EFFECT OF DIRECT CONTACT WITH Aβ ON NEURITE OUTGROWTH IN AN IN VITRO MODEL OF DYSTROPHIC NEURITES

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Alzheimer's disease (AD) is a progressive neurological disorder, and is the most common form of dementia in the elderly. One of the key pathological features in AD is the presence of dystrophic neurites surrounding amyloid plaques, which is thought to contribute to the cognitive decline in AD. In this study, an in vitro assay was created to examine the effect of direct contact of hippocampal neurons with a substrate-bound amyloid beta (Aβ) deposit. Evidence strongly suggests that Aβ is the main neurotoxic agent underlying AD. Hippocampal neurons were seeded on and around Aβ deposits and morphological features were compared using immunocytochemical staining techniques between neurons that were in contact with and neurons that were not in contact with the Aβ deposit. This study showed that contact with Aβ40 and Aβ42 induced the collapse of growth cones ($P<0.0001$ for both Aβ40 and Aβ42) and increased pathogenic tau species phosphorylated at ser202/thr205 ($P<0.0001$ for both Aβ40 and Aβ42). Neuronal contact with Aβ42, but not Aβ40, increased the level of the neurodegenerative marker, ubiquitin ($P<0.0001$). A calcium imaging technique was also used to examine the effect of Aβ on glutamate activity, and revealed that neurons grown in contact with substrate-bound Aβ deposits exhibited an altered sensitivity to glutamate. These neurodegenerative changes are similar to those reported in dystrophic neurites observed in vivo and in vitro. Therefore, the Aβ-contact assay was found to be a robust model which may be useful for studying the mechanisms of neuritic dystrophy in vitro.

POS-TUE-210

CALCIUM OSCILLATIONS IN GLIAL PRECURSORS INDUCED BY AMYLOID β

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Alzheimer's disease is caused by the amyloidogenic peptide, Aβ, which causes dysregulation of calcium in neurons and astrocytes. Excitotoxicity associated with disrupted calcium signalling is thought to underlie the symptoms and pathology of Alzheimer's disease. However, the mechanism of Aβ-induced calcium influx in brain cells is unknown. Recent work in our lab has shown that an amyoidogenic form of transthyretin, which causes familial amyloidotic polyneuropathy, triggers calcium influx into dorsal root ganglion cells via a transient receptor potential (TRP) channel. We therefore asked whether Aβ acts in a similar manner to induce calcium dysregulation in CNS-derived cells. We grew cultures of dissociated cortical cells isolated from newborn mice for 1 week in vitro, and then treated the cultures with 1μM oligomeric $Aβ_{1-42}$ for 2-6h. Cells were loaded with 2μM Fluo-4 AM and calcium fluorescence images were captured on a Zeiss LSM 510 confocal microscope. Nearly twice as many cells in $Aβ_{1-42}$-treated cultures displayed dynamic calcium activity compared to vehicle-treated control cultures (155% of control, n=8 cultures). $Aβ_{1-42}$ induced a shift from a spontaneous pattern of calcium activity to a regular spike pattern (55% of active cells vs 33% in vehicle-treated cultures). Immunostaining of imaged cells revealed that this was not attributable to MAP2+ neurons or GFAP+ astrocytes, and that significant populations of glial precursors were present in the cultures. Live staining suggests that the Aβ-responsive cells may be NG2 cells. In conclusion, $Aβ_{1-42}$ induced an increase in the number of cells in cortical cultures with active calcium signalling, and promoted a pattern of regular spike activity. These active cells may be glial precursors, possibly NG2 cells.

POS-TUE-211

POST-SYNAPTIC SCAFFOLD PROTEIN LOSS CORRELATES WITH ALZHEIMER'S DISEASE PATHOLOGY

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Cognitive decline in Alzheimer's disease (AD) conforms strongly to region-specific synapse loss. In brain areas affected by AD, a concurrent decline in NMDA receptors has been reported. Glutamate-evoked excitotoxicity mediated through over-stimulation of NMDA receptors may contribute to synaptic degeneration in AD. NMDA receptor expression and activity at the synapse is highly regulated through binding to the post-synaptic scaffold proteins PSD-93, PSD-95, SAP-97 and SAP-102. Altered scaffolding could underlie NMDA receptor subunit loss in AD. Using absolute quantification Real-Time PCR, we detected no significant differences in expression of scaffolding protein mRNA transcripts between AD cases (n=13) and controls (n=12), in the pathologically affected hippocampus (Hip) and inferior temporal cortex (ITC). In contrast, the pre-synaptic protein synaptophysin was markedly less abundant in the ITC on post-hoc testing ($P<0.05$), consistent with previous reports. No differences in the expression of any of these transcripts were found in the occipital cortex (OC), a region spared from marked cell loss, between AD cases and controls. Proteins were precisely quantified against recombinant truncated protein standards. A significant decline in PSD-95 and SAP-102 protein expression was observed in the ITC on post-hoc testing ($P<0.05$), between AD cases (n=15) and controls (n=15) and for SAP-102 in the OC ($P<0.05$). Both transcript and protein levels declined significantly with the progression from absence of AD neuropathology to moderate stages ($P<0.001$). PSD-93, PSD-95 SAP-97 and SAP-102 immunohistochemical staining in Hip, ITC and OC sections, revealed differences between AD (n=6) and controls (n=6). Our data suggest a possible mechanism for reduction in NMDA receptor subunit expression in AD ITC. This research provides further understanding of the excitotoxic pathology of AD at the molecular level.

POS-TUE-212

NEUROPATHOLOGY OF SENILE PLAQUES AND EXPERIMENTAL HAEMORRHAGIC LESIONS: TEST OF THE MICROVASCULAR HYPOTHESIS OF PLAQUE FORMATION IN HUMAN BRAIN

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A key unresolved issue in understanding age-related dementia (ARD, Alzheimer's disease) is the cause of formation of the senile plaques prominent in the ARD brain. This study tests the hypothesis that plaques form at sites of capillary haemorrhage. Small surgical lesions were made in neo- and hippocampal cortex of young adult rat brain (n=20), and the resulting pathology was examined after 1, 3, 7, 14, 19 and 30d, for haemorrhage (the Perl reaction), cell death (the TUNEL technique) and, using immunohistochemistry, for gliosis, neuronal survival, synaptic density, and the levels and location of amyloid precursor protein (APP) and Aβ. Neocortex from the superior parietal and superior temporal lobes from ARD cases (n=4) was also examined, with the same techniques. Evidence of haemorrhage was found both at experimental lesions and in human plaques; neurones and synapses were depleted at both; micro- and macroglia (astrocytes) invaded both; small, autofluorescent extracellular deposits formed at both. Dying cells were detected only at the experimental sites within a few days of lesion. Aβ was prominent extracellularly at the core of human plaques, in the human plaques and was expressed intracellularly, in neurones and neuroglia around the experimental lesion. These two differences between the experimental lesion sites and human plaques may reflect the relative maturity of plaques, which were observed in the post-mortem brain, as long as 7 years after diagnosis, whereas the experimental lesions were < 1 month old. The similarities give support to the idea that plaques form at the sites of intracerebral haemorrhage and that ARD may, therefore, be a vascular dementia.

POS-TUE-213

DECREASES IN RECEPTOR-ASSOCIATED PROTEIN IN ALZHEIMER'S DISEASE HUMAN BRAIN TISSUE CORRELATE WITH INCREASED PLAQUE LOAD

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Beta amyloid (Aβ) accumulation is a critical event underlying the pathogenesis of Alzheimer's disease (AD). Previous studies have shown that the receptor-associated protein (RAP) assists in maturation of a number of proteins involved in metabolism of the beta-amyloid precursor protein. In addition, RAP binds Aβ and can inhibit its neurotoxic effects *in vitro*. However, no studies have assessed the association between RAP and Aβ deposition in AD brain tissue. Brain tissue from 7 AD and 8 age-matched control cases with short postmortem delays was obtained from the Prince of Wales Medical Research Institute Brain Bank. Ten µm sections were cut from paraffin-embedded formalin-fixed tissue blocks of the hippocampus and peroxidase immunohistochemistry performed using anti-RAP (mouse monoclonal 7F1) and anti-Aβ (IE8) with cresyl violet counterstaining. All cases were staged for the severity of Aβ plaque deposition according to CERAD criteria. Analysis revealed that most RAP immunoreactivity was neuronal and intracellular. RAP-immunoreactive and immunonegative CA1 neurons were counted in standardised samples (11x11 eye piece grid at 200x magnification, 5% rater agreement) of the hippocampus and the percentage of RAP-immunoreactive neurons calculated. Analysis revealed 89 ± 3% (± SEM) of control CA1 neurons contained RAP immunoreactivity whereas only 38 ± 11% of AD CA1 neurons were RAP-immunopositive (t-test p= 0.003). The numbers of RAP-immunoreactive neurons negatively correlated with the severity of Aβ plaque pathology (Spearman correlaton = -0.8, p=<.0001). These data demonstrate significant decreases in neuronal RAP in association with Aβ deposition in AD brain, supporting the concept that RAP is protective against Aβ toxicity and aggregation.

POS-TUE-214

CHANGE IN GLUTATHIONE EXPORT PARAMETERS BY ACTIVATED ASTROCYTES - A NOVEL TARGET FOR ALZHEIMER'S DISEASE?

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Purpose: Astrocytes possess numerous neurosupportive functions, including the synthesis of glutathione (GSH). In pathological conditions, astrocytes are able to switch from metabolic support cells, to immunologic cells, capable of producing free radicals and cytokines, which can further exacerbate pathological conditions, including neurodegenerative disorders such as Alzheimers disease (AD). Since AD is characterized by astro- and microglia activation, it is proposed that reduced astroglial GSH export could render neurons vulnerable to inflammatory and oxidative attack, possibly contributing to neurodegeneration. **Methods**: Thiols were measured by HPLC after pre-column derivitisation with 4-aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole. Cell viability was measured by the resazurin assay. **Results:** U373MG human astroglia were activated using various concentrations of the proinflammatory cytokines, IL-1β and TNF-α. It was shown that cytokine-activated astroglia initially (2-20hrs) increase the export of GSH in a cytokine concentration-dependent fashion (10 ng/ml IL-1β+TNF-α induced increased GSH export compared to 0-1 ng/ml; n=9). After extended activation (30-72hrs) a decrease in GSH export was observed in response to 10ng/ml IL-1β+TNF-α compared to the untreated control and to astroglia treated with lower concentrations (0.01-1 ng/ml; n=9). This decrease in astroglial GSH export is accompanied by a depletion of intracellular GSH. Furthermore, pre-treatment of astroglia with lipoic acid significantly increased GSH efflux, even in astroglia challenged with 10 ng/ml IL-1β+TNF-α (n=6). Additionally, astroglia-conditioned media is able to protect SHSY5Y neurons against hydrogen peroxide toxicity in a manner that correlates with astroglial GSH export (n=6). **Conclusion:** Therefore, low grade inflammation and lipoic acid increase astroglial GSH export and may be able to attenuate neuron death in AD and thus help slow the progression of the disease.

POS-TUE-215

TAU DEPOSITION CORRELATES WITH INFLAMMATION IN ALZHEIMER'S DISEASE

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Monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) are consistently upregulated in Alzheimer's disease (AD) brain and thought to play a role in disease pathogenesis. The major stimulator/s for this inflammatory response remain unclear. The aim of this study was to determine which major AD pathology (Aβ plaque, hyperphosphorylated tau, or neuronal loss) most closely associates with MCP-1 and IL-6 containing cells. Brain tissue samples from the inferior temporal cortex of 8 AD, 6 cases with mild cognitive impairment (MCI) and controls with either Aβ deposition, tau deposition, or no neuropathology (n = 22) were obtained from the Prince of Wales Medical Research Institute Brain Bank. Ten µm sections were stained (peroxidase as well as fluorescence double-labelling) using antibodies against MCP-1 (anti-human MCP-1), IL-6 (anti-human IL-6) and tau (AT8) counterstained with cresyl violet for cellular quantitation. Neuronal density was determined in five counting frames in cortical layer III, and the proportion of immunoreactive neurons determined. Significant neuronal loss was found in AD (154 ± 16/mm^2) and MCI (140 ± 6/mm^2) compared to controls (254 ± 14/mm^2, Kruskal Wallis, posthoc p≤0.05). Immunoreactive glia (semi-quantitatively scored 0-3), cored and non-cored plaques (density and areal fraction) were also assessed. AD cases demonstrated a significant increase in MCP-1- and IL-6-immunoreactive glia compared with controls (average 200% increase, Kruskal Wallis, posthoc p≤0.05). Stepwise multiple regression analyses (p≤0.05) revealed that increasing MCP-1- and IL-6-immunoreactive glia most strongly and consistently correlated with increasing tau. Double labelling demonstrated a relationship between glial activation and neuritic pathologies. These results suggest that tau neuritic changes underlie the increased expression of MCP-1 and IL-6 immunoreactive glia in AD brain tissue.

POS-TUE-216

GENERATION OF TAU-PROMOTER REPORTER CELLS FOR HIGH THROUGHPUT SCREENING

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The microtubule-associated protein tau is the major constituent of the paired helical filament, the main fibrous component of the neurofibrillary lesions of Alzheimer's disease. Its expression is neuronal specific and developmentally regulated. In this study, we generated two luciferase reporter vectors. The expression of luciferase is under the control of tau promoter (pTAU) and ubiquitin C promoter (pubc) respectively. Both constructs were stably expressed in SH-SY5Y and COS-7 cells by lentiviral gene transfer. Reporter cells were analysed by Western blot, immunocytochemistry and a luciferase activity assay. Using luciferin as substrate to determine luciferase activity is well-established, sensitive and effective, and therefore renders this system ideal for high throughput screening. The cell lines are currently used in a large array drug screening to identify compounds that can regulate tau expression. Candidate compounds will then be tested in transgenic mouse models to establish potential usage for treatment of diseases with tau pathology, such as Alzheimer's disease.

POS-TUE-217

QUINOLINIC ACID IN AIDS DEMENTIA COMPLEX WITH IMPLICATIONS FOR AN ALZHEIMER LIKE PATHOGENETIC COMPONENT

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Human immunodeficiency virus-1 (HIV-1) infection of the central nervous system (CNS) can induce a dementing syndrome known as AIDS dementia complex (ADC). The mechanism remains poorly understood, evidence suggesting a relationship to Alzheimer disease (AD). The predominant pathogenesis of ADC is believed to involve activation of brain infiltrating macrophages and microglia. Quinolinic acid (QUIN) is an end product of tryptophan catabolism through the kynurenine pathway (KP) and is produced by activated macrophage/microglia. QUIN acts as an endogenous brain excitotoxin that leads to neuronal dysfunction. The present study investigated the frequency of QUIN, activated macrophage/microglia, and AD markers: amyloid beta (Aβ) and Tau in the hippocampus of 10 HIV-infected individuals. $QUIN^+$ staining was found in 70% (7/10) HIV^+ cases. Activated microglia/macrophages were 100%(10/10). Intra-neuronal Aβ deposits were prevalent in all cases 100% (10/10); with four case 40% (4/10) having Aβ plaque. Hyperphosphorylated Tau deposition was found in 30% (3/10) in all cases. Furthermore, using double staining, the infection of microglia/macrophage was demonstrated by detecting HIV p24 protein in microglia/macrophage in all cases. These microglia/macrophages were observed within and between $QUIN^+$staining. HIV $p24^+$ staining was also shown with Aβ plaque or Tau staining in some cases. Interestingly, $QUIN^+$ staining was co localised with either Aβ plaque or Tau staining in these cases. We conclude that HIV-infected or immune-stimulated microglia/macrophage produce QUIN, and that there is a convergent pathogenetic mechanism with Alzheimer disease.

POS-TUE-218

MICROTUBULE-ASSOCIATED PROTEIN TAU PHOSPHORYLATED AT SPECIFIC RESIDUES IS RECRUITED TO ADF/COFILIN-ACTIN NEURITIC AGGREGATES: PATHOGENIC MECHANISMS IN EARLY ALZHEIMER'S

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Alzheimer's disease (AD) is a devastating neurodegenerative disease characterised by three neuropathological hallmarks: neurofibrillary tangles, striated neuropil threads, both of which are comprised of phosphorylated microtubule-associated protein tau, and β-amyloid plaques. Almost always associated with tau and β-amyloid AD pathologies are aggregates of the actin-associated protein cofilin, which take on a characteristic rod-like structure similar to tau neuropil threads. While mechanisms underlying initiation of the disease remain poorly understood, increasing evidence suggests reduced cellular metabolism and mitochondrial function to be critical early events in the pathogenesis of sporadic AD. In recent work, we have demonstrated that mitochondrial inhibition in primary neuron culture triggers rapid activation of the actin-associated proteins cofilin and actin depolymerizing factor (ADF) which subsequently recruit and co-localize with phosphorylated tau. The resulting rod-shaped aggregates bear striking resemblance to the cofilin aggregates and tau neuropil threads observed in human AD brains. We are now interested in characterizing the phosphorylation patterns of this recruited tau, with emphasis on the Alzheimer-related phospho-epitopes. RESULT: Here we demonstrate that recruitment of tau to ADF/cofilin-actin aggregates is indeed dependent on its phosphorylation at specific residues. Interestingly, we observe a predominant recruitment of tau phosphorylated in the Microtubule Binding Domain (Ser262/Ser356), epitopes widely regarded as the first to be phosphorylated in AD. We propose that the formation of rod inclusions containing ADF/cofilin and phosphorylated tau most probably represent a very early event in AD neurodegeneration and as such, this work identifies a useful target for the development of AD therapeutics and treatment.

POS-TUE-219

N-CADHERIN, β-CATENIN AND GEPHYRIN LEVELS WITH PATHOLOGICAL SEVERITY IN ALZHEIMER DISEASE

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Alzheimer disease (AD) shows regional-specific loss of neurones and synapses. N-cadherin is a synaptic junction protein which bridges pre- and postsynaptic excitatory terminals via β-catenin attachment to the cytoskeleton. Gephyrin is an inhibitory postsynaptic scaffolding protein which associates with $GABA_A$ and glycine receptors. Brain tissue from pathologically confirmed AD and control cases was obtained at autopsy with informed written consent and frozen at $^-$80°C in 0.32 M sucrose. Areas investigated included hippocampus and inferior temporal cortex, which are susceptible to AD pathology, and occipital cortex, which is relatively spared. Levels of N-cadherin, β-catenin, and gephyrin were measured by "in-gel" immunodetection on crude membrane preparations of frozen human brain tissue. Significantly higher levels of N-cadherin and β-catenin were observed in AD cases (n = 15) than in controls (n = 15), particularly in the hippocampus. Gephyrin levels were significantly reduced in AD cases compared with controls. Cases were scored according to pathological severity accounting for neuronal loss, tangle and plaque load, and gliosis. A score of 0 indicated no pathology; 1, mild or modest pathology; 2, moderate pathology; and 3, severe pathology, for each area studied. N-cadherin and β-catenin levels showed a significant, positive correlation with increasing pathological score. The levels of synaptophysin and GFAP were measured by indirect ELISA, to gauge the extent of synapse loss and reactive astrocytosis respectively. Synaptophysin levels were significantly lower, and GFAP levels were significantly higher in susceptible areas of AD cases than controls. GFAP levels correlated strongly and positively with increasing pathological severity, but synaptophysin correlated negatively and poorly with increasing pathological severity. These results could indicate a dysfunction of excitatory synapses in AD with differential pathology.

POS-TUE-220

CHARACTERISATION OF BRAIN DERIVED APOLIPOPROTEIN-E PEPTIDES USING IMMUNOPRECIPITATION AND MASS SPECTROMETRY

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Apolipoprotein-E (apoE) is a polymorphic protein with an important role in neurobiology. There are three major isoforms of apoE (E2, E3, E4) and possession of the epsilon 4 allele is the greatest genetic risk factor for late onset Alzheimer's disease (AD). Previous work by our group has demonstrated that apoE undergoes proteolysis in the human brain and is present as a series of tris buffered saline (TBS) soluble peptides. ApoE proteolytic peptides were detected at significantly greater concentrations in apoE3 subjects compared to apoE4 subjects and this was independent of AD status. In the current study, immunoprecipitation was used to purify several apoE peptides from the brain homogenate of an apoE3 subject. The amino acid sequences of these peptides were deduced using mass spectrometric analysis. Currently, the two most abundant peptides, of approximately 25 and 26.5 kDa, have been characterised in detail with respect to the presence or absence of key functional regions of the apoE protein. The lipid-binding C-terminal domain was absent from the 25 kDa peptide, but present in the 26.5 kDa peptide, whereas the N-terminal domain was completely intact in the 25 kDa peptide and partially present in the 26.5 kDa peptide. Interestingly, the LDL-receptor binding region of the N-terminal domain was present in both peptides and the protease-sensitive inter-domain hinge region of apoE was intact only in the 26.5 kDa peptide. This data suggests that the major apoE fragments detected in the human brain are N-terminal peptides that contain the functionally important LDL receptor-binding region. The sequence analysis information will also be useful for identifying the enzymatic cleavage sites, and thus the responsible enzymes, for apoE proteolysis in the brain.

POS-TUE-221

COMPARTMENTALIZED EXCITOTOXICITY IN PRIMARY MOTOR NEURONS: A NOVEL MODEL OF ALS PATHOLOGY

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by distal axon degeneration prior to symptoms. Excitotoxicity has been implicated as a major factor in ALS pathogenesis. We have linked excitotoxicity in primary motor neurons to ALS-like distal axon degeneration. It remains unclear if toxicity is directed through the distal axon or somatodendritic compartment. To investigate the site of toxicity we have constructed an in vitro model utilising compartmentalized chambers. Growth of primary cortical neurons in these chambers leads to distinct somatodendritic and distal axon separation as evidenced by immunolabelling with MAP2 (dendritic marker) and tau (axonal marker). Cultures within the chambers (n=5) were focally exposed to glutamate (100μM, 24 hours). Somatodendritic treated neurons demonstrated degeneration of axons and dendrites, whilst axon treated neurons did not. However, evidence of distal axon synaptic maturity was absent in this paradigm. To further develop this model and investigate the role of the cell body/astrocyte and distal axon/muscle interactions in development of axonopathy, chambers were utilised such that motor neuron cell bodies were grown on astrocytes with the distal axon terminals on a substrate of primary skeletal muscle cells (from neonatal rats). This model mimicked many of the interactions of the lower motor neuron, including formation of rudimentary neuromuscular-synapses. Motor neuron growth on different substrates was determined, with significantly ($p<0.05$) diminished survival seen on muscle feeder layers. Mature (21DIV) cultures were subsequently exposed to chronic kainic acid (25μM, 24 hours), resulting in a significantly ($p<0.05$) increased loss of neurons co-cultured with astrocytes in comparison with the other substrates. These data suggests that non-neuronal cells can contribute to neuronal vulnerability to excitotoxicity. Further manipulations of this model may reveal skeletal muscle involvement.

POS-TUE-222

HYPOTHALAMUS IN BEHAVIOURAL-VARIANT FRONTOTEMPORAL DEMENTIA

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Behavioural-variant frontotemporal dementia (bvFTD) is a progressive neurodegenerative brain disorder, characterised clinically by changes in behaviour (including feeding and sleeping disturbances) and cognition. The hypothalamus plays a critical role in homeostatic body functions such as feeding and sleeping, yet neuropathology in this region has not been assessed previously in bvFTD. We aimed to determine the severity of atrophy and cell loss in the hypothalamus in bvFTD, and the relations to feeding and sleeping behaviours. Two cohort studies were performed. Study 1 investigated early bvFTD cases (N=18) and matched controls (N=16) who had undergone structural MRI. Information on feeding and sleeping habits was collected from carer questionnaires. Hypothalamus volumes were traced manually on coronal images. Study 2 investigated postmortem bvFTD cases (N=12) and matched controls (N=6). Fixed coronal hypothalamic tissue blocks were serially sectioned and stained for Nissl substance and immunohistochemically for peptides regulating feeding and sleeping behaviours. Unbiased stereological estimates of hypothalamus volume and the number of neurons and glia were performed. Significant atrophy of the hypothalamus in bvFTD (posterior > anterior) was present in both studies. Behaviourally, patients with high sleep disturbance had significantly reduced anterior hypothalamus whilst patients with high feeding disturbance exhibited significantly reduced posterior hypothalamic volume. Neuronal loss, which was observed only in bvFTD cases with TDP-43, was predominant posteriorly and related to the degree of atrophy. Importantly, however, orexin neurons in the lateral hypothalamus that regulate feeding were spared. These data suggest that feeding disturbances in bvFTD indicates TDP-43 pathology and relates to posterior hypothalamic atrophy caused by neuronal loss.

POS-TUE-223

NEURONAL LOSS IN THE CINGULATE CORTEX IN FRONTOTEMPORAL DEMENTIA

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Frontotemporal dementia (FTD) has clinical variants that are characterised by behavioural (behavioural-variant FTD, bv-FTD) or language (semantic dementia, SD) impairments. The anterior cingulate cortex (ACC) is known to be affected early in bv-FTD, but regional vulnerability in other cingulate cortices and other FTD subtypes has not been evaluated. With approval from institutional ethics committees, paraffin-embedded samples were obtained from cases with cortical volume measurements from the POWMRI and Cambridge University Brain Banks. Samples from the ACC (Brodmann area 24) and posterior cingulate cortex (PCC; BA 23) were cut at 10μm and stained with cresyl violet and immunohistochemically for inclusions (AT8-tau and TDP-43). Non-biased quantitative techniques were used to estimate the neuronal number in bv-FTD (n=12), SD (n=6) and control (n=10) subjects. In the ACC, both groups showed an average 60% reduction in total neuron number compared with controls ($p<0.01$). In SD the PCC was similarly affected ($p<0.01$). However in bv-FTD the reduction failed to reach significance because of the wide variation in number between cases (16-61 million). This wide variation was also reflected in the volume measurements for these regions (2.2-5.9 mL). With respect to inclusion pathology, the ACC exhibited more inclusions than the PCC suggesting neuronal dysfunction in addition to neuronal loss. The greater damage to the PCC in SD may contribute to the different symptoms observed in SD compared with bv-FTD. The findings also reinforce the involvement of the ACC in FTD syndromes.

POS-TUE-224

INCREASED IRON RELATED MR PHASE SIGNALS IN THE STRIATUM IN HUNTINGTON'S DISEASE: A NOVEL MR NEURODEGENERATIVE BIOMARKER

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Introduction: Our objective was to investigate novel in-vivo biomarkers of neurodegeneration in Huntington's disease (HD) for use in future clinical drug trials. MR phase signals are produced by magnetic susceptibility changes and can be used as an MR surrogate measure of iron concentration increases known to occur in HD. We have developed a novel method to investigate and quantify iron concentration in the basal ganglia of HD patients. **Patients and Methods:** Ten symptomatic HD patients (mean UHDRS 27.3) and ten controls were scanned using a T2* imaging sequences on a 3T MRI. The T2* scans were reconstructed using a complex image optimised reconstruction method to produce magnitude and phase images. For each phase image, the corpus callosum, caudate, globus pallidus and putamen were segmented manually. For voxels in each region the X84 outlier rejection rule was applied and the median phase signal was computed (Hz/Tesla). A univariate analysis of variance was computed to identify significant differences between groups. Partial correlations were computed between phase signals and relevant neurological scores, including: UHDRS, HADS, STROOP, SDMT, SCOPI, FrSBe and UPSIT. **Results** Compared to controls, there was a significant increase of the phase signal in HD patients in the globus palidus (+0.20 Hz/Tesla, $p=0.005$) and putamen (+0.25 Hz/Tesla, $p=0.01$). Moreover, there was a significant ($p < 0.005$) positive correlation between the UHDRS score and the phase signal in the putamen. **Conclusion:** We have developed a novel approach to investigate changes of iron concentration in-vivo. This one of the first studies to quantify iron levels in patients with HD suggesting that MR phase signals, as a surrogate measure of iron concentration, may serve as a potential biomarker of neurodegeneration in HD. The finding of a significant relationship between UHDRS motor score and iron concentration give this finding clinical applicability.

POS-TUE-225

THE EFFECT OF SOCIAL DEFEAT ON TYROSINE HYDROXYLASE PHOSPHORYLATION IN RAT BRAIN AND ADRENALS

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Tyrosine hydroxylase (TH) is regulated acutely by protein phosphorylation and chronically by protein synthesis. In these studies we aimed to investigate the phosphorylation of TH in rat brain and adrenals occurring within the first 24 hr in response to the social defeat stress. Intruders were exposed to social defeat (SD+) and then were sacrificed 10 min (n=6), or 24 hr (n=6), after the end point of the protocol. Sham intruders were not exposed to social defeat (SD-) but otherwise were treated identically to the intruders and were sacrificed 10 min (n=6), or 24 hr (n=6), after the end point of the protocol. Adrenals and brains were dissected and TH phosphorylation at serine residues 40, 31 and 19 (Ser40, Ser31 and Ser19) was analysed by western blotting. In the adrenals, pSer40 levels were significantly decreased at 24 hr (2 fold, $p<0.01$) but not at 10 min in SD+ animals compared to SD- animals. In the locus coeruleus and ventral tegmental area TH phosphorylation in SD+ animals was not significantly different from that in SD- animals at any time. In substantia nigra, pSer40 levels were significantly increased at 10 min (1.7 fold, $p<0.01$) and were significantly decreased at 24 hr (1.5 fold, $p<0.05$) in SD+ animals compared to SD- animals. We provide evidence for the first time that TH phosphorylation in rat adrenals and substantia nigra is modulated over time in response to social defeat and this may lead to changes in TH activity.

POS-TUE-226

INVESTIGATION OF THE LOSS OF ASTROCYTES AS THE INITIATION OF PARKINSON'S DISEASE

Tiong V. and Allbutt H.N. Department of Physiology, University of Sydney, NSW 2006.

The cause of sporadic Parkinson's disease (PD) is unknown. There is no evidence of any myelin involvement; there has been no insult to neurons demonstrated that is capable of producing the pattern of degeneration observed and the consensus of the literature is that the activation of microglia is secondary to the neuronal degeneration associated with the condition. One possibility that could give rise to the pathologies associated with the condition is the loss of astrocytes. Astrocytes are the support cells of the brain and have been shown to be vital to the continued function and survival of neurons. It is hypothesized that a loss of astrocytes is the initiating trigger for the pathologies associated with PD. The present study examined the loss of astrocytes as a possible initiating trigger of the pathologies associated with PD by ablating astrocytes in the substantia nigra pars compacta (SNc; n=24). The number of tyrosine hydroxylase labelled neurons (marker for dopamine producing cells) and the metabolic activity of the nucleus using cytochrome oxidase was examined. This group of animals was compared to animals treated with 6-hydroxydopamine (positive control for the loss of dopamine; n=19), saline injected sham (n=16) and naive (n=11) group of rats. The effect was examined at 28 days and 56 days survival time. We found that loss of astrocytes did not appear to alter the number of dopamine producing cells however there was a significant decrease in the level of metabolic activity in the SNc ($p<0.05$). It was suggested that this change in metabolism may reflect early changes involved in the initiation of PD and that this may prove useful in the study of early pathologies.

POS-TUE-227

LIPIDOMIC ASSESSMENT OF PARKINSON'S DISEASE BRAIN

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Parkinson's disease (PD) is a neurodegenerative disorder affecting approximately 1% of the population aged 65 to 69 years, rising to approximately 3% of the population aged 80 years and older. Lewy bodies are neuropathological inclusions found in PD brain that are enriched in aggregated alpha-synuclein (Asyn) and lipids. Asyn is predicted to play a role in lipid homeostasis and Asyn null mice display a CNS lipid phenotype. **Aim:** To determine the lipid profile of anterior cingulate cortex, amygdala, and occipital cortex of human Parkinson's disease and age-matched control brains and correlate any detected changes with expression of Asyn protein. **Methods:** Pilot studies examined the occipital cortex using electrospray ionisation mass spectrometry (MS), high-performance liquid chromatography (HPLC) and western blotting (to detect the different aggregated forms of Asyn). **Results:** Several major sphingomyelin and ceramide species were accurately quantified (SEM < 12%, n = 4) by MS. In total, 23 molecular sphingomyelins and 6 molecular ceramides were identified, with species containing stearate (18:0) or nervonate (24:1) dominating both classes. Cholesterol, alpha-tocopherol and dolichol species were clearly detected by reversed-phase HPLC and a three-step fractionation method was established that can quantify soluble and insoluble fractions of Asyn protein. **Conclusion:** This research establishes reliable MS and HPLC methods that will be used to conduct a lipidomic assessment of the major sphingolipid and sterol species in Parkinson's disease and control brains.

POS-TUE-228

DEVELOPMENT OF AN *IN VITRO* MODEL OF HUMAN DOPAMINERGIC NEURONS

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Parkinson's disease (PD) is a progressive degenerative disorder resulting from a degeneration of dopaminergic neurons in *Substantia Nigra*. The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. The KP is activated in several neuroinflammatory diseases and is likely to be involved in PD pathogenesis. A prolonged activation of the KP leads to production and accumulation of the potent excitotoxin quinolinic acid (QUIN). We hypothesise that the KP in human dopaminergic neurons will lead to the production of neuroprotective KP metabolites and that these neurons will be very sensitive to QUIN toxicity as we previously found in cortical neurons. We aim to establish an $^{in\ vitro}$ model of human dopaminergic neurons to characterize the KP in Tyrosine Hydroxylase^{+}(TH) neurons. We have differentiated human neuroblastoma SH-SY5Y (n=3) and SK-N-SH (n=3) cell lines using 3 different sets of treatments and then characterised them for neuronal and dopaminergic markers. Both cell lines treated with retinoic acid in with serum for 5 days followed by BDNF in serum free media for 5 days exhibited the morphology of dopaminergic neurons with fusiform and multipolar cell bodies and projecting dendrites. We will also assess expression of specific markers. This *in vitro* model will provide an important tool to study the involvement of QUIN in the death of TH^{+} neurons and also the neuroprotective ability of KP inhibitors as potential therapeutic for PD.

POS-TUE-229

SUBSTANTIA NIGRA DOPAMINERGIC CELL RESPONSE TO ROTENONE INDUCED OXIDATIVE STRESS IN VIVO

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Rotenone, a high-affinity inhibitor of the mitochondrial complex 1 electron transport chain causes oxidative damage in dopaminergic neurons via production of reactive oxygen species. We examine oxidative damage related changes in the neuronal and glial cell population of the substantia nigra in response to focal rotenone challenge. Adult male Sprague-Dawley rats (n=3/group) were injected with 6, 3, 1, 0.5, and 0.25µg of rotenone focally into the substantia nigra or the medial forebrain bundle and sacrificed 14 days post infusion. Treatment was compared to a sham and 6-OHDA infusion. Brains were analysed using immunohistochemical techniques to identify dopaminergic neurons, their projections, glial cells, synapses, oxidative stress, and cell stress. Infusion of 6, 3, 1, 0.5, and 0.25µg of rotenone into the substantia nigra causes extensive damage and tissue necrosis and therefore limited in its relevance as an oxidative stress disease model. Infusion of 0.5µg of rotenone targeting the medial forebrain bundle caused a reduction in TH and synaptophysin density in the striatum ($p \leq 0.01$), and a reduction in TH positive cells ($p \leq 0.01$) and an increase in GFAP and IBA-1 positive cells ($p \leq 0.01$) in the substantia nigra. Rotenone at 0.5µg also induced oxidative stress in dopaminergic neurons causing ongoing cell stress. Initial data in rats (n=18) infused with a low focal dose of rotenone produced a progressive reduction in striatal TH density over 60 days ($p \leq 0.01$). These findings are supportive of a chronic model of neurodegeneration, where cellular interactions and pathways involved in degenerating dopaminergic neurons may be elucidated.

POS-TUE-230

PARKINSON'S DISEASE PATIENTS CAN ADAPT TO PERTURBED VISUAL FEEDBACK WITH SUFFICIENT TRAINING

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Parkinson's Disease (PD) patients appear to have difficulty adapting goal-directed movement to perturbed visual feedback in the visuomotor adaptation task paradigm. The mechanisms through which this occurs is unclear. **Purpose:** (1) To investigate if PD patients can achieve visuomotor adaptation to performance levels of controls given sufficient training (2) Evaluate the mechanisms through which PD patients achieve visuomotor adaptation. **Methods:** All participants (14 PD, 14 age-matched controls and 14 young controls) were trained to similar performance levels (movement direction within 3 degrees of the ideal trajectory) in a target-reaching task during practice. Visual feedback of movement trajectory was shown after movement completion. During the test phase, visual feedback of movement trajectory was rotated 30 degrees counter-clockwise. Participants adapted by reducing error in movement direction (directional error). Participants completed 4 blocks of 25 adaptation trials. One no-perturbation trial was interleaved at the end of each block to assess aftereffects. Adaptation rate was calculated by the rate at which directional error decreased (degrees/trial). **Results:** Across all blocks, adaptation rate was fastest in young controls (m =3.40), followed by age-matched controls (m=2.58) and PD patients (m=0.86). Differences in adaptation rate between age-matched controls and PD patients reduced from 0.81 in Block 1 to 0.35 by Block 4. This indicates that PD patients adapt more slowly than controls, and may need more adaptation trials to achieve similar performance levels. Despite the absence of online visual feedback, PD patients may have used online kinesthetic feedback to correct movement error during adaptation. This is supported by the prevalence of submovements in PD movement trajectories, which were largely absent in controls. Submovements are believed to correct movement error online. **Conclusion:** Given sufficient exposure to perturbed visual feedback, PD patients can achieve visuomotor adaptation, however the mechanisms through which this is achieved may be different from controls.

POS-TUE-231

WNT PROTEINS IN REMODELING AND REPAIR OF THE MIDBRAIN DOPAMINE PATHWAYS

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Repairing the injured or diseased brain will most likely rely on similar guidance cues as necessary for development. Recently we showed that Wnts play an important role in the development of midbrain dopamine (DA) pathways. These DA pathways are affected in a number of neurological disorders including Parkinson's disease (PD). Understanding regulators of adult DA axon remodeling may open new avenues to promote repair, in particular cell replacement therapy (CRT) for PD. The role of Wnts in adult remodeling was investigated using models established in our lab that illustrate the plasticity of the DA axons. Chronic haloperidol treatment (a DA receptor antagonist) results in a 40% increase in DA axonal branching and synapses in the target striatum. We chronically administered haloperidol to adult tyrosine hydroxylase-GFP mice (TH-GFP mice, in which all DA neurons are GFP+), isolated and dissociated the ventral midbrain and sorted (FACS) DA neurons (GFP+) from other VM cells (GFP-). Using qRT-PCR analysis DA axonal sprouting was confirmed in haloperidol mice by an upregulation of Gap43 and Synaptophysin compared to control mice. Interestingly, a number of Wnts and Wnt related receptors were also upregulated. A limitation of CRT for PD is the inadequacy of the grafted cells to reinnervate the striatal tissue. We therefore examined the ability of Wnts to promote axonal growth of grafted DA neurons in animal models of PD. Parkinsonian mice receiving fetal grafts as well as adjacent Wnt5a over-expressing grafts showed enhanced neuritogenesis and directional axon growth compared to fetal VM grafts + control cells. These findings suggest that Wnts are regulated in the remodeling brain and can be harnessed to promote repair.

POS-TUE-232

DOPAMINE RECEPTOR EXPRESSION CHANGES IN THE NUCLEUS ACCUMBENS OF RATS DISABLED BY PERIPHERAL NERVE INJURY

Austin P.J. and Keay K.A.
School of Medical Sciences, University of Sydney, NSW, Australia, 2006.

Purpose: Chronic neuropathic pain is characterised by sensory changes, disability and altered affect. We have shown that following sciatic nerve constriction injury (CCI), 30% of rats develop *persistent disabilities* (altered social behaviour, sleep and endocrine function). Altered affect in chronic pain states may result from changes in the mesolimbic dopaminergic 'reward-aversion' circuitry of the nucleus accumbens (NAcc). The **aim** of these studies was to quantify changes in the NAcc by examining dopamine receptor mRNA and protein expression and the density of D2 receptor expressing neurons, in rats with *persistent disability* (n=6) and *no disability* (n=6) following CCI. **Methods:** Rats underwent sensory testing, as well as resident-intruder testing for 5 days prior to, and 6 days after CCI. Following testing, brains were processed for dopamine receptor expression using RT-PCR and Western blots, or D2 immunoreactivity (-IR) analysed stereologically. **Results:** There were decreases in mRNA expression of dopamine receptor sub-types in the NAcc of *persistent disability* animals [$p>0.05$ vs. *no disability*]; specifically D1 (contralateral) and D2 & D3 (ipsilateral). There was a significant decrease in D2 protein expression contralaterally in *no disability* animals [$p>0.05$ vs. *persistent*]. There were topographically specific decreases in the density of D2-IR neurons in the contralateral NAcc (2.5 mm to bregma) of *no disability* [$p>0.05$ vs. control] and in the ipsilateral NAcc (1.0 mm) of *persistent disability* animals [$p>0.01$ vs. control]. **Conclusion:** A subpopulation of rats, displaying sensory and affective disabilities, show highly lateralised neural (mal-)adaptations in reward-aversion circuitry of the NAcc after unilateral CCI. These changes appear to have major consequences on social behaviour, and may be involved in manifestation of disability and altered affect following nerve injury.

POS-TUE-233

PARKINSON'S DISEASE AND INSTABILITY WITHIN THE PARK2 LOCUS

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Genomic alterations in PARK2 (*PRKN*) are the most common cause of familial early-onset autosomal-recessive Parkinson's disease (PD). *PRKN* shares a bidirectional promoter with *PArkin Co-Regulated Gene (PACRG)*. The genes span an exaggerated genomic interval of 2 Mb as both genes encode super expanded introns, and dominate the 3rd most fragile site in the human genome - FRA6E. The significance of this genomic architecture has not been investigated. We hypothesise that the super expanded introns of these genes are a consequence of an ancestral rearrangement, and underlie the genetic instability within FRA6E. To investigate the genomic architecture of *PRKN* and *PACRG* we identified orthologues in model organisms that are experimentally relevant to human biology. Emphasis was placed on identifying the chromosomal location, genomic size and conservation of the bidirectional promoter. The genomic structure and bidirectional promoter of the PRKN-PACRG locus was conserved in mammals. In contrast, while both genes were located in the head-to-head orientation in birds the bidirectional locus was not maintained. Within the fish, *PRKN* and *PACRG* were identified on separate chromosomes and while the *PACRG* introns were consistently super-expanded *PRKN* introns are not. These findings suggest that proximity to *PACRG* or the associated genomic region may have contributed to the expansion of *PRKN* introns. Identifying elements involved in the expansion of the PRKN-PACRG locus may aid in understanding the underlying basis of the instability of FRA6E and the role of *PRKN*-proven genomic alterations in the aetiology of PD.

POS-TUE-234

CRITICAL ROLE FOR THE PARAVENTRICULAR THALAMUS IN COCAINE-PRIMED 'RELAPSE': MODULATION BY COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT

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Purpose: Recent studies suggest that the paraventricular thalamus (PVT) is involved in the reinstatement of alcohol-seeking (relapse-like behaviour). The present study aimed to extend these findings and investigate whether the PVT might also mediate cocaine-seeking behaviour. Further, hypothalamic neurons expressing the neuropeptide *cocaine- and amphetamine-regulated transcript* (CART) densely innervate the PVT and have been implicated in drug-motivated behaviours. It is not known, however, whether CART signaling within the PVT plays a role in the relapse phase of the addiction cycle. Thus, the effect of PVT-directed CART injections on cocaine-primed drug-seeking was determined. **Methods:** Rats were trained to self-administer cocaine and were then extinguished to a set criterion. Following extinction training, animals received either PVT-directed saline (n=9), tetrodotoxin (TTX, 5ng/0.25µl; n=7), or CART (1.25µg/0.25µl; n=5) followed by a cocaine-priming injection (10mg/kg, i.p.). A second group of rats received PVT-directed CART (0.5µg/0.25µl, n=4; or 1.25µg/0.25µl, n=4) or saline injections in the absence of a cocaine-priming injection. **Results:** Following a cocaine-prime, TTX-treated animals exhibited significantly attenuated drug-seeking behaviour ($p<.05$) compared to saline treated rats. PVT-directed CART injections significantly potentiated cocaine-seeking behaviour ($p<.01$). In contrast, treatment with either dose of CART alone had no effect on drug-seeking behaviour (p's>.05). **Conclusions:** These data suggest that the PVT plays a critical role in modulating cocaine-primed reinstatement of drug-seeking behaviour and that CART signaling in this region contributes to this response. Together, the present findings indicate that whilst CART plays a role in relapse-like behaviour, it is insufficient to produce a recovery of responding on its own.

POS-TUE-235

NEURAL REMODELLING IN MICE WITH TARGETED ABLATION OF D1 DOPAMINE RECEPTOR-EXPRESSING STRIATAL NEURONS: A MODEL OF BASAL GANGLIA NEURODEGENERATION

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BACKGROUND: Dopamine responsive medium spiny neurons in the striatum are preferentially lost in Huntington's (HD) and Parkinsonian syndrome. A transgenic mouse line with selective ablation of dopamine receptor D1+ striatal neurons was generated using the Cre-LoxP system under the control of DARPP-32 (dopamine and adenosine 3',5'-cyclic monophosphate (cAMP)-regulated phosphoprotein, 32kDa) promoter. METHODS: Brain tissues from adult mice (WT n=7, MUT n=7) were analysed for Drd1, Drd2 and related neurochemical markers using *in situ* hybridization and immunohistochemistry. Tissues were immunolabelled for astrocytes (GFAP), medium spiny neurons (DARPP-32, CB, PPE), interneurons (GABA, NPY, VAChT), dopaminergic cells (TH) and terminals (DAT). The concentration of extracellular dopamine and DOPAC was measured by HPLC. RESULTS: Striatal Drd1a, substance P, and dynorphin mRNA expression was reduced uniformly throughout the entire rostrocaudal extent of the dorsal striatum, while Drd2 and enkephalin mRNA was upregulated. Atrophy and astrogliosis was present in the striatum but not in the cortex and only striatal DARPP-32+ cells were reduced. CB+ cells were reduced, while GABA+/NPY+ interneurons and PPE+ projection neurons were increased and DAT+ dopaminergic terminals were reduced in the striatum. Dopamine concentration was reduced in the striatum and cortex, but DOPAC concentration was unchanged. CONCLUSION: Selective ablation of Drd1a-expressing striatal neurons demonstrated compensatory upregulation of Drd2-mediated indirect pathway and neural remodelling in a subset of striatal neurons.

POS-TUE-236

THE HUMAN Q133K TDP-43 MUTATION, BUT NOT THE WILD-TYPE TDP-43, INHIBITS NGF-MEDIATED DIFFERENTIATION OF PC-12 CELLS - MIMICKING THE EFFECTS OF SILENCING TDP-43 EXPRESSION

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The TAR-DNA-binding protein TDP-43 has recently been detected in cytosolic ubiquinone-positive inclusions in several neurological diseases and mutations in the gene encoding for this protein can cause an autosomally dominant inherited form of motor neuron disease. The normal function of TDP-43 suggests that modifications to this protein could impair neuronal plasticity. In this study we investigated the consequences of introducing the human TDP-43 mutation Q133k on NGF-mediated differentiation in PC-12 cells. The outcome of these experiments was compared to the response to NGF in cells overexpressing human wild-type TDP-43 and to cells in which TDP-43 expression had been silenced using a microRNA generating plasmid. PC-12 cells were transfected using Nucleofection. This approach resulted in 81 ± 4 % of the cells expressing the transgene (n=3). Differentiation was defined as having one or more neurties exceeding two times the diameter of the cell body. Exposure to 200 ng/ml NGF resulted in neurite outgrowth in 76 ±11% of the cells at 24 hours and 86 ± 9% after 48 hours (n=3). In cells expressing the Q133k mutation, the proportion of differentiated cells was decreased by 60 ± 2 % compared to mock transfected cells ($p<0.001$, Student T-test, n=3). Overexpression of wild type TDP-43 did not affect differentiation. The effect of the Q133K mutation on NGF-mediated differentiation was mimicked by silencing TDP-43 expression. In those experiments, neurite outgrowth was inhibited by over 80% compared to controls($p<0.001$;ANOVA;n=3). Together these results suggest that the observed effect of the Q133k mutation resulted from a lack of function of TDP-43.

POS-TUE-237

THE ANXIOLYTIC COMPOUND BNC210 EXHIBITS AN ANTIDEPRESSANT EFFECT WITHOUT SYMPTOMS OF PHYSICAL DEPENDENCE

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BNC210 is a novel compound that displays acute anxiolytic activity in three rodent species and several models of anxiety. BNC210 is free of sedative side effects and does not cause abuse liability or development of tolerance. Here we present data to show that, in addition to its anxiolytic effects, acute administration of BNC210 at 100 mg/kg produces an antidepressant effect in the rat Forced Swim Test (FST), the primary screening test for antidepressants. Increased efficacy in this test was observed when BNC210 was dosed daily for 14 days, with significant antidepressant action seen at 30 mg/kg/day. Sudden discontinuation of antidepressant medication may produce withdrawal effects caused by physical dependence to the drug. In the 5 days following the chronic dosing period, rats were observed for changes in food intake, body weight and body temperature. Abrupt withdrawal of BNC210 did not produce any changes in these parameters indicating that it does not produce physical dependence and supporting its suitability for chronic use to treat anxiety and depression. Many antidepressant drugs have been shown to increase neurite outgrowth. At 0.01 nM and higher concentrations, BNC210 was found to enhance neurite outgrowth from rat primary cortical neurons. The concentrations at which BNC210 exerts its effects in this system correlate with its in vivo potency and measured brain concentrations.

POS-TUE-238

CHARACTERIZATION OF TERTIAPIN-Q ACTIVITY ON INWARDLY RECTIFYING K^+ CHANNELS

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A large number of cells including cardiac myocytes and neuronal cells express the inwardly rectifying K^+ channels (Kir3.x or GIRK). This family of K^+ channels plays a pivotal role in regulating neuronal excitability and heart rate. GIRK channels can be modulated by several G-protein coupled receptors (GPCRs) including opioid, adrenergic, muscarinic, dopaminergic and $GABA_B$ receptors. The activation of GIRK channels involves many intrinsic factors including Mg^{2+} and Na^+, pH, phosphatidylinositol 4,5-bisphosphate (PIP_2) and intracellular proteins such as Gi/o proteins. It has been shown that Tertiapin-q (TPNq), a peptide derived from honey-bee toxin venom, inhibits two members of the inwardly rectifying K^+ channel superfamily, the GIRK1/4 and the ROMK1 channels, with nanomolar affinities. In contrast, naringin, a flavonoid glycoside, is an activator of the GIRK1/4 channels. We hypothesize that naringin and TPNq bind to the same site on GIRK1/4 channels. To test this hypothesis, the effects of naringin at concentrations ranging from 10μM-1mM were evaluated in the presence of a constant concentration of TPNq (3nM) on Xenopus oocytes expressing wildtype GIRK1/4 channels. Channel activity was measured using two-electrode voltage clamp recordings. Results indicated that the response of naringin in the presence of TPNq showed a shift to the right compared with the activity of naringin alone. This indicates a possible competitive inhibition by TPNq against naringin. To determine whether TPNq can cause a right-ward shift of the EC_{50} of GABA on $GABA_B$ receptor, oocytes expressing both $GABA_{B(1b,2)}$ and GIRK1/4 were evaluated. TPNq inhibited the maximal GABA (100 μM) response by 30% while the EC_{50} concentration of GABA (3 μM) was unaffected, indicating a non-competitive inhibition. We further explored the binding site of TPNq on GIRK channels. For these studies the homomeric $GIRK1^{F137S}$ mutant was expressed in oocytes and the effect of TPNq was evaluated. It was found that TPNq had a significant lower affinity on GIRK mutants compared to wildtype GIRK1/4 channels. 3μM of TPNq inhibits 50% of the barium sensitive current compared to 10nM necessary for GIRK1/4 wildtype. Further GIRK1 mutants will be studied to determine which amino acids are important for TPNq activity on GIRK1 and whether these mutations also affect naringin's activity.

POS-TUE-239

CANINE SAND MAZE: A NON-AVERSIVE SPATIAL MEMORY RETENTION TASK FOR USE IN A CANINE MODEL OF ALZHEIMER'S DISEASE

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Canine cognitive dysfunction (CCD) is an age-related cognitobehavioural syndrome in older dogs. Pathological similarities with human dementia and the superior prediction of pharmacological responses have heightened interest in CCD as a model for human Alzheimer's disease. A major limitation to further research is the lack of a quick and accurate spatial memory test as current methods require in excess of 40 days of training. Experimental set up was based on a non-aversive appetitive appropriation of the Morris Water Maze. A 4.5m diameter circular pool was filled with sand/powdered food reward mix to a depth of 10cm. A food reward was positioned in a static location for all learning trials. Dogs were given 4 habituation trials followed by 16 learning trials which alternated between the reward being half buried and fully buried to a depth of 4cm. After a 90 minute break a probe trial was conducted in which the reward was buried 1/4 rotation around the pool. Time to learned annulus for healthy old (>8yrs, n=11) and young (1-4yrs, n=11) breed-matched dogs was compared. Average probe times were 6.88s and 38.53s for young and old dogs respectively (p=0.017). After correction for differences in motivation and learning times, probe times remained significantly different between groups (p= 0.031). The Canine Sand Maze is a quick and non-aversive tool for determining short-term spatial memory in dogs. It is sufficiently sensitive to detect differences in memory function between young and healthy aged dogs.

POS-TUE-240

DEVELOPMENT OF EPHA4 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF CNS INJURY

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Purpose: Robust regeneration of the human CNS after neurotrauma rarely, if ever occurs and there is no effective drug treatment. Following spinal cord injury, EphA4-/- mice undergo axonal regeneration with functional improvement. EphrinA5 is a ligand of EphA4, therefore we investigated whether ephrinA5 mimetic peptides can block EphA4 activity *in vitro* and *in vivo*. **Methods**: Peptides based on the ephrinA5 CD and GH loop structure were synthesised and tested. *Phosphorylation assay*: cortical astrocytes (n=3) were treated with peptide for 15 mins followed by soluble ephrinA5-Fc for 20 mins. EphA4 phosphorylation was quantitated by Western analysis. *Growth cone assay*: hippocampal neurons (n=3) were treated with peptide for 30 mins, +/- ephrinA5-FC to induce growth cone collapse and the percent of neurites with growth cones determined. *In vivo administration*: following spinal cord hemisection in adult wild type mice (in each group, n=7), a GH loop peptide was administered IP for two weeks, followed three weeks later by anterograde tract tracing and motor function testing. **Results**: GH loop peptides were partially effective at blocking EphA4 phosphorylation, dependent on the peptides length and structure. CD loop peptides alone were ineffective at blocking phosphorylation, but when combined with GH loop peptides they were more effective than GH loop peptides alone. While some GH loop peptides inhibited ephrinA5-Fc induced growth cone collapse, they were ineffective at inducing regeneration or improving motor function following spinal cord injury, due to clearance in the kidneys. **Conclusion**: Ephrin mimetic peptides partially block EphA4:ephrin interactions, but their small size prevents them from reaching the CNS injury site *in vivo*.

POS-TUE-241

GENOTYPE-PHENOTYPE INTERACTION IN CHINESE MALE HEROIN-DEPENDENT SUBJECTS

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The objective of this study is to compare cold-pain response (pain threshold, tolerance and net-pain tolerance) among current opioid users (n=48), long-term opioid abstainers (at least 1 year abstinence; n=34), and healthy controls (n=63). All subjects were male Chinese. We further investigated whether there is any phenotype-genotype interaction amongst these subjects in studying personality traits (neuroticism and extraversion measured by NEO PI-R) and gene polymorphisms of the opioidergic and dopaminergic systems. Results showed that pain threshold of ex-users resembled those of current users and their pain tolerance matched that of controls, resulting in a net tolerance (time that pain is endured) that fell between these two groups. The overall Neuroticism score was highest in current users compared to the other two groups ($p = 0.0001$), with the subscale N3:Depression ($p<0.0001$) and N6:Vulnerability ($p=0.0002$) being the main contributory factors. Extraversion score was of current users were lower ($p = 0.048$) than the controls. COMT val158met polymorphism was shown to have a significant interaction ($p=0.007$) with the 'N3:Depression' subscale of Neuroticism in the current users. The present study observes that chronic opioid use causes cold-pain hyperalgesia in current users, which may be explained by their highly neurotic personality, and further supports the notion of pain-response recovery in former opioid addicts is a prolonged process that may take months to re-establish. This negative affect may be in part due to the influence of the COMT val/met genotype of these subjects. Pain management focused in the first 6 to 12 months of opioid abstinence may be an effective measure to prevent opioid-induced hyperalgesia-related relapse.

POS-TUE-242

LIFETIME MENTAL ACTIVITY PROMOTES STRUCTURAL AND FUNCTIONAL BRAIN INTEGRITY

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Lifespan mental activity (LMA) is an important modifiable protective factor in the development of dementia. Individuals with a rich history of education, occupational complexity and cognitive life style activities are almost one-half the risk for developing dementia. The Life_Experience_ Questionnaire (LEQ) was developed and validated to quantify these lifestyle characteristics. This is the first study to correlate whole-brain structural or functional MRI with LMA on mild cognitive impairment individuals. Subjects (N=23, aged 65 to 80, 27.8% male) comprised an initial subsample of the SMART (Study of Mental Activity & Regular Training for the Prevention of Cognitive Decline in At Risk Older Individuals). LEQ scores were normally distributed (mean = 94.1+/-18.5). Those in the bottom (N=9) and top tertile (N=9) were categorized as Low and High LEQ respectively. High LEQ individuals exhibited several areas of reduced brain atrophy including temporal and frontal regions ($p<0.05$). There were also differences in the topographical pattern of hippocampal functional connectivity. Further, we observed areas of significantly increased connectivity with the posterior cingulate appeared in several regions in the High LEQ group. So LMA promotes a series of brain changes in the older brain. Some structural differences may account for decreased functional connectivity. To sum up, LMA may therefore lead to a structurally healthier and functionally more efficient brain in later life.

POS-TUE-243

MULTISENSORY FACILITATION AS A FACTOR INFLUENCING IQ IN CHILDREN

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The ability to consolidate multiple sensory inputs into a unified percept is imperative to perceptual learning and the acquisition of cognitive and intellectual abilities. This study investigated whether multisensory integration and its facilitative effect on motor actions is related to the intellectual abilities of children. The Wechsler Intelligence Scale for Children (WISC-IV) was used to assess the general intellectual abilities of 90 children, who showed good or poor multisensory facilitation during an audiovisual detection task when performed in quiet conditions and in the presence of auditory background noise. All children included in the study had Full-Scale IQs (FSIQ) above 80. Children who demonstrated good multisensory facilitation in quiet and noise showed above average FSIQs (p <.01), while those with poor multisensory facilitation in noise showed significantly lower FSIQs and Verbal Comprehension Index (VCI) scores. Stable and consistent multisensory facilitation across quiet and noisy conditions is likely to optimise the ability to form appropriate associations between sounds and corresponding objects or events, leading to heightened general intellectual abilities, as assessed by the WISC-IV. Interestingly, in some children characterised as having slow motor responses, the likelihood of gain from multisensory integration improved in the presence of auditory background noise.

POS-TUE-244

EVIDENCE OF A ROLE FOR INTERSTITIAL CELLS OF CAJAL IN REGULATING NORADRENERGIC PERISTALSIS IN GUINEA-PIG VAS DEFERENS

King D.A., Chung E.S.Y., Sanai F., McParland B.E. and Lloyd H.G.E. School of Medical Sciences (Pharmacology), The University of Sydney, NSW 2006.

Sperm transport through the vas deferens requires coordinated, unidirectional contractile activity, but direct evidence of such peristalsis is scarce. It is possible that elevated intraluminal pressure stimulates rhythmic contractions in vas deferens, hence, we used pressure myography to examine the mechanisms underlying peristalsis in this tissue. Using guinea-pig prostatic vas deferens, rhythmic pressure changes that resembled peristalsis were recorded only when tissues were simultaneously stimulated with phenylephrine, an α_1-adrenoceptor agonist, and increased intraluminal pressure (from 0 to approximately 20 cmH_2O, achieved by fluid injection). The removal of either phenylephrine or pressure caused rhythmic activity to cease (n = 4 tissues). Prazosin (1 μM), an α_1-adrenoceptor antagonist, reduced the amplitude of rhythmic contractions by approximately 60% (n = 4 tissues), but did not reduce frequency. We have previously reported that imatinib (50 μM), which disrupts the pacemaker function of interstitial cells of Cajal (ICC) in the gastrointestinal system, also inhibits pressure-dependent rhythmic contractions in isolated vas deferens (n = 7 tissues). Further analysis of those tissues where some rhythmic contraction persisted (n = 4 tissues) revealed that the frequency of contractions, but not the amplitude, was reduced, consistent with an effect on pacemaker cells. Importantly, we have demonstrated that imatinib does not reduce the amplitude of direct contractile responses to phenylephrine, indicating little or no effect on L-type Ca^{2+} channels (n = 4 tissues). Taken together, these results suggest that imatinib-sensitive ICC play a role in determining the frequency of noradrenergic peristalsis in vas deferens. Further experiments are warranted to explore the relevance of ICC in male fertility.

POS-TUE-245

HFI-1 REDUCES DAMAGE, BEHAVIOURAL DEFICITS AND NEUROPATHIC PAIN IN A RAT MODEL OF SPINAL CORD CONTUSION

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Spinal cord injury (SCI) predominantly occurs in young adults leading to permanent paraplegia and quadriplegia, and increased costs to society. Previously we have demonstrated that HFI-1, a mexiletine analog that has a sodium channel blocking pharmacophore linked to an antioxidant moiety, reduces deficits in model of mild SCI. We have subsequently assessed its efficacy in a model of spinal cord contusion. Male Hooded Wistar rats were anaesthetised (2% isoflurane/98% oxygen), and laminectomy performed at spinal level T10. Contusion injury was produced using the Infinite Horizon spinal cord impactor with a 2.5 mm diameter impounder dropped from a height of 6 mm at a force of ~150 kdyn. Mexiletine (12.5mg/kg, i.p.; n=7), HFI-1 (6mg/kg; 30mg/kg, i.p.; n=8) or vehicle (n=9) were administered at 3h after the injury and twice daily thereafter for 7 days. Behavioural tests were conducted weekly. At 6 weeks, rats were anaesthetised and transcardially perfused, to fix the spinal cords. Sections were cut and processed to examine the size of the cyst and modulatory effects of HFI-1 on lesion formation. HFI-1 treatment significantly decreased behavioral deficits as assessed by BBB scale, ladder walking test and inclined ledged beam *(P<0.05)*. Additionally, we utilised the plantar test to assess neuropathic pain, and observed mexiletine and HFI-1 treatment to reduce thermal hyperalgesia at 21 days post-SCI *(P<0.01)*. Mexiletine and HFI-1 reduced volume of damage following SCI by ~25% and ~45%, whilst axonal damage, assessed by sera phosphorylated neurofilament-H levels, was significantly reduced following HFI-1 (~70%) and mexiletine (~30%) treatment. These data indicate that HFI-1 may be a potential neuroprotective drug for the treatment of SCI.

POS-TUE-246

ZIC3 REGULATES CRITICAL GENES INVOLVED IN THE ESTABLISHMENT OF THE NEUROECTODERM LINEAGE AND NEURONAL DIFFERENTIATION

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The transcription factor *Zic3* (**Zi**nc finger protein of the **C**erebellum **3**) is involved in a spectrum of brain development processes from the establishment of neuroectoderm (Nakata et al, 1997), to patterning of the dorsal neural tube (Aruga, 2004; Inoue et al, 2004; Purandare et al, 2002), development of mature dorsal neurons and axon targeting within the retina of the developing visual system (Zhang et al, 2004). However little is known to date about the *Zic3*-regulated networks involved in these processes. Our investigation into the functions of *Zic3* during early embryonic stem cell (ESC) neural differentiation has identified critical genes involved in brain development. We report that *Zic3* target genes include regulators of neurogenesis, CNS patterning, and neuronal function. Overexpression of *Zic3* in early ESC differentiation led to changes in transcript levels of these target genes (>2-fold; FDR<0.05). We further present a *Zic3* DNA-binding sequence by motif-discovery algorithms in promoter regions enriched by chromatin-immunoprecipitation, and demonstrate that promoter regions encompassing the *Zic3* DNA binding motif are functionally responsive to *Zic3* ($p<0.05$). A detailed knowledge of *Zic3* transcriptional circuitry is fundamental to a comprehensive understanding of embryonic brain development and neuronal function in the adult brain. Our work has elucidated a set of *Zic3*-regulated genes that influence the critical decisions made during ES cell neural differentiation, and provide clues to pathways potentially regulated by Zic3 in the establishment of neuroectoderm and embryonic brain development.

POS-TUE-247

ALTERATION IN TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS FUNCTION IN A MODEL OF CHRONIC PAIN

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Introduction: Deletion of TRP-V1, -V4 and -A1 channels reduce mechanosensitivity of healthy colonic afferents (1) however; their role in visceral hypersensitivity is unknown. **Aims:** To determine the relative contribution of TRP-V1, -V4 and -A1 in colonic mechano- and chemo-sensation in a model of chronic post-inflammatory visceral hypersensitivity. **Methods:** Using an in vitro mouse colon preparation, we determined the effect of TRP channels agonists on mechano- and chemo-sensory function of splanchnic afferents. Mechanical hypersensitivity of high-threshold colonic afferents was induced by rectal administration of 0.1mL (130µg/mL) TNBS (2). Afferents were studied in control conditions or at 28 days post-TNBS administration when mucosal pathology had fully recovered. **Results:** (i) The TRPV1 agonist Capsaicin (3µM) caused desensitization to mechanical stimuli in both healthy and post-inflammatory afferents (n≥14; $P<0.05$ each). The chemosensory response to capsaicin was more intense and of shorter duration in TNBS recovery than healthy afferents. However, the number of fibres responding to capsaicin was greatly reduced in post-inflammation (37.8% mesenteric and 52.7% serosal). (ii) The TRPV4 agonist 5,6-EET (10µM) increased mechanosensitivity in healthy afferents ($P<0.05$), this was not seen post-inflammation (n≥12 each). The chemosensory response to 5,6-EET post-inflammatory was smaller than control afferents. (iii) The TRPA1 agonist AITC (40µM) increased mechanosensitivity in both healthy and post-inflammatory afferents ($P>0.05$), with no major changes in chemosensory response (n≥15 each). **Discussion:** Surprisingly TRPV1 channels are less functional in chronic visceral hypersensitivity, which contrast with the accepted role of TRPV1 in other pain models. TRPV4 channels contribute to mechanical hypersensitivity and post-inflammatory hyperalgesia, whilst TRPA1 plays an important mechanosensory role in multitude of conditions. [1]Brierley, 2009. [2]Hughes, 2009.

POS-TUE-248

IS THERE A DORSAL COLOUR AREA IN HUMAN VISUAL CORTEX?

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High resolution functional MRI enables the reconstruction of retinotopic maps in human visual cortex, but the organisation of these maps remains controversial in a number of areas. There is a current debate focussed on the human homologue of macaque V4, specifically whether V4 is divided between ventral and dorsal components, as in macaque, or whether there is an entire hemifield represented ventrally. Macaque V4 has been shown to be strongly responsive to colour. Here we compared responsivity to colour between the human ventral V4 and its putative dorsal component. We acquired high resolution functional images of human occipital cortex while participants (n=6) viewed rotating wedge and expanding ring stimuli (standard stimuli for mapping visual field polar angle and eccentricity), and coloured vs black and white stimuli. We chose to use coloured vs black and white movie excerpts as more naturalistic stimuli than Mondrian patterns or gratings, which have been used by previous studies. We found a robust colour preference in ventral V4 and surrounding areas, and little or no colour preference in the vicinity of its suggested dorsal counterpart. Our results thus argue against the existence of a dorsal component of V4 and for a ventral representation of the entire hemifield. Furthermore, our results suggest that our stimulus is a useful localiser of ventral visual areas when used in conjunction with retinotopic mapping procedures.

POS-TUE-249

THE ROLE OF cAMP-RESPONSE-BINDING-ELEMENT PROTEIN (CREB) IN THE NUCLEUS ACCUMBENS IN RELAPSE TO METHAMPHETAMINE ADDICTION

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Rationale: Methamphetamine abuse is a large problem within society however little is known about the underlying neurobiology that contributes to relapse to drug use. Recent studies have suggested a role for the cAMP-response-binding-element protein (CREB) in drug reward processes which can be activated by the release of cAMP-dependent-kinase PKA. Using the reinstatement model of drug-seeking behaviour, this study aimed to elucidate the role of the CREB activation within the Nucleus Accumbens (NAc) in mediating drug-induced relapse to methamphetamine-seeking behaviour. *Methods:* Male Sprague Dawley rats (374 ± 6g, n=12) were surgically implanted with a jugular vein catheter and bilateral cannulae into the NAc while under isoflourane anaesthesia. One week following surgery, rats were trained to self-administer intravenous methamphetamine at 0.1mg/kg/infusion on a fixed ratio schedule for 14 days. Following 14 days of behavioural extinction rats underwent 3 reinstatement test days where they were treated with an intracranial infusion of the PKA inhibitor Sp-cAMPs (10 nmol/0.5µl/side, 20 nmol/0.5µl/side) or aCSF (0.5µl/side) 30 minutes prior to a methamphetamine priming injection (1mg/kg, i.p.). *Results:* Treatment with the PKA inhibitor Sp-cAMPs produced a dose-dependent decrease in methamphetamine-induced drug seeking behaviour when compared to infusions of aCSF. *Conclusions:* This data suggests that PKA mediated activation of CREB in the NAc are involved in the relapse to methamphetamine use in rats.

POS-TUE-250

QUANTIFICATION OF NMDA RECEPTOR NR1 SPLICE VARIANT EXPRESSION IN THE CORTEX OF HUMAN ALCOHOLICS

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Ethanol is a modulator at the N-methyl-D-aspartate class of glutamate receptors (NMDAR) in the brain. NMDAR comprise a combination of NR1 and NR2 subunits. NR2 subunits are encoded by four different genes. In contrast, a single gene with a number of splice-variants encodes the NR1 subunit. These variants are generated by the absence or presence of exons 5, 21, 22 and 22' and influence the response of NMDAR to ethanol. In animal studies the receptor adapts to sustained ethanol exposure through altered expression of its subunits. We used real-time RT-PCR against engineered standards to assay the four C-terminal NR1 subunit mRNA splice variants in dorsolateral prefrontal and primary motor cortex tissue obtained at autopsy from chronic alcoholics with and without co-morbid cirrhosis of the liver (average daily ethanol consumption > 80 g), and from matched controls. Autopsies were performed under informed written consent by authorized pathologists for the Australian Brain Bank Network. The level of expression of the NR1-2 variant was significantly lower than that of all other variants, independent of area or pathology. The NR-3 and NR-4 variants were the most highly expressed. All variants were expressed at a markedly lower level in alcoholics than in controls in both areas. This was also true for the cirrhotic alcoholics, with the exception of the NR1-4 variant in the motor cortex, where levels were similar to controls. The data show that chronic alcoholism can influence the expression of NR1 subunits and that for these subunit isoforms, cirrhosis only has a minor influence.