POS-TUE-001

EARLY LIFE ENVIRONMENT PRODUCES LONG-LASTING EFFECTS ON $GABA_A$ RECEPTORS

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Background: Clinical and epidemiological studies suggest a relationship between early post-natal environment and long-term neurobiological and psychological development. Post-natal handling models in rodents comparing groups exposed to either brief daily handling sessions (EH) or no human intervention (NH) prior to weaning, indicate that NH models result in an anxious and stress-reactive adulthood phenotype similar to that observed in psychiatric disorders such as schizophrenia. **Purpose:** As $GABA_A$ receptors are rapidly affected by adulthood stress and implicated in schizophrenia, this study examined the effects of early-life environment on adulthood $GABA_A$ receptor expression, distribution and stress reactivity. **Methods:** Male and female mice were exposed to 15 minutes daily handling (EH) or no handling (NH) over PND1-14. Following behavioural testing in adulthood, mice were exposed to either a brief 3 minute swim stress, or remained undisturbed in the home cage immediately prior to death. Brain sections were then examined for high and low affinity [^{3}H]GABA binding at the $GABA_A$ receptor orthosteric site using quantitative receptor autoradiography. **Results:** Behaviourally, adult male and female NH mice were more anxious on the elevated plus maze and showed impaired reward-seeking in the sucrose-preference model of anhedonia compared with the EH group (n=12 per group). These behaviours were associated with reduced [^{3}H]GABA binding and enhanced stress-induced changes in [^{3}H]GABA binding in a number of brain regions of NH compared with EH mice (n=6 per group). **Conclusions:** Such findings suggest that early post-natal experience in rodents has long-lasting effects on $GABA_A$ receptor expression and plasticity and are of potential importance for understanding psychiatric disorders in which early-life environment and adulthood stress reactivity are implicated.

POS-TUE-002

NORADRENALINE INACTIVATES RELEASE SITES AT A GLUTAMATERGIC BASKET SYNAPSE IN THE CENTRAL AMYGDALA: PRESYNAPTIC INHIBITION VIA A CHANGE IN N

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The lateral division of the central amygdala (CeAL), receives direct input from the brainstem parabrachial nucleus (PB), which terminate in large basket terminals around CeAL cell bodies. The CeAL is also innervated extensively by ascending noradrenergic input from the brainstem. Purpose: This study investigated the interaction between these CeAL inputs. Methods: Using stereotaxic injection of DiI into the PB, we made recordings in acute brain slices from CeAL cells targeted by PB fibres, stimulating large all or none type responses by electrical stimulation of the afferent DiI-labelled fibre tract. Results: We found that noradrenaline, acting via presynaptic α_2-adrenoceptors inhibited release at PB-CeAL synapses without changing paired pulse ratio (PPR) an indicator of changes in presynaptic release probability (P). Variance-mean analysis of the effect of noradrenaline was consistent with a reduction in the number of release sites (N) contributing to evoked release in noradrenaline. $GABA_B$-mediated presynaptic inhibition of the same synapses was accompanied by a significant increase in PPR, consistent with a change in release probability. Consistent with this we found that presynaptic $GABA_B$ activation reduced evoked presynaptic calcium influx (as imaged by loading the PB terminals with the low-affinity calcium indicator Mag-green AM), whereas calcium influx was unaffected by noradrenaline. Finally, we found that noradrenaline, acting via NEM/pertussis toxin sensitive $G_{i/o}$, inhibited release directly via $G_{\beta\gamma}$ binding the botulinum toxin-sensitive SNAP-25 component of the SNARE complex. Conclusion: We conclude that noradrenaline, acting via presynaptic G-protein linked receptors and $G_{\beta\gamma}$ signalling inhibits release directly from a subset of release sites at PB-CeAL synapses.

POS-TUE-003

INHIBITORY SYNAPTIC TRANSMISSION AND CANNABINOID EFFECTS DIFFER IN MOUSE SUPERFICIAL AND DEEP DORSAL HORN NEURONS

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Neurons in the superficial (SDH; laminae I-II) and deep dorsal horn (DDH; laminae IV-VI) of the spinal cord process noxious and innocuous peripheral inputs. Many features of the SDH and DDH differ, suggesting they play different roles in nociceptive processing. **Purpose:** We compared $GABA_A$ergic and glycinergic synaptic transmission, as well as the effect of the type-one cannabinoid receptor agonist methanandamide (mAEA) on inhibitory synaptic transmission in the two regions. **Methods:** Mice (C57Bl/6, P17-27) were anaesthetised (Ketamine; 100 mg/kg, i.p.) and decapitated. Transverse slices were prepared from L3-L5 segments and voltage-clamp recordings were made from SDH and DDH neurons (- 70 mV, 23°C). Spontaneous $GABA_A$ergic and glycinergic inhibitory postsynaptic currents (sIPSCs) were isolated in strychnine (1 μM) and bicuculline (10 μM), respectively. Miniature IPSCs were recorded in TTX (1 μM). **Results:** $GABA_A$ mIPSC amplitude and decay times differed in the SDH vs. DDH (26.6 ± 0.8 vs. 40.3 ± 4.2 pA, and 17.2 ± 3.4 vs. 13.4 ± 1.2 ms; n = 7 and 6). The same was true for glycinergic mIPSCs (44.7 ± 6.3 vs. 63.3 ± 6.6 pA, and 11.6 ± 1.2 vs. 5.1 ± 0.6 ms; n = 10 and 11). Application of mAEA (5 μM) reduced $GABA_A$ sIPSC amplitude and frequency in the SDH (51.4 ± 4.7 vs. 35.3 ± 4.6 pA; 0.34 ± 0.13 vs. 0.10 ± 0.03 Hz, n = 5), but not in the DDH. In contrast, application of mAEA reduced glycinergic sIPSC frequency in both regions. **Conclusion:** The expression levels and kinetics of $GABA_A$ and glycine receptors, and the action of cannabinoids on inhibitory synaptic transmission differ in the two nociceptive processing regions.

POS-TUE-004

GLYCINERGIC AND GABAERGIC SYNAPTIC TRANSMISSION IN MEDIAL VESTIBULAR NUCLEUS NEURONS DURING VESTIBULAR COMPENSATION

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Behavioural recovery after removal of a peripheral vestibular apparatus by unilateral labyrinthectomy (UL) is referred to as “vestibular compensation”. It has been proposed that much of this compensation occurs in vestibular nuclei, the primary target for incoming vestibular afferents. Reciprocal commissural inhibitory inputs between opposing medial vestibular nuclei (MVN) are believed to play an important role in mediating the compensatory process. **Purpose.** We have investigated whether GABAergic and glycinergic quantal synaptic transmission changes during vestibular compensation. **Methods.** After recovery from UL, as described by Gacek and Kheterpal (1998), mice were anaesthetised at either 4 hours, 2 or 7 days using Ketamine (100mg/kg, i.p) and decapitated. Brainstem sections were prepared as previously described (Camp et al., 2006). Glycine and $GABA_A$ miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of appropriate antagonists: tetrodotoxin (1μM), CNQX (10μM), bicuculline (5μM) and strychnine (1μM). **Results.** mIPSCs were recorded from 157 MVN neurons. Our results show no significant changes in GABAergic mIPSC properties at any time point after UL. In contrast, four hours after UL glycinergic mIPSC amplitude and frequency were significantly increased compared to controls (amplitude: 70.2±16.1pA vs 37.3±6.6pA; frequency: 0.7±0.2Hz vs 0.2±0.03Hz, n=28, $p<0.05$). These changes were not due to alterations in receptor subunits because mIPSC kinetics did not change. **Conclusions.** Our results suggest that GABAergic synaptic transmission has a limited role in vestibular compensation during the time period studied. In contrast, glycinergic synaptic transmission plays a significant a role in altering neuronal excitability immediately after UL. *References:* Gacek, RR & Kheterpal U. (1998). Laryngoscope, 108, 671-8 Camp, AJ. et al. (2006). J Neurophysiol, 95, 3208-18.

POS-TUE-005

THE INHIBITION OF GLYCINE RECEPTORS BY β-CARBOLINES: SUBUNIT SPECIFICITY AND A POSSIBLE INVOLVEMENT OF THE ALCOHOL BINDING SITE S267

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Purpose: Glycine receptor (GlyR) chloride channels mediate inhibitory neurotransmission in the central nervous system. β-carbolines represent a large family of structurally-related compounds, some of which are naturally found in human body. β-carbolines display high affinities at γ-aminobutyric acid type A receptors (GABAARs) and are believed to act via a non-classical benzodiazepine site. Despite the effects of β-carbolines depend significantly on the composition of GABAARs, the subunit specificity on GlyRs are yet to be determined. Here we examined four β-carbolines, harmane (HM), norharmane (NHM), 1,2,3,4-Tetrahydro-9H- pyrido[3,4-b] indole (INDOLE) and 6-methoxyharmalan (MH) on homomeric alpha1, alpha2 and alpha3 GlyRs. **Methods:** GlyRs were recombinantly expressed in HEK293 cells and the effects of the drugs were investigated using whole-cell patch clamp electrophysiology. All dose-responses were performed on at least four cells. **Results:** All compounds tested demonstrated inhibition at micromolar concentrations in a glycine concentration-dependent manner. While INDOLE displayed similar potency at all alpha subunits, HM showed a weak preference for alpha1 and alpha2 subunits relative to alpha3 subunit. 100 μM NHM almost completely inhibited alpha2 subunit current but only blocked 50% of the current induced by alpha1 or alpha3 subunits. MH produced weak inhibition at all three subunits. In comparison to the dramatic lost of picrotoxin sensitivity upon the incorporation of beta subunit, the sensitivities to all four compounds had little change, if any, in the presence of beta subunit. A Ser267Cys mutation of the alpha1 subunit dramatically reduced the inhibition (>50%) by 100 μM INDOLE, despite modest decreases (~20%) to HM, NHM and MH seen at same concentrations, implying the possible involvement of this residue in drug-receptor interaction. **Conclusion:** The results imply a specific interaction between Ser267 and INDOLE. This provides a novel insight into how these drugs work.

POS-TUE-006

NEUROGLIAFORM CELLS PROVIDE LOCAL SYNAPTIC INHIBITION IN THE MOUSE PIRIFORM CORTEX

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The piriform cortex (PC) is an anatomically-simple three-layered cortex that processes olfactory information. How it achieves this processing is largely unknown. Previously we have shown that five main classes of GABA-releasing inhibitory interneurons are present in the PC, distinguished by their morphology, immunolabelling and intrinsic electrical properties. The purpose of the current work was to further examine the functional properties of one of these classes of interneurons, the neurogliaform (NG) cells. **Methods:** Experiments used 300 μm-thick coronal slices of PC from GAD67-GFP mice (14-18 days old) in which neurons expressing the GABA synthetic enzyme, GAD67, are labelled with GFP. Targeted whole-cell recordings were accomplished using standard methods. **Results:** NG cells (n = 38) were identified by their bright GFP fluorescence, compact dendritic and axonal arbors, and delayed firing during a current step at rheobase (latency to first spike = 294 ± 19 ms, n = 19). Although NG cells were present in all three layers of the PC, some of their properties showed a clear inter-laminar gradient (e.g. halfwidth of afterhyperpolarisation: 29.1 ± 3.2 ms, n = 5, in layer Ia, increasing to 75.5 ± 14.5 ms, n = 4, in layer III). In pair recordings (n = 5), 40 Hz trains of action potentials evoked in NG cells produced strongly depressing IPSCs both in layer II principal neurons and in other NG cells. **Conclusions:** By virtue of their compact morphology, NG cells can provide localised feedforward or feedback synaptic inhibition to principal neurons in the PC. Laminar gradients in the properties of NG cells may enable them to perform a variety of roles in olfactory processing.

POS-TUE-007

LOW-DOSE DOMOIC ACID PRECONDITIONING ENHANCES LTP AND LTD INDUCTION IN RAT HIPPOCAMPAL CA1

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For the past decade the focus on domoic acid (DA) has largely been on the effects of high dose exposure on seizures, neuronal degeneration and ionic conductance changes. Here we examined the effects of acute low dose DA and low dose DA preconditioning on LTP and LTD in hippocampal region CA1 *in vitro*, and on performance in the Morris water maze. Acute low dose DA (50 nM, 10 min) enhanced LTP (n=6 slices) but completely blocked LTD induction (n=7). In contrast, DA preconditioning (50 nM, 30 min wash-in + 30 min wash-out) markedly enhanced both LTP (n=7) and LTD (n=5). The fact that preconditioning enhanced both, well after the toxin had been washed from the preparation, suggests that metabotropic KA receptors are involved in metaplasticity processes. Acute low dose DA (0.25 mg/kg s.c., 30 minutes prior; n=10) resulted in significant impairment of performance in the Morris Water Maze but did not adversely affect new learning the following day, suggesting that low dose DA disrupts memory and learning by reversible shifts in neuronal excitability and plasticity. There were no differences between DA preconditioned (0.25 mg/kg s.c., 90 minutes prior; n=10) and saline treated animals (n=10) in any of the trials on either Days 1 or 2. Taken together, our findings suggest that a disruption in the *balance* of net LTP and LTD impairs memory and learning, while enhancement of *both* LTP and LTD does not correspond to enhanced memory and learning.

POS-TUE-008

AVERSIVE STIMULUS RESETS MEMBRANE POTENTIAL OSCILLATION IN THE AMYGDALA

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Purpose: A large body of evidence points to the amygdala as a critical structure in the acquisition and expression of Pavlovian fear conditioning. It has been proposed that during pairing of a conditioned stimulus, like an odour or a tone, with an aversive unconditioned stimulus, like a footshock, learning takes place as a result of the simultaneous excitation of afferent projections to the amygdala. The present project focuses on the cellular mechanism that underlies fear-conditioning induced memory. **Methods**: in vivo whole cell recording in urethane anaesthetized rats. **Results**: We characterized different populations of amygdala projection neurons based on their firing properties. All neurons displayed a slow oscillation of near 0.3 Hz (n=30). The phases of these oscillations can be divided into a down-state, when the cell's membrane potential is more negative, and an up-state when the cell is more depolarised and therefore closer to spike threshold. We show that this fluctuation is synaptically driven, probably by cortical inputs. An aversive stimulus (footshock, 3 to 5 mA, 1ms), when administered in the down-state, can induce an up-state and the phase of the oscillation is reset to the onset of the footshock (FS) induced up-state. Interestingly, FS occurring in the up-state does not have any detectable effect. **Conclusion**: These results show that an aversive stimulus can synchronize the activity of all projection cells in the amygdala. This oscillation and its implication in the transfer of information between the cortex and the amygdala will be particularly important for our understanding of emotion based memory.

POS-TUE-009

CALCIUM WAVES INVADE SPINES

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Purpose: The majority of synaptic contacts between excitatory neurons are made on dendritic spines. Spines compartmentalize calcium and other second messengers, and this compartmentalization is thought to mediate the input specificity of synaptic plasticity. Synaptically activated calcium rises can also occur in the dendrite. We have previously shown that in projection neurons of the basolateral amygdala (BLA), synaptic activation of metabotropic receptors leads to a focal rise in free calcium in the dendrite (15-50 µm from the soma) that propagates as a wave along the dendrite and invades the soma and nucleus. Here we investigated whether dendritic calcium waves invade the spines as they propagate along the dendrite. Methods: Rats (21-28 d) were anesthetized with a lethal dose of halothane, decapitated, and 350 µm slices were prepared. Whole-cell patch-clamp recordings and high-speed fluorescence images were made from BLA projection neurons loaded with the calcium indicator Fluo5F. Results: Calcium waves were evoked by local tetanic stimulation (100 Hz 1s) in the presence of APV and NBQX. A detectable increase in spinal calcium was seen in the majority of spines during the propagation of the calcium wave. The spinal calcium signal was delayed relative to that in the shaft (301 ± 71 ms; n = 17; $p < 0.01$), and was generally smaller in amplitude. The extent to which calcium waves invaded the spine head was correlated with the rate at which the spine fluorescence recovered after photobleaching (FRAP) and inversely correlated with the spine neck length ($r = -0.76$; $p < 0.01$). Conclusion: These results indicate that dendritic signals can invade spines and that the degree of invasion is dependent on the diffusional coupling between the spine and its parent dendrite. Thus the biochemical segregation of the spine head and parent dendrite also serves to exclude global calcium signals from the spine head. Supported by Queensland Smart State Fellowship (JP) – NHMRC Program Grant (PS).

POS-TUE-010

IDENTIFICATION OF CAMKII BINDING PROTEINS IN BRAIN SENSITIVE TO CAMKII PHOSPHORYLATION STATE

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PURPOSE: Calcium/calmodulin stimulated protein kinase II (CaMKII) is an important regulator of synaptic function. CaMKII is regulated by multi-site phosphorylation and targeting to cellular locations through interactions with specific proteins. CaMKII phosphorylation at Thr286 is well characterised. Recently we identified Thr253 as a new phosphorylation site in vivo that enhances CaMKII binding to post-synaptic densities (PSDs) (Migues et al. J Neurochem 98:289–99). We hypothesise that phosphorylation at Thr253 or Thr286 differentially regulates CaMKII function in vivo by altering the binding of CaMKII interacting proteins. **METHODS:** To test this we used a modified western blot overlay binding assay, recombinant FLAG-tagged CaMKIIα (wild-type and phospho-mimic mutants Thr253Asp or Thr286Asp) and subcellular fractions from rat brain enriched in PSDs, plasma membranes, nuclei or cytosol. **RESULTS:** CaMKII binding proteins were differentially distributed among subcellular fractions from rat cortex and cerebellum. We have identified at least twenty distinct proteins whose ability to bind CaMKII was selectively affected by phospho-mimic mutations ($n > 3$). The binding of wild-type CAMKII to one protein at ~15kDa in fractions from cerebellum and cortex enriched in PSDs was blocked by phospho-mimic mutation at 253 but unaffected by 286. In contrast, the binding of wild-type CaMKII to one protein at ~180kDa in cortical plasma membranes was enhanced by phospho-mimic mutation at 253 but unaffected by 286. Further bands were unaffected by phospho-mimic mutation at 253, but were affected by 286; still others were affected by both mutations. **CONCLUSIONS:** These results strongly suggest that phosphorylation of CaMKII at Thr286 and Thr253 independently alters binding to specific proteins.

POS-TUE-011

INITIATION AND PROPAGATION OF ACTION POTENTIALS IN CEREBELLAR PURKINJE CELLS STUDIED WITH VOLTAGE IMAGING

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Purkinje cells are the sole output neurons of the cerebellar cortex transmitting inhibitory signals to the deep cerebellar nuclei. Each Purkinje cell receives and integrates excitatory input from two different sources; parallel fiber input which modulates simple spikes and climbing fiber input which produces complex spikes. Here we investigate the site of initiation and the characteristics of propagation of action potentials produced by these two excitatory inputs. Using voltage sensitive dye imaging, we illustrate that both simple and complex spikes are initiated in a localised region of the proximal axon. The fluorescence signal underlying the simple and complex spike voltage waveform was detected first at axonal distances 22.5 ± 7.5 µm and 15 ± 2.5 µm from the axon hillock (respectively), indicating these are the sites of action potential initiation ($n = 4$). Once initiated, the simple spike propagates faithfully along the axon whereas the individual spikelets of the complex spike fail to propagate to distances greater than 80 µm from the hillock. Therefore the waveform differences of the simple and complex spike as observed at the soma are maintained in the proximal axon but are converted to similar waveforms at more distal locations (> 80 µm). Since the Purkinje cell is the sole output of the cellebellum, the site of initiation and the propagation of the simple and complex spike are critical to understanding cerebellar cortex function.

POS-TUE-012

ACTION POTENTIAL GENERATION REQUIRES A HIGH AXON INITIAL SEGMENT SODIUM CHANNEL DENSITY MAINTAINED BY ANCHORING TO THE ACTIN CYTOSKELETON

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The axon initial segment (AIS) defines a specialized region in neurons where action potentials are initiated. It is commonly assumed this requires a high density of voltage-gated sodium channels. Paradoxically, patch-clamp estimates suggest the AIS sodium channel density is similar to that at the soma and proximal dendrites. Using antibody staining, whole-cell voltage-clamp and sodium imaging we estimate the AIS sodium channel density is 30 to 45 times that in the proximal dendrites. Anchoring of sodium channels to the cytoskeleton can explain this discrepancy, as disruption of the actin cytoskeleton increased the amplitude of sodium currents measured in patches from the AIS. Consistent with experimental findings, computational models required a high AIS sodium channel density (~3,000 pS/µm^2) to account for observations on action potential generation and backpropagation. In conclusion, action potential generation in cortical pyramidal neurons requires a high AIS sodium channel density maintained by anchoring to the actin cytoskeleton.

POS-TUE-013

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF PARVALBUMIN EXPRESSING NEURONS IN THE DORSAL HORN OF THE SPINAL CORD

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Superficial and deep dorsal horn (SDH and DDH) neurons receive a variety of peripheral inputs in the innocuous and noxious ranges. One barrier to a greater understanding of how these neurons process such inputs has been the neuronal heterogeneity in both regions. Furthermore, little is known about the characteristics of dorsal horn neurons with different neurotransmitter phenotypes (excitatory or inhibitory). The calcium binding protein parvalbumin serves as a marker of the GABA/glycine neurotransmitter phenotype in the SDH. **Purpose:** In this study we used a transgenic mouse, expressing enhanced green fluorescent protein (eGFP) in parvabumin-expressing neurons, to record selectively from inhibitory interneurons. **Methods:** Mice (5-7 weeks) were anaesthetised (Ketamine; 100 mg/kg, i.p.), decapitated, and transverse slices were prepared from the spinal cord. Patch clamp recordings were made from visualized neurons expressing eGFP. **Results:** Two populations of eGFP-expressing neurons were identified: one located throughout inner lamina II (SDH); and another located in the medial portion of lamina V (DDH). Voltage-clamp recordings showed eGFP-expressing neurons in the SDH (n = 12) had higher input resistances and more hyperpolarized RMPs than those in the DDH (n = 4). Spontaneous excitatory postsynaptic currents had smaller amplitudes, slower kinetics, and occurred at lower frequencies in SDH (n = 8) versus DDH (n = 4). The majority of eGFP-expressing neurons in both regions, however, exhibited the non-selective cation current Ih. **Conclusion:** Parvalbumin-positive neurons in both the SDH and DDH are relatively homogenous populations with few shared characteristics. This suggests a specific role for each in the spinal processing of sensory information.

POS-TUE-014

THE BIS-T SERIES OF NANOMOLAR POTENT DYNAMIN INHIBITORS BLOCK ASSEMBLY OF DYNAMIN HELICES

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Dynamin I is involved in synaptic vesicle endocytosis (SVE) in neurons, while dynamin II mediates endocytosis of activated receptors in most cells. The dynamin GTPase activity is required for endocytosis, which is stimulated by its assembly into two types of rings. Single dynamin rings are formed by self-assembly or stimulated by Grb2, while dynamin helices are formed around liposomes or microtubule templates. The helices have much greater GTPase activity than the rings. We have developed dynamin I GTPase inhibitors that also inhibit endocytosis. The Bis-T analogues, Bis-T-22 and Bis-T-23, are the first nanomolar potent dynamin I inhibitors identified. They have an in vitro IC50 of 558 and 265 nM respectively when dynamin is stimulated by phosphatidylserine liposomes to form helices. Bis-T-22 was poorly membrane permeable in most cell lines, but Bis-T-23 reversibly blocked receptor-mediated endocytosis in COS7 cells at an IC50 of 23 μM. Bis-T-22 was non-competitive with GTP and with phosphatidylserine, therefore it does not target the dynamin GTPase nor PH domains. Instead we found that Bis-T affects assembly into helices. Bis-T-23 did not inhibit the intrinsic activity of dynamin, which does not involve ring formation. Nor did it inhibit Grb2-stimulated dynamin activity, which involves formation of single rings. Since helix formation requires a transition from a ring to a helix comprised of multiple "rings" we propose that the Bis-T inhibitors affect the ring-to-ring interactions required for dynamin to transform from a ring to a helical conformation. Such inhibitors are important tools for biological studies of endocytosis and may lead to clinically import drugs for epilepsy or mood disorders.

POS-TUE-015

HYPERPOLARIZATION-ACTIVATED INWARD CURRENT IS REGULATED DEVELOPMENTALLY IN RAT TRIGEMINAL SENSORY NEURONS

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Purpose: Hyperpolarization-activated inward current (I_h) contributes to neuronal excitability in sensory neurons. We have recently identified hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels 1, 2 and 4 in rat trigeminal ganlglia (TG) neurons. The aim of this study was to examine the functional properties of I_h in developing TG neurons. **Methods:** I_h was investigated in acutely dissociated TG neurons from 1 to 35 postnatal (P) day-old rats using whole-cell patch, voltage-clamp electrophysiology. I_h was activated by giving voltage steps from -40 to -130mV in -10mV increments. The half maximal activation voltage and activation kinetics of I_h were measured. Protein expression of HCN1 and 2 in developing TG neurons were detected by Western blot. Data are presented as mean ± S.E.M. **Results:** The half maximal activation voltage was shifted to more depolarized potentials from -93.9 ± 2.7 mV (P1, n=13) to -82.1 ± 7.0 mV (P35, n=6). In addition, the activation kinetics of I_h got faster with age (795 ± 239 ms (P1, n=13) to 237 ± 31 ms (P35, n=6) at -90mV). Western blot analysis of the whole ganglion showed increase in band intensity for both HCN 1 and 2 (638% and 1477% respectably, P1 to P35, n=1). **Conclusions:** This study showed I_h was activated more quickly and at more depolarized potentials, which coincided with increased HCN channel proteins in older rat trigeminal sensory neurons.

POS-TUE-016

A MECHANISM FOR QUINOLINIC ACID INDUCED CYTOTOXICITY IN HUMAN PRIMARY ASTROCYTES

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Purpose Quinolinic acid (QUIN) causes excitotoxicity in neurons through overactivation of the N-methyl D-Aspartate (NMDA) receptor. Recent evidence suggests that astrocytes are also susceptible to QUIN cytotoxicity by an unknown mechanism. This study investigated the putative role of NMDA receptor activation and oxidative stress in QUIN induced cytotoxicity in astrocytes. **Methods** Cultures of primary human foetal astrocytes and neurons were exposed to QUIN at concentrations between 0 and 1200 nM for 24 hours. Intracellular NAD levels and poly(ADP-ribose) polymerase (PARP) activity were quantitated on culture homogenates. Cell death was assessed by quantitation of lactate dehydrogenase (LDH) activity in culture supernatants. All experiments were done in at least triplicate. **Results** Our results showed that QUIN increased intracellular concentrations of the essential pyridine nucleotide nicotinamide adenine dinucleotide (NAD+) at low, physiological, concentrations (<50 nM) in cultures of both neurons and astrocytes (n=4). However at concentrations above 150 nM, QUIN was shown to activate the NAD+- dependent DNA repair enzyme PARP resulting in a dose dependant decrease in NAD+ and increased LDH activity in both astrocytes and neurons, (n=4). Treatment of astrocyte and neuron cultures, (n=3), with the NMDA ion channel blockers, MK-801 and memantine, and the nitric oxide (NO•) synthase inhibitor, N (G)-nitro-L- arginine methyl ester, L-NAME, prevented PARP activation and NAD+ depletion. Treatment with the competitive NMDA receptor antagonist AP-5 also ameliorated NAD+ depletion and PARP activation at higher concentrations. **Conclusion** These results indicate for the first time that astrocytes are susceptible to QUIN induced cytotoxicity via an apparent NMDA receptor mediated process, analogous to that previously identified for neurons.

POS-TUE-017

SK CHANNELS REGULATE SYNAPTIC TRANSMISSION IN THE RAT MEDIAL PREFRONTAL CORTEX

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Purpose: The prefrontal cortex (PFC) is essential for performing executive and cognitive functions. Disorders of the PFC underlie a variety of neurological disorders including schizophrenia, bipolar disorder and depression. SK channels, a type of calcium-activated potassium channel, have also been implicated in the etiology of schizophrenia. The current project aimed to examine the functional role of SK channels in the PFC. Methods: Visualised whole cell recordings were made from layer 5 pyramidal neurons from 300 µm coronal brain slices containing the PFC (obtained from 3 week old rats anaesthetized with isoflurane). Recordings were made from slices perfused in oxygenated aCSF containing picrotoxin (100 µM) at 32°C. Synaptic responses were evoked by stimulating layer 2/3 (L2/3) or layer 5 (L5). Results: Blockade of SK channels with apamin (100 nM) enhanced EPSPs evoked by 27 ± 2% (n=5) and 37 ± 8% (n=8) following stimulation of L2/3 and L5 respectively. Bicuculline methiodide (50 µM) potentiated EPSPs to a similar degree. Enhancement of EPSPs by apamin or bicuculline was blocked by loading cells with 10 mM BAPTA (n=6), a caesium internal (n=2), or by pre-application of the NMDA receptor antagonist AP5 (30 µM; n=6). NMDA receptor-mediated EPSPs evoked in 0.1 mM extracellular magnesium and NBQX (10 µM) were potentiated by apamin to a similar degree (□40%, n≥3) as EPSPs in control aCSF. Application of AP5 in cells loaded with 10 mM BAPTA revealed that NMDA receptors contribute □30% to the EPSP amplitude at L2/3 (n=6) and L5 (n=5) inputs. Conclusion: Calcium influx through NMDA receptors during basal synaptic transmission at L2/3 and L5 inputs to L5 pyramidal neurons maximally activates SK channels, which shunt excitatory synaptic transmission in the medial PFC.

POS-TUE-018

STUDIES ON THE MOLECULAR MECHANISM OF NOTEXIN IN FACILITATING SPONTANEOUS SYNAPTIC CURRENTS

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Purpose: Here we explore the molecular mechanism of Notexin, a presynaptic phospholipase A2 (PLA2) neurotoxin isolated from Notechis scutatus scutatus venom on the potentiation of synaptic transmission in neuromuscular junction. **Methods:** All of our studies were carried out at developing neuromuscular synapse in Xenopus cell culture by using whole-cell patch clamp recording. **Results:** Bath application of notexin dose-dependently increase the frequency of spontaneous neurotransmitter release (n=15). The facilitatory effect on SSC frequency was unaffect while notexin was chemically modified at arginine residues located in the proximity of its catalytic and toxic site (Notexin-Arg20 and Notexin-Arg80) to weaken its PLA2 activity (n=5 and 4 for Notexin-Arg20 and Notexin-Arg80, respectively). Pretreatment of the potent PLA2 inhibitors aristolochic acid (n=6) and glycyrrhizic acid (n=4) also fail to hamper notexin-induced SSC frequency facilitation, indicating that the PLA2 activity was not responsible for notexin-induced SSC frequency facilitation. Elimination of calcium from culture medium significantly abolish notexin's facilitatory effect (n=5). However, bath application of calcium channel inhibitor either cadmium (n=4), nifedipine (n=6), verapamil (n=4) or ω-conotoxin (n=2) is incapable of impeding notexin-induced synaptic facilitation. **Conclusions:** Overall, our current results suggest that the influx of Ca2+ from extracelluar space is involved in facilitating spontaneous synaptic current elicited by notexin.

POS-TUE-019

CONVERGENT G-PROTEIN COUPLED RECEPTOR SIGNALLING PATHWAYS INHIBIT POTASSIUM CHANNELS IN SYMPATHETIC NEURONS

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Purpose: The excitability of neurons is influenced by the activity of a wide range of potassium channels. Potassium channels can be activated by changes in membrane potential, calcium concentration or other intracellular signalling pathways accompanying neuronal activity. Conversely, the activity of many potassium channels is inhibited by the stimulation of G-protein coupled receptors (GPCRs), especially those operating via the Gq subunit. Methods: Guinea-pigs of either sex (195-250g) were killed by stunning and exsanguination according to protocols approved by Flinders Animal welfare Committee. Coeliac ganglia were isolated with splanchnic nerves attached and maintained in HEPES-buffered salt solution at 32-35°C. Membrane potential was recorded via intracellular microelectrodes in bridge mode. Drugs were applied by superfusion. Results: The long-lasting after- hyperpolarisation (LAH) following an action potential seen in vasomotor neurons was completely inhibited by substance P (0.1-1 µM) acting via NK1 receptors or angiotensin II (5-50 nM) acting via AT1 receptors, as well as arginine vasopressin, bradykinin and non-peptide agonists such as oxotremorine (0.1-1 µM) acting via muscarinic receptors or an unknown endogenous neurotransmitter. Substance P, angiotensin II and oxotremorine also blocked the potassium channels responsible for M-current, when it was present. The inhibitory effects of substance P, angiotensin II, and the unknown endogenous transmitter on potassium channel activity were potentiated by wortmannin (30 µM) under conditions where it inhibited PI4-kinase leading to depletion of PIP2 from cell membranes. Conclusion: Multiple GPCRs inhibit the same populations of potassium channels by depleting membrane of phospholipids essential for their maintained activity. Since these molecular interactions occur over small spatial domains, all the elements are likely to be closely packed within membrane microdomains.

POS-TUE-020

IDENTIFICATION OF ALTERNATIVE SPLICING OF THE TRPC3 ION CHANNEL SUBUNIT IN MOUSE AND GUINEA PIG CEREBELLUM

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Purpose: The canonical transient receptor potential type 3 (TRPC3) subunits assemble to form cation permeable ion channel which is a primary Ca^{2+} entry pathway in many cell types. The TRPC3 channel is highly expressed in cerebellum, and is likely to regulate Ca^{2+} levels in the neurons. The aim of this study was to investigate the expression profile of the TRPC3 channels within the cerebellum of mice and guinea pigs. **Methods:** Total RNA from mouse (n=2) and guinea pig (n=2) cerebellum was used for first strand cDNA synthesis. This template was then probed by PCR using primers that encompassed the complete open reading frame of the TRPC3 gene. The resulting TRPC3 amplicon from each species was isolated by agarose gel purification and verified using restriction digest. **Results:** In both species we identified a splice variant of the TRPC3 cDNA involving deletion of exon 9. This exon encodes a highly conserved Calmodulin and IP_3 Receptor Binding motif (CIRB), which is believed to contribute to regulation of the channel activation. By microdissection of cerebellar tissue, we found that the proportion of the spliced and un-spliced TRPC3 isoforms varied within different regions of the mouse cerebellum. **Conclusion:** The deletion of the CIRB motif in the splice variant of the TRPC3 gene may decouple the channel activation from intracellular Ca^{2+} stores. Tissue-dependent regulation of the CIRB motif splicing may therefore have ramifications for store- versus non-store dependent activation of this channel.

POS-TUE-021

DOPAMINERGIC NEURONS DERIVED FROM MOUSE EMBRYONIC STEM CELLS: PHENOTYPING BASED ON RECEPTOR EXPRESSION & FUNCTION

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Purpose: The possibility exists that directed differentiation of mouse embryonic stem cells is capable of yielding enriched populations of dopaminergic neurons, but at present there is little understanding of the pharmacological properties of these cells; or whether such cells represent a pharmacologically, phenotypically similar population. **Methods:** In this study we used a simple culture protocol to generate dopaminergic neurons and offer a preliminary pharmacological investigation of these cells using Ca^{2+} imaging and [^{3}H]- dopamine release studies. **Results:** In fluo-4 AM loaded cells, 13-17 days post-plating, and after the addition of tetrodotoxin some of the population of mouse embryonic stem cell derived neurons responded to adenosine triphosphate, noradrenaline, acetylcholine and L-glutamate with elevations of Ca^{2+} influx (n=10). Within the microtubule-associated protein and tyrosine hydroxylase positive cell population adenosine triphosphate, noradrenaline, acetylcholine and L-glutamate elicited positive elevations of Ca^{2+} in 74, 66, 58 and 67% of the population (n=10); cells could be further subdivided into three major pharmacologically distinct populations based on the combinations of agonist they responded to. Acetylcholine (30 µM) and noradrenaline (30 µM) were the only agonists to elicit significant tritium overflow from [^{3}H]- dopamine loaded cells. The acetylcholine effect was blocked by atropine (1 µM) and tetrodotoxin (1 µM) and elevated by haloperidol (100 nM). The noradrenaline effects were reduced by cocaine (10 µM), but not by tetrodotoxin (100 nM). **Conclusion:** These data indicate that the dopaminergic neurons derived from mouse embryonic stem cells represent a heterogeneous population possessing combinations of purinergic, adrenergic, cholinergic and glutamatergic receptors located on the cell soma.

POS-TUE-022

DEVELOPMENTAL VITAMIN D DEFICIENCY ALTERS DOPAMINE SIGNALLING IN ADULT RAT BRAIN

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Purpose: Based on epidemiological evidence low prenatal vitamin D may lead to increased risk of neuropsychiatric diseases in later life. We have shown in a rodent model of DVD deficiency that baseline locomotion and locomotor response to amphetamine are enhanced, particularly in females. One possible explanation for these behavioural findings is that dopamine receptor and/or transporter (DAT) function may have been altered during development. **Method**: Striatum and nucleus accumbens (NAc) were dissected from 10-week old DVD-deficient and control Sprague-Dawley rats (n=8 per group). Synaptic membrane fractions were prepared and incubated with increasing concentrations of radioligands ([^{3}H] SCH23395 for DA1 receptor, [^{3}H] raclopride for DA2 receptor; [^{3}H] GBR12935 for DAT) until equilibrium, with appropriate inhibitors to determine non-specific binding. Bound radioactivity was trapped on Whatman GF/B filters and counted. **Result**: DA1 receptor binding in NAc of male DVD deplete rats was reduced 40% compared to controls (1.4 ± 0.3 pmol/mg protein, $P<0.05$). DA1 density was unchanged in the striatum. DA2 receptor and DAT binding in both regions were unaltered by DVD deficiency. In female DVD-deficient offspring dopamine receptors were unaltered in both regions. However, striatal DAT density was increased by 44% in DVD-deficient females (Bmax= 26.6 ± 2.7 pmol/mg protein, $P<0.05$) and DAT affinity in NAc was double that of controls (Kd= 24.2 ± 3.6nM, $P<0.05$). **Conclusion**: These results confirm that DVD deficiency alters dopamine signalling in adult rat brain. Increased DAT density or heightened affinity in terminal fields relevant to locomotion in female DVD-deplete rats may help to explain the sexually dimorphic effects of amphetamine in this model.

POS-TUE-023

EFFECTS OF REPEATED EXPOSURE TO MDMA ON THE LOCOMOTOR ACTIVATING EFFECTS OF MDMA, DA AGONISTS AND ANTAGONISTS

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Purpose: Repeated exposure to amphetamine-like stimulants results in sensitisation to the behavioural and neurochemical effects. This sensitised response has been suggested to contribute to the development of compulsive drug use and abuse. **Methods:** In the present study, (*all groups n=6)* rats were pre-treated with MDMA (5.0 or 10.0 mg/kg/day for 5 days). Following a 2-day withdrawal period, the locomotor activating effects of MDMA (0-10.0 mg/kg), SKF 81297, MDMA (0-10.0 mg/kg) and SCH23390, Apomorphine (0-4.0 mg/kg) and MDMA (10.0 mg/kg) and Eticlopride (0-0.2 mg/kg) were measured. **Results:** Pre-treatment with 10.0 mg/kg but not 5.0 mg/kg MDMA shifted the dose-effect curve for hyperactivity to the left, consistent with an increased potency. There was no alteration in the dose-effect curve for hyperactivity produced by SKF 81297 in either pre-treatment group and no alteration in the dose-effect curve for repression of activity produced by the D1 antagonist SCH23390. The dose-effect curve for apomorphine-produced hyperactivity was shifted upward following pre-treatment with both 5.0 and 10.0 mg/kg MDMA consistent with an increased efficacy. Administration of the D2 antagonist eticlopride did not alter the dose response attenuating effect in doses likely to antagonise the D2 postsynaptic receptor. **Conclusion:** These data suggest that under some pre-treatment conditions, sensitisation to the behavioural effects of MDMA is produced. These effects are not, however, likely to be due to sensitisation of dopamine D1-like or D2-like receptor mechanisms since (1) response to a selective D1-like agonist or antagonist was not altered under conditions that rendered rats more sensitive to MDMA and (2) the enhanced response to apomorphine suggested an increased efficacy. Further, the increased efficacy of apomorphine was produced under conditions that failed to result in a sensitised response to 5.0 mg/kg suggesting that the D2 postsynaptic receptor does not underlie the development of sensitisation to the hyperactive response produced by MDMA.

POS-TUE-024

DIFFERENTIAL RESPONSES OF GLUTAMATE TRANSPORTERS, ASTROCYTES AND MOTONEURONS TO COMBINED OXIDATIVE STRESS AND EXCITOTOXICITY IN MURINE SPINAL CORD CULTURES

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Our hypothesis is that both oxidative stress and excitotoxicity are required to produce an injury profile paralleling the neurochemical pathology of amyotrophic lateral sclerosis (ALS): motoneuron death, astrogliosis and loss of glutamate transporters (EAATs). Astrocytic EAATs mediate most glutamate uptake in brain and their function is compromised by oxidative stress producing excitotoxicity and further reactive oxygen species, exacerbating injury. **PURPOSE**: This study investigated the pattern of injury produced by the combination of oxidative stressor-AMPA agonist to motoneurons and astrocytes in spinal cultures. METHODS: Spinal cord cultures (n=3 separate cultures) (embryonic d12.5 C57BL/6 mice) were maintained in Neurobasal medium containing B27 supplement. Cells were treated at 12-13 days for 24-48 hours with the NO donor (SIN-1; 1.67/3mM to produce oxidative stress) or the AMPA receptor agonist fluorowillardiine (FW; 3-20µM + cyclothiazide 100µM, to produce excitotoxicity). Neurochemical and cytochemical procedures were employed to analyse cellular responses to these insults. **RESULTS:** Treatments caused time and concentration dependent changes in EAAT activity ([3H]D-aspartate uptake) and cellular viability (MTT assay). SIN-1 impaired cellular viability significantly more rapidly than EAAT activity. SIN-1 alone resulted in significantly less cell injury than when in combination with FW. Injury modes were analysed by annexin V and propidium iodide labeling. Drug concentrations employed for combined insults produced early, extensive labeling with annexin V and were considered to induce apoptosis. These insults (1.67mM SIN-1 + 20µMFW and 3mM SIN-1+ 10µMFW) produced early astrogliosis, later decreases in [3H]D-aspartate uptake and slow death (neuritic loss & blebbing) of motoneurons. **CONCLUSIONS:** Under conditions mirroring injury patterns in ALS, different phases of astrocytic-motoneuron crosstalk likely contribute to motoneuron pathology and reactive astrogliosis.

POS-TUE-025

ROTTLERIN, PKC AND THE REGULATION OF ASTROCYTIC GLUTAMATE TRANSPORTERS

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Purpose: In astrocytes, glutamate uptake is performed through the action of high-affinity Na+-dependent glutamate transporters (EAAT1, EAAT2). These transporters are localized on the plasma membrane, although little is known about the long and short term regulation of the trafficking of these transporters to the cell surface. Protein kinase C (PKC) has been shown to regulate the activity and cell surface expression of several different neurotransmitters including EAATs. Rottlerin, a PKC δ inhibitor, is considered to inhibit glutamate transport activity by removing transporters from the cell surface and accelerating intracellular degradation. **Methods:** In this study the effects of rottlerin (100 μM, 0-24 h) on EAAT activity and expression were examined in primary cultures of mouse astrocytes. **Results:** [^{3}H]D-aspartate uptake was decreased to ~55% of control by 1 h treatment with rottlerin and further decreased to ~25% following 24 h treatment (n=4). A small reduction in cell viability was observed with 24 h treatment. Once rottlerin was removed (post 6 h treatment), [^{3}H]D-aspartate uptake returned to control levels within 1 h (n=3). Flouorescent labeling of F-actin revealed that rottlerin caused a breakdown of stress fibres in astrocytes within 1 h of treatment, and a total rearrangement of the cytoskeleton at 24 h. Reduced cell surface expression of EAATs was also observed, thus further immunocytochemistry was used to investigate the co-localization of EAATs with intracellular markers. **Conclusions:** These results suggest that PKC is involved in the trafficking and cell surface expression of astrocytic EAATs and thus in regulating glutamate uptake in the brain.

POS-TUE-026

EFFECT OF SELECTIVE NERVE BLOCK ON REFLEX TENDON AND CUTANEOUS INHIBITION OF GASTROCNEMIUS

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Transcutaneous tendon electrical stimulation and cutaneous nerve stimulation produce a similar pattern of strong reflex inhibition of the ongoing voluntary EMG activity in both heads of the human gastrocnemius muscle (GA). The type of nerve fibers involve in the inhibition process is controversial. The aim of the experiment was to determine the type of nerve fibers responsible for the reflex inhibition of the ongoing voluntary EMG activity following cutaneous and tendon electrical stimulation. Eight healthy volunteers with no neurological disorder were recruited for the experiment. Subjects were positioned on their right side on a stretcher adjustable for height. The right knee was fully extended and the right foot was stabilized to a supporting frame. The ankle joint was kept at 90 deg throughout the experiment. Bipolar surface EMG electrodes were placed 2 cm apart over the two heads of GA. A stimulus intensity of 60 mA was used to obtain tendon reflex inhibition. The GA tendon was stimulated using small metal plates located on the midline and adjacent to the musculotendonous junction and the adjacent anterior surface of the leg. Cutaneous afferents from the sural nerve were stimulated below the fibular malleolus. All shocks were constant current stimuli of 0.2 ms duration and maximum intensity of 35 mA. An inflated cuff was used to selectively block the tibial nerve at the knee The inhibitory response to tendon and cutaneous stimulation were then assessed before, during and after the nerve block during a small (15% of maximum) voluntary contraction. The nerve block was applied for approximately 30 minutes during data recording. It was found that for all subjects, tendon inhibition was completely absent within 25-30 mins, but cutaneous inhibition was partially blocked during this time period ($P<0.005$, repeated measure ANOVA). Cutaneous inhibition was found 63.23+/-12.3 % at 30 mins. The much longer time to block the cutaneous inhibition suggest that tendon inhibition is mediated by different nerve fibers, possibly large diameter fibers.

POS-TUE-027

NECK MUSCLE CONDITIONING ALTERS PERCEPTION OF HEAD AND NECK POSITION

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Purpose: Muscle spindles play an important role in perception of head-neck position. The sensitivity of muscle spindles to stretch depends on how they have been conditioned beforehand. When a muscle is passively shortened, spindles are unable to take up the new shortened length and fall slack. This leaves them in a desensitised state. Our hypothesis is that neck muscles conditioned so that intramuscular receptors are in a desensitised state result in increased errors in head-neck position sense. **Methods:** Two experiments on human subjects (n=7 and n=10) were conducted. Subjects were blindfolded, and their head-neck moved actively five times into flexion/extension. The head-neck was then moved passively to a pre-determined target head position. This process was repeated three times so that the subject familiarised themselves with the target position. The dorsal neck muscles were then conditioned to leave neck intramuscular receptors either tight (hold short conditioning) or slack (hold long conditioning). The end point of the conditioning procedure returned the head to a neutral position. From here the head-neck of the subject was passively moved toward the target head position and subjects were asked to identify when they reached that position. **Results:** There was a statistically significant difference between the hold long (12.5 +/- 1.8 deg) and hold short (10.5 +/- 0.8 deg) estimates of target position at 10 degrees. Similar results were obtained for other target positions. **Conclusion:** Neck muscle conditioning alters perception of head neck position in a predictable way. It is suggested that hold long conditioning decreases neck intramuscular afferent input to the CNS which disturbs head-neck kinaesthetic sensibility.

POS-TUE-028

CHARACTERISTICS OF NEURONS WITH INPUT FROM DEEP NECK STRUCTURES

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Purpose: Little is known about the processing of sensory information arising from the deep tissues of the neck in spite of their significant involvement in chronic neck pain (1). The aim of this study was to determine the location and response characteristics of neurons with input from deep neck structures as a first step to determining the role of these neurons in chronic neck pain. **Methods:** Experiments were performed on 18 adult Wistar rats (250-600g) of either sex, pre-medicated with atropine (0.02mg/kg sc) and anaesthetized with a mixture (1:1) of urethane (1g/kg) and chloralose (0.1g/kg) given ip and supplemented (iv) to ensure the withdrawal and palpebral reflexes were abolished. Venous and arterial canulas were used to provide fluids and record arterial blood pressure. Rats breathed spontaneously via a tracheotomy. Standard extracellular electrophysiological recording strategies were used to record unitary nerve activity in the upper cervical spinal cord and medulla. Six rats were paralysed with gallamine triethiodide (35mg/kg iv) and artificially ventilated during the recording session. Paralysis was allowed to wear off, to check the level of anesthesia which was supplemented as needed. **Results:** 25 units responded to mechanical and or noxious chemical stimulation of deep neck tissues, 12 of these responded to electrical stimulation of C2 dorsal primary rami. **Conclusion:** These preliminary data demonstrate that neurons receiving input from the deep structures of the neck can be found in the upper cervical spinal cord and medulla, and like those with input from superficial tissues, tend to receive convergent input suggesting they serve to process information not just relay it. **Reference** 1) Barnsley L et. al. 1995 *Spine* 20: 20-25.

POS-TUE-029

THE SIZE, NEUROCHEMISTRY AND SEGMENTAL DISTRIBUTION OF SENSORY NEURONS INNERVATING THE RAT TIBIA

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Purpose: The aim of this study was to determine the size, neurochemistry and segmental distribution of sensory neurons innervating the rat tibia. **Methods:** The soma of sensory neurons innervating bone were labeled with injections of a retrograde tracer (Fast Blue) into the periosteum, medullary cavity or trabecular bone of the rat tibia. The segmental distribution of labeled neurons was determined from counts in L1-L6 DRG (n=3 for each injection location); the size of labeled neurons determined in L3 DRG (n=3 for each injection location); and the neurochemistry of labeled neurons in L3 DRG examined using SP and CGRP immunohistochemistry and IB4 binding (n=3 for periosteum and trabecular bone injections; n=2 for medullary cavity injections), and NF200 immunohistochemistry (n=1 for each injection location). **Results:** L3 DRG contained the greatest percentage of labeled neurons. The size distribution at L3 revealed that labeled neurons were predominantly in the small and medium size range. CGRP immunoreactivity was observed in the small and medium population, and in some of the few large neurons. SP immunoreactivity and IB4 binding was confined almost entirely to the small neuron population. NF200 immunoreactivity was only observed in the medium and large neuron population. Although there appeared to be more CGRP immunoreactive and IB4 binding neurons labeled following trabecular bone injections, no other differences in the size, neurochemistry or segmental distribution of labeled neurons were observed following injections into the periosteum, medullary cavity and trabecular bone. **Conclusion:** The periosteum, medullary cavity and trabecular bone are all innervated by sensory neurons that have size and neurochemical profiles consistent with a role in nociception. This role may be mediated by both peptidergic and non-peptidergic mechanisms.

POS-TUE-030

FUNCTIONAL ASSESSMENT OF LOCOMOTION IN DORSAL ROOT INJURED RATS: A COMPARATIVE STUDY USING THE LADDER RUNG WALKING TEST

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Purpose: Progress in the development of animal models of neural injury requires tests that are sensitive enough to characterise distinct levels of impairment. This study aims to assess the value of the ladder rung walking test (LRWT) as an analytical tool to rate levels of forepaw sensory impairments in dorsal root injured (DRI) rats. **Methods:** 18 AAW rats were used in this study. The rats were divided into 4 groups: 4-root DRI (C5-8; n=3), 3-root DRI (C5-7; n=3), 2-root DRI (C5-6; n=6) and control (n=6). The lesions were carried out under general anaesthesia with ketamine/xylazine (80/10mg/kg ip). Dorsal roots were exposed unilaterally and crushed with fine-tipped forceps for 3x 10 seconds rostral to the dorsal root ganglion. The root lesions were confirmed histologically. All animals had three training sessions on the LRWT apparatus prior to filming. Two levels of difficulty were created by varying the positions of the metal rungs from regular to irregular. Performance was measured using a paw placement rating scale which assesses correct placements versus errors made. **Results:** Gait impairment was proportional to the severity of the lesions with 2, 3 and 4 root-lesioned animals showing significantly different percentages of errors per step ($p<0.05$). All groups performed worse on an irregular compared to a regular pattern. **Conclusion:** Although the LRWT is considered primarily a motor task it is sensitive enough to discriminate between varying levels of sensory impairment and hence can be used for assessment of sensory impairment.

POS-TUE-031

RAT MODEL OF DORSAL ROOT INJURY (DRI): A COMPARISON OF 2- AND 4-ROOT LESIONS ON PAIN AND SKILLED-REACHING PERFORMANCE

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DRI disrupts afferent inputs from the periphery and often leads to sensory deficits and neuropathic pain, but a thorough characterisation of the sensory deficits produced by such injury is still lacking. **Purpose:** This study aimed to compare the deficits resulting from 2-root (C7-8) and 4-root (C5-8) dorsal root crush injuries made at levels which innervate the forepaws. **Methods:** Seventeen AAW-rats were divided into 4 groups: 4-root DRI (n=3), 2-root DRI (n=6), sham-operated (n=2) and control (n=6). Animals underwent DRI under anaesthesia with ketamine/xylazine (80/10mg/kg, i.p). Dorsal roots were exposed and crushed 3x10 seconds with fine-tipped forceps. Performance on skilled-reaching for sucrose pellets, as well as mechanical and thermal pain responses were measured before and up to 6 weeks after the lesions. **Results:** Postoperatively, only the 2-root DRI rats developed mechanical allodynia which persisted throughout the course of the study with a transient thermal hyperalgesia at week 1 and 6 only. In contrast, a decreased in sensitivity to mechanical and thermal stimulation were observed in 4-root DRI rats. No significant changes of pain responses were found in sham animals, suggesting the allodynia and hyperalgesia were due to the DRI. Decreased percentage of successful skilled-reaching could only be measured in 2-root DRI rats as 4-root DRI rats showed severe impairment in aiming and were unable to grasp the pellets. **Conclusion:** These results suggest that 2-root DRI represents a good model to assess treatments for neuropathic pain and for the restoration of the sensory component of the skilled-reaching performance. On the other hand, the 4-root DRI could be a useful model when complete forepaw deafferentation is needed.

POS-TUE-032

CHANGES IN FAST A-TYPE POTASSIUM CURRENT INACTIVATION HELPS PRESERVE NETWORK ACTIVITY IN THE SUPERFICIAL DORSAL HORN OF SPASTIC MICE

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The spastic mouse has a mutation in the inhibitory glycine receptor that disrupts synaptic input in motor and sensory pathways. **Purpose:** We examined how altered inhibitory drive affects neuron excitability and signal processing in the superficial dorsal horn (SDH) of spastic mice using both in vitro and in vivo patch-clamp recording techniques. **Methods:** For in vitro experiments, mice (C57Bl/6 or spa/spa; mean age P23) were anaesthetised with Ketamine (100 mg/kg, i.p.) and decapitated. Transverse slices were prepared from the lumbar cord and membrane properties and excitability of spastic and wildtype SDH neurons (n=91 and 97, respectively) were compared. For in vivo experiments, patch-clamp recordings were made from SDH neurons (n = 32 and 37, respectively) in urethane-anaesthetised (2.2 g/kg, i.p.) wild type and spastic mice (mean age P37). **Results:** Apart from a modest reduction in resting membrane potential (approx. 3 mV), membrane and action potential (AP) properties of neurons in the spastic mouse were identical to those of wildtype mice. There was, however, a substantial reorganization of AP discharge patterns during current injection. The prevalence of delayed firing neurons increased by 14% in spastic neurons and there was a depolarising shift in the steady-state inactivation of the rapid A-current. To assess the consequences of these changes on network activity, we next examined in vivo responses to innocuous (brush) and noxious (pinch) hindpaw stimulation. The prevalence of high-, low-, and sub-threshold responses during hindpaw stimulation was similar in wildtype and spastic mice. **Conclusion:** We suggest changes in rapid-A current function compensate for reduced inhibitory synaptic input and help preserve network function in the SDH of the spastic mouse.

POS-TUE-033

ASCENDING PROJECTIONS FROM THE RAT LUMBOSACRAL SPINAL CORD TO THE BRAIN: THREE DIMENSIONAL RECONSTRUCTIONS FOR CORRELATING ANATOMY AND FUNCTION

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Purpose: Ascending projection neurons can be identified in rat spinal cord following the injection of retrograde tracers into discrete brain regions. In this study, functional groupings of projection neurons in somatic (L4/L5) and autonomic (L6/S1) regions of the spinal cord were identified. **Methods:** Female Wistar rats aged 3-4 months were deeply anaesthetised with ketamine (60 mg/kg) and zylazine (10 mg/kg i.p.). Rats were then placed in a stereotaxic frame and 200 nl of 4% Fluoro-Gold was microinjected into the periaqueductal gray, Barrington's nucleus, medial hypothalamus, or gracile nucleus ($n \geq 2$ for each region). One week after injection, rats were perfused and the placement of injections confirmed. Segments L4–S1 were sectioned horizontally at 40 µm and processed for immunohistochemistry using anti-FluoroGold antibody and visualized using diaminobenzidine. Each segment was then reconstructed using Neurolucida, to show the distribution of FluoroGold positive neurons. **Results:** Serial reconstructions of the lumbosacral segments revealed neurons projecting centrally that lay in a series of clusters distributed longitudinally. The dimensions and morphology of neuronal groups differed according to the location of tracer injection in the brain. For example, groups of neurons projecting to the periaqueductal gray were located mainly in the superficial and intermediate dorsal horn and the lateral spinal nucleus in all of segments L4-S1, while neurons projecting to Barrington's nucleus were concentrated in the intermediate dorsal horn of L6 and S1. **Conclusion:** These three-dimensional reconstructions of lumbosacral projection neurons extend previous reports and provide a more precise map of ascending pathways from these functionally distinct caudal segments.

POS-TUE-034

PRIMARY AFFERENT FIBRES HAVE EXTENSIVE PROJECTIONS ACROSS MULTIPLE SEGMENTS OF THE SPINAL CORD DORSAL HORN, AS SHOWN USING IN VITRO ANTEROGRADE TRACING TECHNIQUES

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Introduction: In mice, some nociceptive neurons express the glutamate transporter VGluT2 but not the peptides SP or CGRP or the lectin IB4. We are investigating the central projections of these neurons into lamina I-III of the dorsal horn. Anterograde labelling in vitro was used to distinguish primary afferent endings from those of interneurons or projection neurons. **Methods:** C57/Bl6 mice (n=9) were killed by inhaled isoflurane and heart removal. Spinal cords and attached dorsal root ganglia were removed. The peripheral ends of single lumbar dorsal roots were isolated, 5% neurobiotin was applied, and the preparation incubated for 18-23 hours at 35°C. Spinal cord segments spanning mid-thoracic to sacral levels were serially sectioned and labelled immunohistochemically. **Results:** Primary afferent fibres were labelled in all preparations with most restricted to the ipsilateral dorsal horn. Larger diameter axon bundles ran in Lissauer's Tract at the level of the dorsal root entry zone and in the dorsal funiculus cranial to the entry zone. Smaller diameter varicose fibres ran through laminae I-III. Few labelled fibres occurred in the deep dorsal horn or around the central canal; none occurred in the ventral horn. Labelled primary afferent fibres projected at least twelve segmental levels cranial to the level where neurobiotin was applied. Fewer labelled fibres projected to segments caudal to the application site. **Conclusion:** In vitro anterograde tracing using neurobiotin is a successful method for labelling the central endings of primary afferent fibres in the mouse spinal cord. The next aim is to use immunohistochemical markers to distinguish the different nociceptive fibre populations.

POS-TUE-035

CORTICAL REORGANIZATION FOLLOWING SPINAL CORD INJURY AND NEUROPATHIC PAIN

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Purpose: Although the loss of motor control is arguably the most debilitating consequence of spinal cord injury (SCI), more than half of all SCI subjects report the presence of on-going chronic pain. Chronic pain can also develop after limb amputation and recent neuroimaging studies have revealed that limb amputation pain is associated with functional reorganization of the primary somatosensory cortex (S1). Although S1 reorganization has been shown to occur following SCI, no study has investigated whether this reorganization is correlated to pain. The aim of this investigation is to use functional magnetic resonance imaging (fMRI) to investigate cortical organization in SCI subjects and to determine if any reorganization is correlated to on-going pain. Methods: Fifteen healthy, 8 SCI subjects with pain and 7 SCI subjects without pain were recruited. S1 activation during innocuous brushing of the lip, thumb and little finger were assessed using fMRI (57 slices, TR = 3s, matrix size = 128*128, TE = 30ms). The Euclidian distances between an anatomical marker and the maximally activated voxel in S1 were calculated. Results: SCI patients displayed S1 reorganization with the maximally activated voxel during little finger stimulation displaced medially compared with controls. Furthermore the degree of this medial shift was correlated to pain intensity. Conclusion: SCI pain is associated with S1 reorganization. We suggest that a better understanding of the underlying mechanisms responsible for this reorganization could help in the search for novel and more effective treatments of SCI pain.

POS-TUE-036

NEURONAL PENTRAXIN-1 ELEVATION IN EXCITATORY NERVE TERMINALS IN SPINAL CORD DORSAL HORN IS ASSOCIATED WITH CHRONIC INFLAMMATORY PAIN

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Neuronal pentraxin 1 (NP1), a secreted neuronal synaptic protein, has been hypothesised to function during synapse formation and remodelling, particularly following pathogenic events such as ischemic injury. One proposed mechanism involves NP1 mediated clustering of AMPA-type glutamate receptor subunits. **Purpose:** We have used immunohistochemistry to characterise the localisation of NP1 in rat spinal cord (SC) and to determine whether NP1 immunostaining is altered during a model of chronic inflammation. **Methods:** Male SD rats were given an injection of complete Freund's adjuvant (CFA) to the left hind paw then transcardially perfused at various time intervals (0-14days) post-CFA injection. Lumbar SC was processed for immunohistochemistry using an antigen retrieval protocol. **Results:** Dense NP1 immunostaining was localised to lamina I of the dorsal horn and the spinal lateral nucleus. Moderate NP1 immunostaining was visualised in laminae II and V. There was a significant increase ($p < 0.05$) in the intensity of dorsal horn NP1 staining at 5 days post-CFA injection (n=8), ipsilateral to inflammation compared to control (n=8). NP1 immunostaining was comparable to control at day 14 (n=3). In double labelling experiments of lumbar SC, 5 days post-CFA injection, NP1 was colocalised with VGlut2 (n=6) synaptic labelling and highly colocalised with cGRP (n=4) immunoreactivity. In dorsal horn, NP1 immunoreactivity was also colocalised with synapsinII (n=4), but not synapsinI (n=4) immunostaining. However, there was no observable overlap with NP1 and GluR1 (n=6), GluR1-831 (n=2) and GluR2 (n=4) AMPA subunit immunoreactivity. **Conclusion:** Inflammation-induced increased NP1 in cGRP positive, glutamatergic terminals (VGlut2 positive) may be associated with changes in AMPA receptor density in dorsal horn synapses of nociceptive neurons.

POS-TUE-037

CHANGES IN PAIN PERCEPTS FOLLOWING REPEATED INTRAMUSCULAR INJECTIONS OF HYPERTONIC SALINE: A LONGITUDINAL STUDY

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Purpose: Intramuscular injection of hypertonic saline is a popular model of experimental muscle pain, with the pain being characterised as a dull, poorly localised ache that is often perceived in local and referred site (or sites). We tested the hypothesis that noxious inputs from the same area of muscle produce pain in a pattern that, for a given subject, is consistent over time. **Methods**: A bolus 1 ml intramuscular injection of 5 % hypertonic saline was made into the same site of the tibialis anterior muscle on the same day each week for four weeks, following an initial training session 1 week previously in which an intramuscular injection was made into flexor carpi radialis. Ten subjects mapped the areas of primary and referred pain, and rated the intensities on a visual analogue scale, every 30 s until the cessation of pain. **Results**: Over four weeks, nine subjects experienced a decrease in the intensity and duration of primary pain. In five subjects the expression of this local pain was perceived to migrate away from the area around the injection site; in three subjects the pain migrated distally over the four successive injections. **Conclusions**: We conclude that activation of muscle nociceptors in a discrete area of tissue generates pain that, with repeated injections over four weeks, is perceived to originate in areas often remote from the site of primary stimulus, and that the local component of this pain decreases over subsequent stimuli.

POS-TUE-038

COMPARISON OF DORSAL HORN REMODELLING IN COMPLETE AND INCOMPLETE SPINAL INJURY MAY REVEAL MECHANISMS THAT UNDERLIE NEUROPATHIC PAIN

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Purpose: Humans with spinal cord injury (SCI) commonly develop chronic neuropathic pain that is difficult to treat. As remodelling of nociceptive circuitry in the dorsal horn above an injury could factor in the development and maintenance of neuropathic pain, we examined structural changes in rat spinal cord after 1) clip compression injury that induces mechanical forepaw allodynia and 2) complete spinal transection, which does not induce allodynia. **Methods**: Six groups ($n \geq 4$) comprising 35g clip-compression injury (CC), complete transection (both at T13) and sham-operation, were studied 2 or 12 wks post-injury. Images of T12-T10 dorsal horn were used to measure the density of immunohistochemically identified peptidergic (CGRP+) and non-peptidergic (GFRα1+ and GFRα2+) primary afferent terminals; and descending projections of supraspinal catecholamine (TH+) and serotonergic (SERT+) neurons. **Results**: Transection and CC resulted in a transient decrease in CGRP+ fibre density, indicating reduced dorsal horn innervation. In contrast, there was a delayed decrease in GFRα1-immunoreactivity indicating reduced innervation after CC, but no change after transection. However, GFRα2+ fibre innervation was reduced after both transection and CC, as indicated by decreased GFRα2-immunoreactivity. A decrease in TH+ fibre density was permanent after transection, but transient after CC, indicating recovery. The density of SERT+ fibres in the dorsal horn did not change in either model. **Conclusions**: We have shown that non-peptidergic afferent fibres and descending catecholamine projections undergo different patterns of remodelling after transection versus clip-compression, which may in part account for the development of allodynia in rats undergoing clip compression.

POS-TUE-039

IDENTIFICATION OF SUPRASPINAL PATHWAYS INVOLVED IN THE TRANSMISSION OF NOCICEPTIVE INPUT OF BONY ORIGIN

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Williams, MC and Ivanusic, JJ Department of Anatomy and Cell Biology, University of Melbourne, VIC 3010 Purpose: In the present study, three ascending spinal pathways (spinothalamic, post synaptic dorsal column, and spinoparabrachial) involved in the relay of cutaneous and/or visceral input were investigated to determine if they were also involved in the relay of nociceptive information from bone. Methods: Fluorogold (FG) was injected into the ventral posterolateral nucleus of thalamus, gracile nucleus or the parabrachial nucleus of deeply anaesthetised adult male Sprague-Dawley rats to identify neurons of the spinothalamic, post synaptic dorsal column and spinoparabrachial pathways, respectively. Seven days later these rats were re-anaesthetised and a noxious mechanical stimulus, bone drilling, was applied to the tibia to induce c-Fos expression in the lumbar dorsal horn. Results: Bone drilling (n=4) resulted in a marked increase in the number of Fos-like immunoreactive (Fos-LI) nuclei in both L3 and L4 relative to sham surgery (n=4). There was no colocalisation of FG and Fos-LI in any spinal dorsal horn neurons following injection of FG into the ventral posterolateral nucleus (n=4) or gracile nucleus (n=4). However, colocalisation was observed in rats that received parabrachial FG injections. In the parabrachial group, there was an increase in the percentage of neurons with colocalisation of FG and Fos-LI in the bone drill group (n=4) relative to a sham surgery group (n=4) (significant at L4 but not at L3; $p<0.05$). Conclusions: These results suggest that nociceptive input from the rat tibia is relayed through the spinoparabrachial pathway but may not be relayed through the spinothalamic or the post synaptic dorsal column pathways. The implications for this work suggest that the pathways for bone nociception appear to be different than those of cutaneous and visceral nociception.

POS-TUE-040

N-ARACHIDONOYL AMINO ACIDS INHIBIT RECOMBINANT T-TYPE CALCIUM CHANNELS

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Purpose: *N*-arachidonoyl amino acids are endogenous fatty acid-amino acid conjugates structurally related to the endocannabinoid anandamide. N-arachidonoyl dopamine (NADA) and N-arachidonoyl serotonin (NA-5HT) display antinociceptive properties which cannot be fully explained by interactions with known proteins. T-type calcium channels (I_{Ca}) are an emerging analgesic target present in nociceptive sensory and central nervous system neurons with prominent roles in pain modulation. This study aimed to investigate the effect of NADA and NA-5HT on recombinant Ca_v3 channels, which form native T-type I_{Ca}. **Methods:** Human $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ were stably expressed in HEK293 cells and channel currents studied with whole cell patch clamp recordings. Each data point is from 6 or more cells. **Results:** NADA and NA-5HT completely inhibited Ca_v3 channels at 10μM. The EC50 values for NADA inhibition of $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ were 520nM, 1.4μM and 350nM respectively. NA-5HT inhibited $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ with EC_{50} values of 50nM, 650nM, and 350nM respectively. EC_{50} concentrations of NADA caused a significant hyperpolarising shift ($P < 0.01$) in the half-inactivation potential of each channel ($Ca_v3.1$: -8.7 ± 1mV; $Ca_v3.2$: -8.2 ± 3mV; $Ca_v3.3$: -8.8 ± 2mV) and the half-activation potential of $Ca_v3.1$ (-6.1 ± 2mV). NA-5HT had similar effects on $Ca_v3.1$. **Conclusions:** Both NADA and NA-5HT inhibit recombinant human T-type I_{Ca}. NA-5HT is the most potent inhibitor of $Ca_v3.1$ channels yet described. Channel inhibition results at least partly from an increased number of inactivated channels at modestly hyperpolarized membrane potentials, leaving fewer channels available to open. The ability of NADA and NA-5HT to inhibit T-type I_{Ca} suggests a possible role in modulating nociception.

POS-TUE-041

OPIOID TOLERANCE IN TRIGEMINAL GANGLION NEURONS FROM CHRONICALLY MORPHINE TREATED ARRESTIN3 KNOCKOUT MICE

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Purpose: μ-Opioid receptor (MOR) agonists are effective analgesics but prolonged use can lead to tolerance. Chronic morphine treatment (CMT) reduces the efficacy of MOR signalling in mouse trigeminal ganglion (TG) neurons. Arrestin3 was reported to mediate receptor adaptations to chronic opioid treatment so we re-examined opioid tolerance in TG neurons from arrestin3 knockout mice. **Methods:** Adult male mice were injected subcutaneously with slow release morphine emulsion (300mg/kg) or vehicle 3 times over 5 days. I_{Ca} was recorded from acutely isolated TG neurons on day 6 or 7 using standard patch clamp techniques. mRNA levels for key receptor regulatory proteins were determined using quantitative real time PCR. Each experiment was repeated in 6 cells or animals. **Results:** Morphine inhibited I_{Ca} with similar potency and maximal effect in naïve animals of each genotype (+/+ 230nM, 43%; -/- 380nM, 44%). There was no constitutive MOR activity in TG neurons from either genotype. The maximal inhibition of I_{Ca} by morphine was reduced to a similar extent in CMT animals of either genotype (to 18% in +/+; 21% in -/-). There were no differences in the expression of mRNA for MOR, arrestin2, G protein receptor kinase 2 or dynamin 1 in TG of naïve animals of each genotype, and CMT produced no changes in mRNA levels. **Conclusions:** In adult sensory neurons acute opioid signalling is not affected by arrestin3 deletion, and adaptations that accompany CMT also do not require arrestin3. This is consistent with increasing evidence that arrestin does not play an obligate role in the acute or chronic agonist-induced regulation of MOR signalling.

POS-TUE-042

CHANGES IN MEMBRANE PROPERTIES OF MOUSE SUPERFICIAL DORSAL HORN NEURONS DURING EMBRYONIC AND POSTNATAL DEVELOPMENT

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Superficial dorsal horn neurons process noxious, thermal and tactile stimuli. Their output is shaped by combined action of synaptic inputs and intrinsic membrane properties. Early in development protective reflexes are exaggerated, peripheral receptive field size is large, and different classes of sensory axons penetrate the SDH sequentially. It is not clear how intrinsic membrane properties of SDH neurons change during development. **Purpose:** We quantified membrane, action potential (AP), discharge, and subthreshold current properties of SDH neurons from E15-P25. **Methods:** Mice (C57Bl/6) were anaesthetised with Ketamine (100 mg/kg, i.p.) and decapitated. Transverse slices were prepared from lumbar spinal cord and patch-clamp recordings were obtained from SDH neurons (32°C). **Results:** Data were assigned to six age categories (E15-17, n = 51; P0-5, n = 60; P6-10, n = 49; P11-15, n = 54; P16-20, n = 49 and P21-25, n = 52 neurons). Resting membrane potential became more hyperpolarized and input resistance decreased during development. AP properties such as spike amplitude and afterhyperpolarization amplitude increased, whereas spike half-width decreased. Neurons were categorized into five groups according to their AP discharge following current injection. Before P6-10 single spiking neurons dominated the sample (> 50%), and delayed firing was rarely (1/160) observed. In older animals (P11-24) initial bursting dominated (> 45%). The changes in AP discharge were accompanied by changes in the prevalence of major subthreshold currents. The rapid A-type potassium current dominated (> 50%) in all age groups, however, its prevalence decreased during development and stabilized after P10**. Conclusion:** Together, these data suggest the membrane, AP, and discharge properties of SDH neurons undergo major alterations during development and reach a mature profile after P10.

POS-TUE-043

VOLTAGE DEPENDENT CURRENTS IN TYPE I AND II HAIR CELLS AND CALYX TERMINALS OF PRIMARY AFFERENTS IN AN INTACT MOUSE VESTIBULAR PREPARATION

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Type I and II hair cells in vestibular end-organs convert mechanical stimuli into neural signals, which are transmitted via primary afferents to the CNS. Past studies have emphasized isolated cell preparations, but this approach disrupts hair cell/afferent interactions and cell currents. **Purpose**. We developed an intact preparation of the mouse crista to study hair cells and primary afferents, *in situ*. **Methods**. Vestibular end-organs were isolated from euthanized mice (ketamine 100 mg/kg; i.p.). Voltage dependent currents were measured using whole-cell patch-clamp method in type I hair cells, the cup-like (calyx) afferent terminals that surround them, and type II hair cells within the crista ampullaris. **Results**. Outward potassium currents were activated positive to -72 mV in type I hair cells (n = 3; holding potential, Vm = -60 mV), similar to that observed in isolated type I hair cells. Preliminary data recorded from one calyx terminal (Vm -60 mV) revealed comparative outward potassium currents that activated positive to -75 mV. In contrast, outward potassium currents in type II hair cells (n = 23; Vm -60 mV) activated at voltages more positive than -45 mV, and inward potassium currents activated negative to -70 mV. No currents were activated between the ranges of -70 to -45 mV in type II hair cells. **Conclusions**. Our intact *in vitro* preparation is a novel approach and we preserve the relationship between hair cells and their associated primary afferents within the crista. This is the first step in developing a viable preparation to determine how information is passed between the hair cell and the primary vestibular afferent.

POS-TUE-044

COCHLEOTOPIC ORGANISATION OF THE CENTRAL AUDITORY PATHWAY IN THE NEONATALLY DEAFENED CAT

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Purpose: Using single- and multi-unit recordings it has been shown that long periods of deafness and / or chronic intra-cochlear electrical stimulation can effect the cochleotopic organisation of the primary auditory cortex (AI), despite lower auditory centres (including the cochlear nucleus and inferior colliculus) appearing to develop a near normal cochleotopic organisation. **Methods:** Nine animals were neonatally deafened and four of these animals were subsequently implanted with a multi-channel scala tympani electrode array. The implanted animals received unilateral electrical stimulation to restricted sections of the basal turn of the cochlea for periods of up to 11 months via a clinical speech processor and cochlear implant. Analyses of electrically evoked potentials, recorded from multi-channel electrode arrays inserted into AI, were used to determine the most efficacious intra-cochlear stimulating electrode for each cortical location. **Results:** Cochleotopic organisation, defined as a systematic change in the most efficacious electrode across the rostral-caudal dimension of AI (Pearson correlation; $p < 0.05$), was observed in three of the five unstimulated deafened animals and in three of the four chronically stimulated animals. Interestingly, chronic intra-cochlear electrical stimulation resulted in a significant increase in the rostral-caudal extent preferentially activated by each intra-cochlear electrode (Student-T test; $p < 0.05$). **Conclusion:** These results, based on evoked potential recordings and therefore dominated by the thalamo-cortical input volley, indicate that the basic cochleotopic organisation of the cortical input is not dependent on afferent activity. This is at odds with multi-unit recordings from AI, suggesting a difference in the cochleotopy of cortical input and local activity within AI.

POS-TUE-045

FACTORS AFFECTING NEURAL RESPONSE TELEMETRY RECORDINGS IN THE CHRONICALLY STIMULATED CAT

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Purpose: There is an increasing tendency to provide cochlear implants to patients at a young age; however, determining stimulation levels in such young patients can be challenging. Therefore, systems to record the electrically evoked compound action potential (ECAP) have been developed, including Neural Response Telemetry (NRT). Many factors may affect the ability to record ECAPs, including electrode impedance, electrode location, and evoked auditory brainstem responses (EABRs) thresholds. **Methods:** Two months after neonatal deafening, profoundly deaf cats (n=8) were implanted with intracochlear electrode arrays and received unilateral electrical stimulation. Electrode impedance was monitored daily, NRT was measured every two weeks and EABRs were recorded every month for each electrode (n=56). **Results:** It was possible to record EABRs from 49 electrodes (87%) and NRTs from 28 electrodes (50%). When both an EABR and NRT was present there was no difference between EABR and NRT thresholds (Student T-test; p = 0.342). For 21 electrodes (37%) it was possible to record EABRs but not NRTs. When compared to electrodes for which it was possible to record both EABRs and NRTs, these electrodes did not have significantly different electrode impedances (Student T-test; Access Resistance: p=0.403; Total Impedance: p=0.705), EABR thresholds (Student T-test; p=0.130) nor were they located within different regions of the cochlea (Chi-square; p=0.720). In no case was it possible to record an NRT but not an EABR. **Conclusion:** It was possible to record NRTs in only 57% of cases where there was an EABR. However, electrode impedance, electrode location, and threshold were not contributing factors. Improvements in the ability to objectively determine threshold and maximum comfortable levels of stimulation are still required as they may contribute to an improved clinical performance among subjects implanted at a very young age.

POS-TUE-046

APOPTOSIS PATHWAYS GENE EXPRESSION CHANGE WITH AGE IN CBA MOUSE COCHLEA

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Apoptosis is an endogenous programmed cell death (PCD). Reports estimate that either too little or too much apoptosis contributes to a significant number of medical illnesses. Apoptotic pathways in the cochlea have been investigated, and these findings point to the possibility of different pathways playing a role in hair cell death in age related hearing loss - presbycusis. **Purpose:** We studied the expression of 318 genes in different apoptotic pathways in the CBA mouse cochlea. **Methods:** Mice were divided into 4 groups: young adult control with good hearing (N=8), middle-aged with good hearing (N=17), old with mild presbycusis (N=9) and old with severe presbycusis (N=6). We found evidence implicating 35 pro- and anti-apoptotic genes in the etiologies of presbycusis. We also investigated the correlation between gene expression and hearing measurements, including Distortion Product Otoacoustic Emissions (DPOAEs) and Auditory Brainstem Responses (ABRs). In the present investigation, we validated the gene expression of 31 of these significant genes using the TaqMan® Low Density qPCR Array (TLDA). **Results:** Eight of the 31 genes identified with the microarray analyses showed comparable results for both the genechip and the qPCR. These genes are: Atf3, Bcl2, Bcl2l1, Casp4, Capn2, Dusp9/Mapk4, Tnfrsf12a and Tnfsf13b. **Conclusion:** It is hoped that increased knowledge of cell death pathways in the aging auditory system may lead to interventions to slow or prevent the progression of presbycusis, and concomitant declines in complex sound processing with age.

POS-TUE-047

INVESTIGATING TONOTOPIC CHANGES IN COCHLEAR NUCLEUS, INFERIOR COLLICULUS AND AUDITORY CORTEX IN AN ANIMAL MODEL OF TINNITUS

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Tinnitus is defined as the perception of sound in the absence of an external source. Although subjective, idiopathic tinnitus is frequently associated with a hearing loss, there is much support for the idea that the generator of tinnitus is central in origin. However, there is no consensus as to where, or at what level of the auditory system this may occur. **Purpose:** To address this issue we examined neuronal changes at three different levels of the auditory system in a noise-trauma model of tinnitus. Our emphasis was to simultaneously record multi-unit responses from cochlear nucleus, inferior colliculus and primary auditory cortex, using multi-channel electrodes. In another series of experiments, conventional electrodes were used to map the auditory cortex of noise-trauma and normal animals. **Methods:** Rats (n=8) were exposed to a 16 kHz bandpass noise for 1hr. Ten unexposed rats served as controls. In the multi-electrode recording experiments, frequency response functions were generated from presentation of 44 frequencies (20-80 dB SPL, 20 repetitions, 1 Hz presentation rate. **Results:** Normal animals showed an ordered, caudal-to-rostral increase in best frequency in the cortex, which was absent in the noise-trauma animals. The majority of multi-unit clusters in the noise-trauma cortex showed two distinct best frequency response areas (9-12 kHz and 30-35 kHz), which bordered the spectral range of the noise-trauma stimulus. Tonotopic changes were less obvious in the inferior colliculus, and virtually absent in the cochlear nucleus. **Conclusion:** Our results suggest a significant role of auditory cortex in noise-trauma induced tinnitus.

POS-TUE-048

ELECTRICAL STIMULATION MAINTAINS SPIRAL GANGLION NEURONES FOLLOWING REMOVAL OF EXOGENOUS NEUROTROPHINS

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Purpose: Exogenous neurotrophins (NT) rescue spiral ganglion neurons (SGNs) from degeneration, however, to be effective they must be supplied continuously[1]. We previously reported significant rescue advantage when NT is combined with chronic electrical stimulation (ES)[2]. Here, we examine whether chronic ES can maintain SGN survival long after cessation of NT delivery. **Methods:** Ten adult guinea pigs were deafened and unilaterally implanted with an intracochlear electrode array and drug delivery system. Brain derived neurotrophic factor (BDNF) was delivered to the cochlea for 4 weeks in combination with ES. One cohort (n=5) received ES for 6 weeks (a 2 week period after the cessation of BDNF delivery; ES_6); a second cohort (n=5) received ES for 10 weeks, (a 6 week period following cessation of BDNF delivery; ES_{10}). Cochleae were harvested for histology and SGN density determined for each turn for comparison with normal controls (n=4). **Results:** The withdrawal of BDNF resulted in a rapid loss of SGNs in turns 2-4 of deafened/BDNF-treated cochleae; this was significant as early as 2 weeks following cessation of the NT when compared with normal controls ($p<0.05$). Importantly, while there was a small reduction in SGNs in turn 1 (i.e. adjacent to the electrode array) after NT removal, this reduction was not significant compared with normal controls. **Conclusions:** These results demonstrate that chronic ES can maintain SGNs after initial rescue using exogenous NTs. This finding has implications for the clinical application of NTs and supports earlier work demonstrating a rapid SGN loss after NT removal[1]. [1]Gillespie et al., 2003 J Neurosci Res 71, 785-790. [2] Shepherd et al., 2005 J. Comp. Neurol. 486, 145-158.

POS-TUE-049

MICROSURGICAL ACCESS FOR CELL INJECTION INTO MAMMALIAN COCHLEA

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Purpose: The potential use of stem cells to repair hearing loss requires surgical access to the cochlea. Here we refine a microsurgical technique, with minimal damage to hearing, for cell injection into the mouse cochlea. A computerised signal-to-noise ratio (SNR) detection method was developed to test the effect of surgery on hearing (Axograph Scientific). **Methods:** CBA/CaH mice aged 3-32 postnatal weeks were used in this study (n=30). Cochleostomies were performed with minimal trauma surgery on live mice (n=9) or mice sacrificed according to ethical guidelines (n=15). A postauricular incision was made, the bulla was opened with a microdrill and the cochleostomy was performed with a 29-gauge needle. Green fluorescent cells (ZsGreen-MCF10A cells), paint or normal saline were injected via a glass needle inserted into the cochleostomy. The effect of surgery on auditory function was investigated with auditory brainstem responses (ABR) to click and tone stimuli. **Results:** Mouse cochleostomies were consistently performed with preservation of the facial nerve and middle ear. Histological analysis with fluorescent microscopy revealed that cells were successfully injected into the scala media and other cochlear compartments. The mean ABR threshold for combined click and tone stimuli was 35 dB greater after sham surgery with minimum hearing loss (15 dB) achieved with a small sized cochleostomy (≤ 0.4 mm) and by sibling matching to control mice (control 33 ± 4 dB, surgery 48 ± 3 dB). **Conclusion:** The surgical techniques provide a basis for future injections of stem cells into the cochlea of deaf mice and the exploration of a cell-replacement therapy for the treatment of hearing loss.

POS-TUE-050

DUAL SITE STIMULATION IN THE VENTRAL COCHLEAR NUCLEUS: A NEW INSIGHT FOR PENETRATING AUDITORY BRAINSTEM IMPLANTS

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Purpose: Several clinical studies have indicated that present users of the surface Auditory Brainstem Implant (ABI) only obtain limited sound perception and not all electrodes stimulated provide acoustic sensation. Our recent study (Shivdasani et al., J Neurophysiol, 2007, in-press) using penetrating multichannel electrodes in the ventral cochlear nucleus (VCN) and the inferior colliculus (IC) has indicated that VCN stimulation of a single site within an isofrequency lamina can achieve lower thresholds and a high degree of frequency-specific IC activation as compared to surface stimulation, however not in all cases. In this study, we hypothesized that simultaneous stimulation of two VCN sites in similar isofrequency laminae would further lower thresholds of IC activation. **Methods:** Experiments were conducted on urethane-anaesthetized rats (n = 9). Data were analysed in response to pure tones from 12 and 52 multiunit clusters in the VCN and IC, respectively. Each individual VCN site and pairs of sites (n = 6) that had similar characteristic frequencies were stimulated while recording IC responses. **Results:** IC sites responded to dual site stimulation with significantly lower thresholds and dynamic ranges as compared to single site stimulation (Threshold = 9.9 ± 3.2 μA dual, 14.4 ± 5.6 μA single, $p<0.0001$; Dynamic Range = 13.9 ± 7.0 μA dual, 18.4 ± 10.0 μA single, $p<0.005$; Mean ± SD). **Conclusion:** This method of dual site stimulation along with an electrode array with more number of sites within a single isofrequency lamina could result in significant improvements in speech understanding if incorporated in an ABI stimulation strategy.

POS-TUE-051

REAL-TIME MEASUREMENT OF SEROTONIN (5-HT) REUPTAKE IN RAT ILEUM

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Purpose: The release of serotonin (5-HT) from the enterochromaffin cell is a critical step in intestinal sensory transduction. Yet it is unclear what contribution the serotonin reuptake transporter (SERT) has on the actions of 5-HT. This project aimed to demonstrate SERT activity in rat ileal mucosa and to assess the effects of SERT inhibition on motility and 5-HT release. **Methods:** Rats (n=14) were anesthetised with pentobarbital and their carotid arteries severed. A segment of ileum was removed, placed in physiological saline and pinned flat, mucosa up in a small organ bath. Electrochemical recordings were made using 7μm carbon fibre electrodes and a force transducer recorded circular muscle tension. 5-HT (5-100μM) was pressure ejected and the time course of 5-HT re-uptake measured. Endogenous 5-HT concentration was calculated from the current produced by exogenous 5-HT. **Results:** In rat ileum, basal release of 5-HT was 6±3μM (n=9). Compression of the mucosa stimulated a total 5-HT release of 16±7μM (time-to-peak: 0.46±0.1s; n=6). Superfusion of the SERT inhibitor fluoxetine (1μM) increased basal oxidation current (control: 0.53±0.26nA; fluoxetine: 1.19±0.59nA; $p<0.05$; n=6) and increased the motility index (from 1.2 to 1.5; $p<0.05$; n=8) but not the frequency of contractions (11 to 13/min). Fluoxetine cause a significant increase in the 50% decay times of exogenous 5-HT from 1.5±0.5s to 2.6±0.9s ($p<0.05$; n=5). **Conclusion:** This is the first characterisation of the real-time release of 5-HT from rat ileum. Further, how SERT shapes the kinetics of exogenous 5-HT was demonstrated and a role for SERT in the control of motility was shown.

POS-TUE-052

IDENTIFICATION OF THE PAIN PATHWAY ACTIVATED BY RECTAL DISTENSION IN MICE

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Introduction: In rodents it is well known that noxious levels of rectal distension activates a stereotypical pain reflex consisting of a contraction of the abdominal muscles - known as a visceromotor response (VMR). Although a variety of ion channels have now been shown to contribute to mechanosensation and the visceral pain arising from the mouse rectum, the actual nerve pathway that conducts visceral pain following rectal distension to the central nervous system (CNS) is not known. In this study, we applied lesions to all the known visceral afferent nerve pathways, to identify which nerve(s) conduct visceral pain to the CNS. Methods: Wild type (C57BL/6) mice were anesthetized by an i.p. administration of Pentobarbitone sodium to induce a sufficient level of anesthesia. Electromyogram (EMG) recordings were made from the transverse oblique abdominal muscles, while intraluminal distension was applied to the distal 5mm of rectum, using a repetitive isobaric distension paradigm (80-100mmHg) to activate a repetitive VMR (n=9). Cutting (n=4), or crushing (n=5) the lumbar colonic nerves had no detectable effect on the VMR. In these same animals, further cutting the right, or left hypogastric nerves also failed to cause any detectable change in the VMR (n=5), at least using our isobaric stimuli. However, cutting the left and right branches of the pelvic nerves completely blocked the VMR. In these animals, even though the pelvic and hypogastric nerves were cut and the distension-evoked VMR was abolished, pinching the tail readily still activated a VMR (n=3). Conclusions: These observations suggest that the visceral pain pathway activated by rectal distension in mice is conducted predominantly, if not solely, through pelvic afferent nerve fibres.

POS-TUE-053

UPREGULATION OF C-JUN PROTEIN IS CAUSED BY AXOTOMY OR DEAFFERENTATION OF PELVIC GANGLION NEURONS

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Purpose: The immediate early gene product, c-Jun, is upregulated within many sensory and sympathetic neurons after axotomy and is considered essential for axonal sprouting during early regeneration. We investigated c-Jun expression in pelvic ganglia to determine if (i) injured parasympathetic neurons show this upregulation, (ii) c-Jun upregulation requires an injury stimulus, or if it can be induced by deafferentation that also elicits axonal sprouting, and (iii) if c-Jun expression is neurturin-dependent. **Methods**: Parasympathetic postganglionic axotomy (penile nerve cut or crush) or ganglion deafferentation (hypogastric and pelvic nerve cut) was performed in adult male rats and mice (n≥4 for all experiments). Pelvic ganglia were processed for immunohistochemistry. In some animals FluoroGold was injected into the cavernous space a week before nerve injury. **Results**: One week after axotomy, many nitrergic FluoroGold neurons and glia expressed c-Jun. Eight weeks later, when structural regeneration was largely complete, neuronal and glial expression of c-Jun returned to control levels. Some noradrenergic neurons expressed c-Jun even though these would not have been injured. Deafferentation was also a potent stimulus of axonal sprouting and c-Jun upregulation in neurons and glia. Neurturin gene deletion did not affect c-Jun upregulation after axotomy or deafferentation. **Conclusion**: Parasympathetic pelvic ganglion neurons and associated glia upregulated c-Jun expression after axotomy. Expression of c-Jun required axonal sprouting rather than injury because it was also induced by deafferentation. Neither axotomy- nor deafferentation-induced c-Jun expression required the neurotrophic factor, neurturin. This study revealed mechanisms involved in structural remodeling of pelvic autonomic nerve circuits that may be modulated to improve regenerative processes.

POS-TUE-054

COCAINE AND AMPHETAMINE REGULATED TRANSCRIPT; A NOVEL MARKER OF VASOMOTOR SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT

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We have examined the distribution of cocaine and amphetamine-regulated transcript-like immunoreactivity (CART-LI) preganglionic fibers in the rat superior cervical ganglion (SCG) and stellate ganglion and their association with postganglionic neurons of identified function. **Methods**: Fast Blue was injected into skin, brown fat, salivary gland and skeletal muscle of rats (n=3 rats in each case). One week later, animals were anaesthetised, perfusion fixed and the ganglia removed. Vascular and non vascular postganglionic neurons were identified by a combination of retrograde labelling and NPY-LI and analysed using a confocal microscope. In a separate series of animals (n=3), the anterograde tracer, biotinylated dextran, was injected into the spinal cord to label the terminals of preganglionic neurons. **Results**: Within both SCG and stellate ganglia, CART-LI terminals were preferentially associated with postganglionic neurons immunoreactive for neuropeptide Y. Confocal analysis revealed that CART-LI varicosities were observed in close apposition with vascular neurons projecting to muscle, skin and salivary gland, where as postganglionic neurons projecting to piloerector muscle and salivary gland acini were not closely associated with CART-LI varicosities. Vascular neurons also received apparent inputs from boutons that lacked CART-LI, in addition to those that contained CART-LI. Analysis of the terminals of biotinylated dextran-labelled preganglionic neurons showed that there were two classes of preganglionic neuron providing input to vasoconstrictor neurons.

POS-TUE-055

EVIDENCE FOR A NOVEL NEURONAL PROJECTION FROM THE OESOPHAGUS TO THE AIRWAYS IN GUINEA PIGS

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Purpose: The aim of the study was to characterize calretinin expressing nerve fibers found to be associated with tracheal parasympathetic ganglia in guinea pigs. **Methods and Results:** Retrograde tracing from the trachea with cholera toxin B (n=4-8) identified parasympathetic preganglionic neurons in the nucleus ambiguus and dorsal motor nucleus of the vagus nerve, sensory neurons in the nodose, jugular and dorsal root ganglia, and sympathetic postganglionic neurons in the superior cervical and stellate ganglia. Although some calretinin-expressing neurons were identified in each of these locations, neurons specifically traced from the airways never expressed calretinin. Calretinin-immunoreactive nerve fibers disappeared from tracheal ganglia following 48 hours in organotypic culture (confirming an extrinsic origin, n=5) but persisted when tracheae were co-cultured with the oesophagus intact (n=5). A population of oesophageal myenteric plexus neurons was immunoreactive for calretinin and co-expressed acetylcholine but not nitric oxide synthase. Electrical stimulation of the oesophagus in vitro (n=5) evoked contractions of the trachea that were significantly ($P<0.05$) inhibited by hexamethonium. **Conclusion:** Taken together, these data suggest that a subset of oesophageal neurons, that express calretinin and contain acetylcholine, provide excitatory input to tracheal cholinergic ganglia in guinea pigs.

POS-TUE-056

EFFECTS OF CHOLECYSTOKININ AND SEROTONIN ON PANCREATIC VAGAL AFFERENT DISCHARGE

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Introduction: Pancreatic secretion is mainly controlled by a vago-vagal reflex. Cholecystokinin (CCK) and serotonin (5-HT) are both released from the gastrointestinal tract and play a critical role in mediating pancreatic secretion via vagal pathways in response to the products of digestion. **Purpose:** This study was designed to investigate the effects of systemic administration of CCK-8 and phenylbiguanide (PBG, 5-HT_3 receptor agonist) on pancreatic vagal afferent discharge (PVAD). **Methods:** Male Sprague Dawley rats anaesthetised with isoflurane (1.5%/100% O_2) were used in all experiments. The pancreatic vagus nerve was identified and placed onto a pair of silver wire hook electrodes to record the PVAD prior to, and after, systemic administration of CCK and PBG. **Results:** CCK (0.1-10 µg/kg, i.v. n = 5) and PBG (1-10 µg/kg, i.v. n = 5) both produced an immediate, significant and dose-dependent increase in PVAD. Bilateral cervical vagotomy did not affect the excitatory responses of PVAD to CCK or PBG (n = 5, $P > 0.05$) suggesting that the discharge was afferent in nature. Lorglumide (CCK_1 receptor antagonist, 10 mg/kg, i.v) blocked and reduced the CCK- and PBG-induced increases in PVAD (n = 3, $P < 0.05$), respectively. On the other hand, granisetron (5-HT_3 receptor antagonist, 1 mg/kg, i.v.) inhibited the PBG-induced increase in PVAD without affecting excitatory PVAD responses to CCK (n = 5, $P < 0.05$). These observations suggest that PBG may, in part, act via CCK_1 receptors to increase PVAD. **Conclusion:** CCK and PBG may both activate vago-vagal reflexes via activation of CCK_1 and 5-HT_3 receptors on pancreatic vagal afferents.

POS-TUE-057

CHOLERA TOXIN INDUCES LONG-TERM INCREASES IN EXCITABILITY IN VIP AND NPY SECRETOMOTOR NEURONS IN GUINEA-PIG JEJUNUM

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Aim. This study investigated long-term changes in excitability in VIP and NPY secretomotor neurons after luminal exposure to cholera toxin (CT). **Methods.** Isolated segments of jejunum were infused with CT (12.5µg/ml, 0.25 - 0.4ml) ± antagonist or saline, tied at each end and incubated for 1.5 hours at 35°C. The tissue was dissected to reveal the submucosal plexus circumferentially adjacent to intact mucosa and then pinned into an organ bath. S neurons in ganglia closest to the intact mucosa were impaled and their synaptic inputs and firing properties examined. Impaled neurons were later identified as VIP^+ or NPY^+ using immunohistochemical techniques. **Results.** 30 S neurons (20 VIP^+, 7 NPY^+, and 3 VIP^-/NPY^- neurons, assumed to be calretinin) from 17 preparations treated with CT and 27 S neurons (19 VIP^+, 4 NPY^+ and 4 calretinin) from 15 control preparations were analyzed. VIP and NPY neurons in CT-treated preparations fired significantly more action potentials and for significantly longer during injection of depolarizing current pulses (0.5 – 3.5 nA, $p<0.05$). This effect was blocked in both cell types when the 5-HT_3 antagonist granisetron (1µM) or the NK1 antagonist SR 140333 (100nM) was incubated with CT. No changes in excitability were observed in calretinin neurons. Synaptic activity did not appear different between control and CT preparations. **Conclusion.** CT induces long-term increases in excitability in VIP and NPY secretomotor neurons in guinea-pig jejunum via a mechanism involving 5-HT_3 and NK1 receptors.

POS-TUE-058

SUBMUCOUS NEURONS FROM MOUSE SMALL INTESTINE EXHIBIT IPSPs

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Purpose: The properties of submucous neurons in guinea-pig small intestine are relatively well defined. The non-cholinergic secretomotor neurons immunoreactive for vasoactive intestinal peptide (VIP) are the only submucous neurons to exhibit inhibitory post-synaptic potentials (IPSPs). The aim of this study was to determine whether submucous neurons from mouse small intestine exhibit IPSPs, and whether such neurons are VIP-immunoreactive. **Methods:** BALB/c mice (male, 19-26g) were killed by cervical dislocation. Preparations of submucosa were dissected and pinned for intracellular recording. Biocytin injection permitted later visualisation of impaled cells and investigation of VIP-immunreactivity. Internodal strands were stimulated focally with 1, 3 and 15 pulses (30Hz) to evoke synaptic potentials in impaled neurons. Values are expressed as mean ± SD (range). **Results:** Stable impalements have been achieved in 9 neurons; all displayed action potentials and/or synaptic potentials. The resting membrane potential of these cells was 52 ± 10mV (36–67mV) and mean input resistance was 188 ± 7MΩ (181–195MΩ). Of the 9 neurons, 6 produced an action potential (64 ± 13mV) evoked by injected current (100–300pA, usually 200pA), 7 exhibited fast excitatory post-synaptic potentials (EPSPs), and 5 neurons exhibited IPSPs evoked by 3 and/or 15 pulses of focal stimulation. The 3-pulse IPSP amplitude was 3.5 ± 0.62mV (2.8–4mV); duration was 1.4 ± 0.17s (1.3–1.6s). The 15-pulse IPSP amplitude was 7.4 ± 0.85mV (6.6–8.3mV); duration was 4.3 ± 2.2s (2.5–6.7s). Three of these neurons were re-identified following immunohistochemical processing – none was conclusively VIP reactive. **Conclusion:** The electrophysiological properties of mouse submucous neurons resemble those of guinea-pig submucous neurons. The neurochemical coding of mouse submucous neurons may differ; further investigation is required.

POS-TUE-059

GLUTAMATE MAY MEDIATE SLOW EXCITATORY SYNAPTIC POTENTIALS IN VIP NEURONS OF THE GUINEA-PIG SUBMUCOUS PLEXUS BY ACTING ON GROUP I MGLURS

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Purpose: Submucosal neurons immunoreactive for vasoactive intestinal peptide (VIP) are important secretomotor neurons in the enteric nervous system. VIP neurons receive a variety of inputs that allow them to display at least 6 different types of synaptic potentials. This study investigated the involvement of glutamate mediating slow excitatory postsynaptic potentials (EPSPs). ***Methods:*** Submucous plexus preparations were dissected from segments of guinea-pig ileum and pinned flat in an organ bath. Standard intracellular recordings were made from neurons identified as VIP neurons, because they exhibited inhibitory synaptic potentials. Slow EPSPs were evoked by applying a single pulse or a train of 3 or 15 pulses (30 Hz) to internodal strands using a focal stimulating electrode. Antagonists were applied into the superfusing solution. ***Results:*** The slow EPSPs evoked by 1 pulse were reduced by the Group I mGluR ($mGluR_1$ and $mGluR_5$) antagonist PHCCC (30 µM) (control 5.7±0.9mV, PHCCC 2.4±0.3mV, washout 3.5±0.7mV, $P<0.05$, n=4). 3 pulse slow EPSPs were also reduced by PHCCC, but this reduction was not significant (control 10.3±2.2mV, PHCCC 3.4±1.3mV, washout 6.3±1.8mV, P=0.1, n=4). Slow EPSPs evoked by 15 pulse trains were significantly reduced by PHCCC (control 9.2±0.7mV, PHCCC 4.8±1.4mV; 7.4±1.4mV, $P<0.01$, n=6). Both 3 and 15 pulse slow EPSPs were unaffected by the specific $mGluR_5$ antagonist MPEP (10 µM, n=4 and n=3 respectively). ***Conclusions:*** We conclude that glutamate may be one of the neurotransmitters mediating multipulse slow EPSPs by acting upon Group I mGluRs. Our results suggest that the action of glutamate may be specifically on $mGluR_1$s.

POS-TUE-060

VARYING ROUTES OF ADMINISTRATION OF 5-HT_3 AND 5-HT_4 RECEPTOR ANTAGONISTS DIFFERENTIALLY AFFECT SEGMENTATION IN GUINEA-PIG SMALL INTESTINE

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Purpose: Segmentation is induced by luminal infusion of decanoic acid in isolated guinea-pig small intestine, an effect mimicked by the serotonin (5-HT) reuptake inhibitor fluoxetine. This study investigated the receptor subtypes involved using both luminal and serosal application of 5-HT_3 and 5-HT_4 antagonists. Methods: Isolated segments of duodenum and jejunum were placed in an organ bath, cannulated and luminally perfused with physiological saline containing decanoic acid (1mM) with either granisetron (5-HT_3 antagonist 1µM), SB207266 (5-HT_4 antagonist 10nM) or a combination of both. In other experiments, the same antagonists were applied serosally. Contractile activity was monitored using spatiotemporal mapping. Results: Luminal infusion of SB207266, granisetron, or both antagonists together, with decanoic acid reduced the frequency of segmentation in duodenum and jejunum when compared to control (all n>6, $p<0.001$). In contrast, in duodenum serosal application of SB207266 alone or combined with granisetron had no effect on segmentation (each n=6) and granisetron alone increased segmentation activity (n=7, $p<0.01$). In jejunum, serosal application of granisetron had no effect (n=7) and application of SB207266 tended to increase segmentation activity, although this effect was not significant (n=8 p= 0.06). Segmentation was only significantly decreased when granisetron and SB207266 were added together serosally in jejunum (n =10, $p<0.0001$). Conclusions: These data suggest that luminal 5-HT_3 and 5-HT_4 antagonists act predominantly in the region of the mucosa to modulate segmentation induced by decanoic acid and hence that the effects of decanoic acid are mediated by release of 5-HT from the mucosa. On the other hand, serosal antagonists appear to have actions on 5-HT mediated transmission within the myenteric plexus.

POS-TUE-061

LOCALIZATION OF TASK CHANNELS IN ENTERIC NEURONS AND ENDOCRINE CELLS

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The currents that provide the resting K^+ flux that sets the membrane potentials at negative values are carried by two-pore domain, K2P channels. **Purpose:** To use RT-PCR to determine which channels are expressed by enteric neurons, and to investigate the localization of one group of these, the TASK channels, in the neurons and endocrine cells of the gut. **Methods:** Tissue from all regions of the alimentary canal of adult guinea-pigs was investigated. RT-PCR was applied to isolated myenteric ganglia. **Results:** The following channels were expressed in myenteric ganglia: TASK1, TASK2, TASK3, TREK1, TREK2, TRAAK, THIK1, THIK2 (n=2-3). Immunohistochemistry revealed TASK1, TASK2 and TASK3 in neurons, but not in enteric glial cells (n=3-4). TASK1 was in all neurons, including the intrinsic primary afferent neurons (IPANs), where we have previously detected TASK1-mediated currents. TASK2 was in very few enteric neurons, but was prominent in the terminals of noradrenergic axons innervating the ganglia. TASK3 was in a minority of Dogiel type I neurons, but did not occur in IPANs. TASK3 immunoreactivity was strong in mucosal endocrine cells throughout the gut. We have examined these in detail in the stomach, and have found that each of the endocrine cell types, gastrin cells, histamine cells, somatostatin cells and ghrelin cells contain TASK3 immunoreactivity. **Conclusion:** TASK channels have specific and selective distribution in the gut. TASK1, but not TASK2 or 3 occurs in IPANs, TASK2 is prominent in sympathetic terminals, and TASK3 is prominent in gastrointestinal endocrine cells.

POS-TUE-062

EFFECTS OF ISCHEMIA AND REPERFUSION ON NEURONS OF THE ENTERIC NERVOUS SYSTEM

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Periods of ischemia are well known to cause damage to nerve cells in the brain, to cardiac muscle and to the lining of the intestine. Damage to enteric neurons is known to occur, but details of the changes have not been published and there is little indication how enteric neurons might be protected from damage. Purpose: To establish a robust technique to produce damage to enteric neurons through ischemia and re-perfusion, and to develop quantifiable histochemical and immununohistochemical methods to measure neuronal changes. Methods: Adult guinea-pigs (n=36) and BalbC mice (n=12) were used. With the animals under anaesthesia, a loop of small intestine was exteriorised. The marginal vessels connecting to adjacent segments were tied off, and the supplying artery was occluded for 30 min. Tissue was taken at 1, 2 and 24 hr. A range of staining techniques was used; those providing relevant changes are included in the results. Results: At 1-2 hours, a strong reaction for activated caspase was observed in a small proportion of neurons, and a weaker reaction in other neurons. In some neurons there was translocation of the nucleotide-binding protein, ELAV, to the nucleus. At 24 hours there was swelling and distortion of NOS neurons, shrinkage of calbindin neurons, and little effect on calretinin neurons. Conclusion: An ischemic insult to enteric neurons causes quantifiable morphological and neurochemical changes in enteric neurons, and differential changes in neuronal subclasses.

POS-TUE-063

EFFECT OF PKA INHIBITION ON THE ACTION POTENTIAL AND LATE AFTERHYPERPOLARIZING POTENTIAL (AHP) IN MYENTERIC NEURONS FOLLOWING INTESTINAL INFLAMMATION

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The inflammation-induced increases in the excitability of neurons might contribute to disorders of motility, secretion and hypersensitivity during and following gastrointestinal inflammation. The late AHP following an action potential controls the excitability of Dogiel type II neurons. **Purpose:** This study aims to investigate the effect of inflammation on the currents and channels underlying the late AHPs in myenteric neurons and regulation of neuronal excitability by PKA and PKC in guinea-pigs with trinitobenzene sulphonate (TNBS)-induced ileitis. **Methods:** Intracellular recordings were made 7 days after induction of inflammation by injecting TNBS (30mg/kg in 30% ethanol) into the guinea-pig ileum. Neurons labelled with biocytin were identified morphologically. Inflammation was quantified histologically and immunochemically. **Results:** Application of PKA inhibitor H-89 (1 μM) increased excitability of Dogiel type II neurons from the inflamed ileum by inhibition of N-type Ca^{2+} channels and block of IK_{Ca} channels underlying the late AHP (n=15), but did not affect neurons from control ileum (n=6). Inhibition of PKA had no effect on voltage-gated K^+ channels. An activator of cAMP, forskolin (10nM), had no effect on N-type Ca^{2+} channels in Dogiel type II neurons from either inflamed (n=4) or control (n=3) ileum. PKC inhibitors, calphostin C (1 μM), bisindolylmaleimide (100nM) and chelerythrine chloride (1 μM), had no selective effect on the excitability of neurons from the inflamed ileum (n=6 for each). **Conclusion:** Intestinal inflammation changes the regulation of N-type Ca^{2+} channels and IK_{Ca} channels in Dogiel type II myenteric neurons by PKA.

POS-TUE-064

HISTOLOGICAL BASIS FOR SYMPATHETIC-SENSORY COUPLING IN SKIN

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PURPOSE: Neuropathic pain may be generated and maintained by sensory nerves alone or by aberrant actions of the sympathetic nervous system (SNS). In some Complex Regional Pain Syndrome (CRPS) patients, sympathetic blockade relieves spontaneous pain, while mechanical hyperalgesia may be rekindled by stimulation of the SNS [1]. We have examined the hypothesis that a histological substrate exists for crosstalk between sympathetic neurons and primary nociceptive afferents in skin. **METHODS**: Fixed skin sections from the hind paw plantar surface of normal controls and a partial sciatic nerve ligation (PNL) rat model of neuropathic pain (*n=6*) were investigated using double-labelled immunohistochemistry and confocal microscopy. Punch biopsies from normal human skin (*n = 2* dorsal hand) were also examined. Tyrosine hydroxylase (TH) and calcitonin gene related peptide (CGRP) immunoreactivity was used to detect sympathetic and sensory fibres, respectively. **RESULTS**: Sympathetic and sensory fibres were seen throughout the dermis in both normal human and rat skin, and in the PNL tissue. Fibres were observed in close proximity in nerve bundles and near blood vessels, and were seen to intertwine in complex patterns. Yen and colleagues [2] observed a similar relationship but only in skin from lesioned rats. **CONCLUSION**: The close physical association between sympathetic and sensory fibres in skin provides a pre-existent histological basis whereby sympathetic-sensory coupling may occur under altered conditions such as neuropathic pain. Further examination of this relationship may offer potential new treatments. 1. Drummond, P.D., et al. Neurology, 2001. 57(7): p. 1296-303. 2. Yen, L.D., et al. J. Comp. Neurol., 2006. 495(6): p. 679-90.

POS-TUE-065

BURSTS OF BROWN ADIPOSE TISSUE METABOLISM UNDERLIE PERIODIC FLUCTUATIONS IN BODY TEMPERATURE IN RATS: RELATION TO TAIL ARTERY BLOOD FLOW

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Purpose: In mammals, including humans, body temperature (Tb) exhibits ultradian rhythms. So far there is no information as to the relative roles of heat production and heat dissipation in ultradradian Tb rhythms. In the present study we investigated this issue in conscious freely moving rats. **Methods:** Sprague Dawley rats (250-450 g) were instrumented (Fluothane anesthesia) with headpieces attached by flexible lines to a swivel device for continuous measurement of Tb (Subcue probe in peritoneal cavity), interscapular brown adipose tissue (iBAT) temperature (Data Sciences International telemetry probe) and tail artery blood flow (Iowa Doppler probe around base of tail artery). At least one week later rats were placed in a temperature controlled environment with reverse day-night cycle lighting and ad libitum access to food and water. Ambient temperature was maintained constant for the 24 hours, with different ambient temperatures (16-30°C) on different days. **Results:** Temperature of iBAT increased by 0.9±0.2°C every 112±6 min (n=4 rats, 16 occasions), in both light and dark cycles, closely correlated with increases in Tb. Onset of iBAT activity was followed within a few minutes by a sudden increase in tail blood flow, occurring when Tb reached a threshold value (36.9±0.1°C). The latency between increase in iBAT temperature and increase in tail blood flow was related to the 24 hour ambient temperature (significant linear regression, $P<0.001$, $n=16$. $R^2 = 0.61$). **Conclusion:**Sudden increases in heat production by BAT make a major contribution to ultradian rhythms in body temperature. Heat loss via the tail circulation contributes to the return of Tb to resting values.

POS-TUE-066

FEAR ACTIVATED PROJECTIONS TO THE VENTROLATERAL PERIAQUEDUCTAL GRAY

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The ventrolateral periaqueductal gray (VLPAG) is crucial in mediating the freezing response to conditioned fear. However, it is unclear how this structure is activated during this form of psychological stress. One popular model proposes that the amygdala activates the lateral hypothalamus (LH) and VLPAG, which separately mediate cardiovascular and behavioural responses to conditioned fear respectively. However, a recent study suggested that the LH also mediated the behavioural response to exposure to a feared context. **Purpose**: To investigate the inconsistency between these two models, and to elucidate how the VLPAG is recruited during conditioned fear. **Methods**: A retrograde tracer, cholera toxin subunit B (CTB) was injected into the caudal VLPAG and Fos expression was examined after exposure to a feared, conditioned context or at rest. Neurons immunoreactive for both CTB and Fos were analysed throughout the brain. **Results**: Volumes and locations of CTB injections were comparable between the two groups (fear group, n=4; rest group, n=4). The areas which displayed the most double labelling in the fear group compared to the rest group were the LH (16.9% of total versus 8.1% of total), the ventrolateral tegmentum (5.1% versus 2.7%), the paraventricular nucleus of the hypothalamus (4.5% versus 2.6%), and the prelimbic cortex (4% versus 0.2%). These group differences were statistically significant ($p < 0.05$). Relatively little double labelling was found in the amygdala (0.9% versus 1.2%) and bed nucleus of the stria terminalis (1% versus 1.2%). **Conclusion**: In conjunction with recent findings, these results indicate that the LH and VLPAG are not functionally dissociated in conditioned fear to context and reveal for the first time a number of structures that affect VLPAG activation during conditioned fear.

POS-TUE-067

ACTIVATION OF PUDENDAL-SPINAL-PELVIC PATHWAYS CONTROLLING FEMALE REPRODUCTIVE TRACT IN GUINEA-PIGS

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Purpose: Neuronal control of blood flow through the pelvic vasculature is essential for normal reproductive and sexual activity, but little is known about these autonomic pathways in females. Therefore, we aimed to map the functional organisation of lumbar and sacral central inputs to pelvic nerve pathways in females. **Methods:** Female guinea-pigs (200-270g) were anaesthetised with 50% urethane (ip). Two types of experiments were carried out: (1) Fos expression was detected immunohistochemically in lumbar and sacral spinal cord following activation of the urethrogenital reflex by urethral balloon distension. Similar experiments were also performed after thoracic spinal cord transection to remove descending inputs. (2) Compound action potentials were recorded extracellularly in pelvic nerves after electrical stimulation of pudendal sensory nerves in isolated preparations perfused via the abdominal aorta. **Results:** Scattered Fos-expressing neurons were observed in lumbar and sacral spinal levels in control animals without stimulation (dorsal horn of sacral cord: I/II: 12±2 cells per section [cps]; III-VI: 14±3 cps, central canal (cc): 5±1 cps, lateral region (L): 6±2 cps, n=5). Urethral balloon distension significantly increased the number of Fos-expressing neurons in the dorsal horn of sacral, but not lumbar, cord (sacral: I/II: 27±11 cps; III-VI: 35±12 cps; cc: 13±6 cps; L: 15±4 cps; n=5). Thoracic cord transection had no significant effect on Fos expression. Electrical stimulation of pudendal nerves (0.3ms, 50V and 1-5 pulses) evoked compound action potentials (100-200μV) in pelvic nerves, which were blocked by transecting the pudendal nerves (n=4). **Conclusion:** We have demonstrated a functional pelvic sensory-motor spinal pathway to the uterus that, we hypothesise, also regulates blood flow to female reproductive organs.

POS-TUE-068

EFFECT OF STRESS AND AROUSAL ON HYPOCRETIN/OREXIN AND MELANIN-CONCENTRATING HORMONE NEURONS DURING THE WAKE-ACTIVE PHASE

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Purpose: Hypocretin (Hcrt, aka orexin) is a neuropeptide produced exclusively by neurons located in the perifornical hypothalamus. Hcrt is thought to maintain wakefulness and may also be involved in high arousal states, such as stress. This study examined the activity of Hcrt neurons (Fos expression) during varying degrees of stress in the wake-phase of the rat, when arousal is already high. The activity of a second perifornical neuropeptide, melanin-concentrating-hormone (MCH), was also examined. **Method:** Three groups of rats were tested between 1am and 3am. Two groups were tested in a context (30min): a group which had previously received electric footshocks in this context (Contextual Fear, high stress), and group which had not (Exposure, low stress). The third group was left undisturbed in the home-cages (Control, no stress). Two hours later the rats were euthanased (200mg/kg pentobarbitone, i.p) and their brains processed for double immunohistochemical detection of Fos and Hcrt or Fos and MCH. **Results:** The percentage of double-labeled Hcrt neurons increased for the Contextual Fear (48%) and Exposure groups (50%) compared to the Control group (37%, $p<0.001$), but did not differ to each other ($p=0.984$). In contrast, the percentage of double-labeled MCH neurons was low for all groups (< 3%, $p=0.064$). **Conclusion:** Hcrt neuron activity further increases during stress in rats that are already awake and active, confirming a role of Hcrt in arousal rather than simply wakefulness. However, Hcrt neuron activity is not specific to stress as high stress (Contextual Fear) was not greater than low stress (Exposure). In contrast, MCH neurons do not appear to be involved in stress and arousal.

POS-TUE-069

SUPRABULBAR CONTROL OF BODY TEMPERATURE AND SKIN BLOOD FLOW DURING CONDITIONED FEAR

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Conditioned fear in the rat will trigger important behavioural and autonomic changes. The changes includes the display of an immobile posture known as freezing and emission of ultrasonic vocalisations (USV) accompanied by a marked increase in heart rate and blood pressure. Other associated autonomic responses include hyperthermia (+1.5°C), tail skin vasoconstriction and a small activation of brown adipose tissue in the interscapular area (iBAT). The neural substrate involved in mediating these three autonomic responses is not known. **Purpose**: To examine the roles of the dorsal tuberal hypothalamus (DTH) and ventrolateral periaqueductal gray (VLPAG) in expression of these 3 autonomic responses as both structures are involved in mediating the fear response. **Methods**: Animals received bilateral microinjections (0.4µl) of muscimol, a GABA-A receptor agonist (0.2µM) or kynurenic acid, a glutamate receptor antagonist (0.1M) in DTH or VLPAG before being tested for conditioned fear to context. Hyperthermia, tail skin vasoconstriction and iBAT thermogenesis were indirectly measured by infrared thermographic recording of skin temperature in the lumbar back, tail and interscapular back, respectively. Freezing and USVs were also recorded. **Results**: Inhibition of the DTH with muscimol or kynurenic acid (n=13) markedly reduced the hyperthermic response ($p<0.0001$) but had no effect on tail skin vasoconstriction ($p>0.53$). In contrast, inhibition of the VLPAG with muscimol or kynurenic acid (n=17) had no effect on hyperthermia ($p>0.34$) but prevented tail skin vasoconstriction ($p<0.025$) and iBAT thermogenesis ($p<0.004$). **Conclusion**: Fear-induced hyperthermia is mediated by DTH while fear-induced tail skin vasoconstriction and iBAT thermogenesis are mediated by VLPAG. This suggests a dissociative role of the DTH and VLPAG in mediating individual autonomic components of fear.

POS-TUE-070

INCREASED PREPULSE INHIBITION IN WOMEN WITH BIPOLAR DISORDER

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Purpose: Gender differences exist in bipolar disorder (BD), for example, men have an earlier onset compared to women, as determined by the first manic episode. Prepulse inhibition (PPI) is a measure of sensory gating or information processing. Few studies have examined PPI in BD and two studies found a disruption of PPI in BD. The role of gender on PPI in BD has yet to be examined and was the focus of the current study. **Methods:** BD patients (12M:15F; average illness length 19 years) were compared with age-matched control participants (16M:16F). PPI of the acoustic startle response included 21 pulses (115dB) and 21 prepulse-pulse trials (prepulses: 74, 78, 86dB) at 2 inter-stimulus intervals (ISI: 60, 120ms). Higher-order cognition was also measured, including memory, attention, language, visuospatial ability and executive function. **Results:** Men with BD tended to show lower PPI at the 60ms ISI compared to control men (18% BD vs 33% controls), but not at the 120ms ISI. In contrast, women with BD had significantly greater PPI than female controls at the 120ms ISI (50% BD vs 34% controls), but not the 60ms ISI. BD patients showed cognitive deficits with immediate memory, language and executive function. Women performed slightly better than men on the language tasks, however there were no sex x group interactions. **Conclusion:** Men and women with BD both show deficits in PPI compared to controls, however in the opposite direction to each other. Therefore, gender differences in BD are also evident by their different responses in PPI, however not in cognition. The functional implications of these findings are unclear and warrant further investigation.

POS-TUE-071

BEHAVIOURAL ANALYSIS OF MGLUR5 KNOCKOUT MICE: GENE-ENVIRONMENT INTERACTIONS

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Introduction: The role of glutamatergic signalling in psychiatric illness, the development of which results from a complex interplay between genetic and environmental factors, is well recognized. The metabotropic glutamate receptor 5 (mGluR5) has been associated with schizophrenia both directly and through downstream signalling via phospholipase C-β1 (PLC-β1). mGluR5 knockout (KO) mice exhibit disrupted cortical development (Hannan et al., 2001, Nat Neurosci.) and show reduced prepulse inhibition, locomotor hyperactivity and cognitive impairment, behavioural abnormalities regarded as schizophrenia endophenotypes. **Method:** Wild-type (WT) and mGluR5 KO mice were assessed for cognitive performance in the Morris water maze, locomotor activity and prepulse inhibition (each group n=4-12). Mice were housed in standard or environmentally enriched conditions to assess gene-environment interactions. In addition, the behavioural phenotype was extended, testing sociability, anxiety and depressive-like behaviour. **Results:** We recapitulated the finding that mGluR5 KO mice show impaired learning in the Morris water maze compared to WT littermates. A beneficial effect of enrichment was observed in mGluR5 KO and WT mice, significantly decreasing the time taken to learn the task and rescuing a memory deficit within KO mice in the probe trial. Enrichment also reversed locomotor hyperactivity seen in mGluR5 KO mice, paralleling our recent finding in PLC-β1 KO mice (McOmish et al., 2007, Mol. Psych). mGluR5 KO mice did not show an overt phenotype on tests of sociability, anxiety and depressive-like behaviour. **Conclusion:** These results demonstrate that behavioural abnormalities relevant to schizophrenia exist in the mGluR5 KO mouse and are selectively modulated by enrichment. Defining the extent of molecular, cellular and behavioural deficits induced by mGluR5 ablation and their environmental modulation will assist in characterising the role of mGluR5-mediated signalling, with relevance to gene-environment interactions in schizophrenia.

POS-TUE-072

VALIDATION OF AN ANIMAL MODEL FOR ATTENTION DEFICIT HYPERACTIVITY DISORDER: A RESPONSE TO ALSOP'S CRITICISMS

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Purpose: The Spontaneous Hypertensive rat (SHR) has been studied extensively and established as one of the most widely used animal models of Attention Deficit Hyperactivity Disorder (ADHD). Recently, Alsop (2007) criticized the methodology of previous research. He postulated that as SHRs are overall more active than their genetic controls, Wistar-Kyoto (WKY), this could exaggerate other group differences. Alsop (2007) re-examined the data of selected papers and found that group differences such as delay sensitivity diminished when overall lever pressing activity was taken into account. **Method:** 12 SHRs (male, 191 – 220g) and 12 WKYs (male, 167 - 202g) had their locomotor activity and their sensitivity to delay measured using a delayed reinforcement (DR) and an extinction (EXT) task. **Results:** As expected, the SHRs maintained higher locomotor activity than WKYs. However, there was no significant difference between the groups on the total lever presses in the DR or EXT tasks. In the DR task, SHRs shifted to selecting the immediate small reward significantly faster than WKYs as the delay increased. WKYs predominantly selected the lever previously associated with the delayed large reward throughout the EXT task, while the SHRs shifted to selecting the lever previously associated with the immediate small reward by the end of the EXT task. **Conclusion:** Contrary to Alsop's (2007) hypothesis, this study demonstrates that increased locomotor activity does not necessarily imply increased lever pressing activity. Additionally, while there were no significant group differences in total lever presses, SHRs were shown to be more sensitive to delay. This study provides further evidence that the SHR is a valid animal model of ADHD.

POS-TUE-073

PAR2 IMPAIRS FEAR-AVERSIVE LEARNING

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Purpose: Centrally expressed serine proteases have significant roles in learning, memory and the development of anxiety behaviours, particularly in fearful or stressful situations. Whilst the thrombin receptor, protease-activated receptor 1 (PAR1) has been implicated in protease-driven memory and learning, little is known about potential similar roles for the trypsin receptor (PAR2). The present study extends our investigations into central roles for PAR2 by examining the effect of PAR2 activation on anxiety and fear-aversive behaviours in Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Methods: Two test-retest paradigms were employed to assess anxiety-like behaviour and fear associated learning over subsequent trials: the elevated plus maze (EPM) and the open field test (OFT). Each test was repeated 3 times with 48hrs between tests. 20 mins prior to testing rats received injections of SLIGRL (PAR2 agonist - 1.5mg/kg s.c.; n=9), or control treatments (scrambled peptide LRGILS, 1.5mg/kg s.c.; n=7, or saline; n=8). Results: No differences in anxiety-like behaviours on initial exposure to the EPM were observed between treatments. In subsequent trials of control-treated groups, exposure to open arms decreased compared with the initial testing ($p<0.05$), which was as expected and indicative of fear-associated learning over the repeated sessions. Following SLIGRL administration, no differences were observed compared to the initial trial, leading to significantly increased open arm exposure on subsequent trials ($p<0.05$), compared to controls. No behavioural differences were observed in the OFT. Conclusions: These data suggest that PAR2 activation using SLIGRL alters experience-based conditioned learning performance. Therefore, we conclude that in a physiological milieu, PAR2 activation, probably via inhibition of neuronally-released trypsin, prevents fear-associated learning that lead to aversive behaviours in rats.

POS-TUE-074

NEONATAL INFECTION PREDISPOSES TOWARD AN ANXIOGENIC PROFILE IN ADULTHOOD: THE TWO-HIT HYPOTHESIS OF PSYCHOPATHOLOGY

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Purpose: The current study assessed the impact of neonatal bacterial exposure on the development of anxiety-like behaviour in rodents subsequently exposed to stress in adulthood. **Methods:** Wistar rats were exposed to lipopolysaccharide (LPS; 0.05mg/kg, ip) or saline on postnatal days 3 and 5. In adulthood, subjects were allocated to either a 3-day stress paradigm or no stress, and then underwent behavioural testing on either the Hide box/Open Field, Elevated Plus Maze (EPM) or Acoustic Startle Response (ASR) (n>6 for all groups). Time and event measures for the Hidebox/Open Field and EPM were analysed using automated tracking software. Startle amplitude and habituation was measured in the ASR test. Blood was collected to measure pre- and post-test corticosterone levels. **Results:** LPS-treated animals spent significantly less time resisting restraint stress compared to controls, indicative of learned helplessness. LPS-treated animals exposed to stress in adulthood exhibited an increased anxiogenic profile across all behavioural tests compared to controls, suggesting that exposure to stress both in early and later life presents greater susceptibility to adult-onset psychopathology. Sex dependent effects were observed with males exhibiting greater anxiety-like behaviour. Alterations in corticosterone responses were demonstrated, with hyposecretion of corticosterone observed for LPS-treated animals exposed to adult stress. Females exposed to neonatal LPS were also seen to reach menarche earlier. **Conclusion:** Neonatal immune activation produces permanent alterations to the HPA and potentially the HPG axis, as well as behaviour. It appears that a two-hit stress model of neonatal bacterial exposure and adult chronic stress predisposes individuals to an increase in anxiety-like behaviour and hyposecretion of corticosterone in response to stress. Early life infection also alters timing of sexual maturation.

POS-TUE-075

HOW CAN STEROIDS DAMAGE THE HIPPOCAMPUS YET BENEFIT THE SPECIES?

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PURPOSE: Stressful experiences cause physiological dysfunction, hippocampal morphological and functional changes, cognitive deficits and result in the development of pathologies. One of the most remarkable phenomena to occur in the animal kingdom is the annual stress induced post-mating death of males at age 11.5 months in several species of small insectivorous marsupials in Australia. During the breeding period the HPA axis is stimulated and plasma cortisol and catecholamines are elevated. An androgen mediated decrease in corticosteroid-binding globulin causes a massive increase in free cortisol that is thought to damage parts of the hippocampus. Hippocampal inhibition of the HPA axis may be impaired causing failure of cortisol feedback. This study examined the effect of testosterone (T) and ACTH upon hippocampal connectivity. METHODS: Comparing groups of marsupial mice Antechinus subtropicus (n=3) and Mus musculus (n=9), castration followed by treatment with T and/or ACTH, was used to mimic the hormonal milieu in the marsupials at the time of their death in the wild, RESULTS: Significant reductions occurred with treatment in both species in dendritic spine density and dendrite length in hippocampal CA3 pyramidal dendrites. CONCLUSION: This effect of ACTH and T upon hippocampal CA3 dendritic connectivity is consistent with a stress related reduction of hippocampal inhibition of hypothalamic CRH leading to an elevation in plasma free GC concentration. This may explain the failure of cortisol feedback seen in marsupial mice males just before their disappearance in the wild. This unusual reproductive strategy has evolved in the marsupial mice to exaggerate the stress response, and its consequent damaging effect upon the CNS, as a benefit enabling the males to mobilise energy reserves to maximise their reproductive effort.

POS-TUE-076

DIFFERENTIAL ROLE OF THE α4* NICOTINIC ACETYLCHOLINE RECEPTORS IN NICOTINE REWARD VERSUS NICOTINE-INDUCED PLASTICITY

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Purpose: A role α4β2 nicotinic acetylcholine receptors in mediating the reinforcing effects of nicotine has been asserted. Mice with a targeted deletion of the α4 nicotinic subunit were assessed in comparison to wildtype littermates in response to nicotine-induced conditioned place preference (CPP), locomotor sensitization, and intravenous self-administration. **Methods**: Mice (n=18 WT, 17 α4-KO) were conditioned with saline and nicotine (0.5 mg/kg i.p.), and their preference for either chamber measured 1 day and 14 days after conditioning. For locomotor sensitization, mice (n=15 WT, 19 α4-KO) were habituated to the monitoring chamber over 3 daily 30 min sessions and then administered either nicotine (0.5 mg/kg i.p.) or saline for a further 5 daily sessions. After a one week withdrawal period, all mice were administered a challenge dose of nicotine (0.5 mg/kg i.p.). Mice were also assessed for self-administration of cocaine and nicotine. **Results**: Both α4 null and WT mice showed a significant increased in time spent in the drug-paired chamber compared in both test sessions, with no effect of genotype. Conversely, a clear effect of genotype was apparent for locomotor sensitization. α4 null mice displayed increased locomotor activity in response to acute nicotine, whereas acute nicotine had a depressant effect in WT mice. While WT mice showed clear sensitization to nicotine following repeated exposure, this was markedly attenuated in α4 null mice. No difference between genotypes was observed in self-administration experiments. **Conclusion**: These results suggest nicotinic receptors containing the α4 subunit are not necessary for the rewarding effects of nicotine. Attenuated locomotor sensitization of the α4 null mice to nicotine suggests a potential role for α4* nicotinic receptors in nicotine-induced plasticity.

POS-TUE-077

THE GENU OF THE CORPUS CALLOSUM, THE MOST ALCOHOL SENSITIVE SUB-REGIONS OF HUMAN BRAIN

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Background: Ethanol is an addictive drug regulates different metabolic pathways and induced cognitive dysfunction in the brain. Neuroimaging analysis revealed that alcohol-induced brain damage appears to be region-specific and major dysmorphology has been observed in the prefrontal cortex, the white matter (WM) including corpus callosum (CC), the vermis and the hippocampus. Molecular mechanisms underlying these structural changes are largely unknown. **Methods:** Human postmortem samples were provided by NSW Tissue Resource Centre. Proteins were extracted from brain tissues (10 control; 7 uncomplicated alcoholic and 6 alcoholic complicated with hepatic cirrhosis) and separated by 2-D gel electrophoresis. Gel images were analysed by Nonlinear Phoretix 2D Expression software followed by protein identification through MALDI-TOF and the MASCOT search engine (http://www.matrixscience.com/) techniques. **Results:** Six separate experiments were conducted using the tissues of prefrontal gray matter (PGM), the BA9 WM, the genu and the splenium of the CC, the vermis and the hippocampus. The relative expression of 44 proteins in the PGM, 28 protein in the BA9, 43 proteins in the splenium, 50 proteins in the genu, 40 protein in the vermis and 17 proteins in the hippocampus were identified in alcoholic tissues compared to control respectively. More than 55% of identified protein expression has been found to be region specific and they were involved in a number of metabolic pathways, including lipid peroxidation, oxidative stress, energy and vitamin cascade pathways, signaling and apoptosis. **Conclusion:** Alcohol induced protein expression is region specific. One or two detrimental metabolic pathways are dominantly expressed all of the regions except genu. Deleterious pathways including lipid peroxidation, oxidative stress, deacetylation, energy and vitamin cascade are prominently expressed in the genu, suggested that multi-factorial detrimental cascades might be involved for degradation of microstructure in this sub-region.

POS-TUE-078

NEUROCHEMICAL CHANGES IN THE REWARD-RELATED BRAIN REGIONS OF MORPHINE PREFERRING RATS

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Purpose: Previous results from our laboratory have shown that following chronic compulsory morphine consumption and withdrawal about 20-30% of rats will voluntarily choose to consume morphine in their drinking water sufficient to reinstate their dependence. We have previously termed these rats high morphine preferring (HMP). In the present study we performed immunohistochemical analysis to analyze the changes in expression of opioid receptors (μ [MOR] and δ [DOR]), dopamine receptors (D_2 and D_3), the cannabinoid CB_1 receptor, and the enzyme fatty acid amide hydrolase (FAAH) in brain regions of HMP rats compared with rats that chose not to consume morphine (termed low morphine preferring or LMP). **Methods**: Male Sprague Dawley rats (n=40) were housed individually and exposed to increasing concentrations of morphine HCl in their flavoured (sucrose) drinking water for 3 weeks. Following a one week drug free period, rats were then given a choice between a morphine containing sucrose solution and a sucrose solution only. 8 days after the choice phase concluded, rats were heavily anaesthetized and brains were fixed for immunohistochemical analysis. **Results**: In HMP rats, there was a significant decrease in MOR, DOR, D_2 and D_3 receptors and FAAH enzyme expression across reward-related specific brain regions compared with LMP rats. On the other hand there was a significant increase in MOR expression in the frontal cortex region. **Conclusion**: The changes in receptor and enzyme expression detected in HMP rats might reflect highly specialized roles for these receptors with possible functional meaning for the plasticity of the opioidergic and dopaminergic transmission.

POS-TUE-079

BASOLATERAL AMYGDALA – NUCLEUS ACCUMBENS SHELL INTERACTIONS INHIBIT CONTEXT-INDUCED REINSTATEMENT OF REWARD-SEEKING

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Reinstatement of extinguished drug-seeking is a competition between two influences on behaviour: a facilitatory influence reflecting control over drug-seeking by the original training contingency, and an inhibitory influence reflecting control over drug-seeking by the extinction contingency. The neural mechanisms for this competition remain poorly understood. We used complementary functional (disconnection lesions) and neuroanatomical (retrograde tracing) approaches to study the role of basolateral amygdala (BLA) – accumbens shell (AcbSh) interactions in inhibiting context-induced reinstatement of extinguished reward seeking. Rats were trained to respond for 4% alcoholic beer in one context (A), extinguished in a different context (B), and tested in context A or B. We disrupted communication between BLA and AcbSh via unilateral excitotoxic lesions (200nl, 0.06M Ibotenic acid) of BLA and AcbSh in either opposite (contralateral) or same (ipsilateral) hemispheres. BLA – AcbSh disconnection augmented reinstatement, producing significantly greater persistence of responding during test. We applied the neuronal tracer Cholera Toxin B subunit (CTb) (40nl, 1% low salt) to AcbSh and examined retrograde-labelled neurons, c-Fos protein induction, and dual c-Fos/CTb labelled neurons in BLA. Two distinct patterns of activation were observed. In rostral BLA there was significant c-Fos induction in CTb-negative neurons associated with reinstatement of reward-seeking. In caudal BLA there was significant c-Fos induction in CTb-positive neurons associated with extinction of reward-seeking. BLA and AcbSh thus have complementary roles in reinstatement of extinguished reward-seeking: rostral BLA is part of the neurocircuitry triggering context-induced reinstatement whereas projections from caudal BLA to AcbSh act to limit or regulate the extent of this reinstatement. This suggests that manipulations which enhance caudal BLA – AcbSh control over behaviour may be useful targets for preventing reinstatement of drug-seeking.

POS-TUE-080

DISTINCT FOS EXPRESSION PROFILE IN BEHAVIOURAL SENSITISATION TO MDMA: A MAPPING STUDY

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Purpose: Behavioural responses to (±)3,4-methylenedioxymethamphetamine (MDMA) have been shown to display a distinctive profile, the acute hyperlocomotion being primarily restricted to the periphery of the open-field while behavioural sensitisation to MDMA reflects a selective increase in activity in the central zone (CZ) of the chamber, suggesting that sensitisation might rely on distinct neuroadaptations. This study was thus undertaken to determine whether acute and sensitised behavioral responses to MDMA can be explained by specific changes in neuronal activation. **Methods:** Rats pretreated with saline or MDMA (10mg/kg, i.p.) for 5 days were challenged 2 days later with saline or MDMA (5 or 10mg/kg, i.p., n=3-10 per group). Behavioural responses in the open-field and emergence test were measured. Neuronal activation was examined by immunodetection of Fos. **Results:** The dose-dependant increase in locomotion in the peripheral zone observed after acute MDMA correlated with an increase in Fos expression in the ventral pallidum and several hypothalamic nuclei. In MDMA-pretreated animals, the increase in MDMA-induced hyperlocomotion in the CZ was associated with an increase in the time spent in the CZ and a decrease in the emergence latency. These responses correlated with changes in Fos expression in structures such as the central nucleus of the amygdala, ovoid nucleus of the bed nucleus of the stria terminalis and lateral habenula. **Conclusion:** This study identifies novel neural substrates potentially involved in the sensitised response to MDMA. Further, these results suggest that the neuronal activation relevant to behavioural sensitisation to MDMA differs from the activation associated with a mere increase in hyperactivity, and might reflect a decreased anxiety.

POS-TUE-081

WAY 100,635, A SPECIFIC 5-HT1A RECEPTOR ANTAGONIST, ATTENUATES MDMA-INDUCED HYPERLOCOMOTION AND C-FOS EXPRESSION IN RATS: A FOCUS ON OXYTOCIN EXPRESSING NEURONS

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Purpose: The popular party drug MDMA (3,4-methylenedioxymethamphetamine, 'Ecstasy') increases extracellular 5-HT levels in the brain and causes powerful prosocial effects. We previously demonstrated that pretreatment with WAY 100,635 prevented MDMA-induced increases in social interaction and plasma oxytocin. The aim of the present study was to further explore this interaction by quantifying the effects of WAY 100,635 on MDMA-induced hyperlocomotion and c-Fos expression. **Methods:** Three groups of male Wistar rats (n=8/group) were given either Saline:Saline; Saline/MDMA; or WAY 100,635/MDMA. Previously habituated rats were injected (i.p.) with either saline (1 ml/kg) or WAY 100,635 (1 mg/kg). 30 minutes later they received either saline or MDMA (10 mg/kg) and placed into a chamber for 60 min in which locomotor activity was recorded. Immediately afterwards, rats were injected with sodium pentobarbitone (120 mg/kg, i.p.), perfused, and their brains processed to visualize Fos protein. **Results:** MDMA increased locomotor activity and this was significantly reduced by pretreatment with WAY 100,635 ($F=36.38$, $df=2,21$ $P<.001$). WAY 100,635 blocked the MDMA-induced Fos expression in the Islands of Calleja, median preoptic nucleus, the Barrel fields of the somatosensory cortex, nucleus of the solitary tract and reduced the number of oxytocin neurons expressing Fos in the supraoptic nucleus and paraventricular hypothalamus. In contrast, WAY 100,635 did not affect the MDMA-induced Fos expression in the caudate putamen, thalamus, or central amygdala. **Conclusions:** These data suggest that $5HT_{1A}$ receptors are involved in many of the behavioural and neurochemical effects of MDMA.

POS-TUE-082

LIMITS TO JUDGING AND ACTING ON THE POSITION OF A RAPIDLY MOVING OBJECT

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School of Psychology, University of Sydney, Brennan MacCallum Bldg (A18), NSW 2006.

Purpose: When asked to judge the location of a moving object at the time of a flash, observers tend to err in the direction of motion (the flash-lag effect). The pattern of errors at most velocities is roughly consistent with the reported position being where the object was 80 ms after the flash, ±90 ms standard deviation (our results and Murakami, 2001). But what about in a more natural sensorimotor task, one that might benefit more from continuous tracking by attention? We investigated this with three experiments using object speeds below and above the limit for attentive tracking. **Methods:** Flash-lag experiment: three subjects fixated a central point while an object revolved about it. At a random time, the central point changed color and subjects attempted to report the position at this time. Sensorimotor synchrony experiment: the same subjects were asked to press a button when a moving object appears aligned with a stationary landmark. Attentive tracking experiment: one of two identical moving objects is cued at the beginning, and subject try to keep track of it and identify it at the end of the trial. **Results:** For the trackable speeds (< 2 Hz) subjects were twice as precise in the sensoriomotor synchrony task (std dev ~50 ms) as in the flash-lag task (~100 ms). But, above the tracking speed limit, this advantage for the sensorimotor synchrony task vanished. **Conclusions:** Position judgments show poor temporal precision in the standard flash-lag effect, but in conditions where the object can be tracked to guide action based on the position, temporal precision is much improved.

POS-TUE-083

VISUOMOTOR TIMING: COMPENSATING FOR SENSORY LATENCIES?

White A.L., Linares D. and Holcombe A.O.
School of Psychology, University of Sydney, Brennan MacCallum Building (A18), NSW 2006.

The brighter a stimulus is, the less time it takes for the neural signal to travel from the retina to visual cortex (e.g. Maunsell et al, 1999). Apparently because of this variation in latency, dim moving objects are perceived to lag bright moving objects. **Purpose:** Does this apparent flaw in perception also afflict our ability to interact with moving objects? **Methods & Results:** In a first experiment (n=4) we replicated previous results (Purushothaman et al 1998; Lappe & Krekelberg, 1998) that the flash-lag effect increases with the luminance of a rotating bar — the brighter a rotating bar is, the further ahead of two stationary flashes it appears to be. In a second experiment with very similar stimuli, the same subjects attempted to press a button in synchrony with the instant the rotating central bar became aligned with two stationary bars. No feedback was provided, and the method of constant stimuli was used with varying values of the luminance of the moving bar and randomized initial position and direction of motion. Contrary to what was expected from the flash-lag data, subjects did not respond any later for dimmer moving objects, over a 100-fold range of luminance. Apparently the processes that guide action compensate for the variation in perceptual neural latencies. Further experiments address whether compensation occurs within both scotopic (night) and photopic (day) vision, and investigate the possible role of interactions between stimuli of different luminance in causing the perceptual distortions of position.

POS-TUE-084

MECHANISMS UNDERLYING THE PERCEPTION OF STRUCTURE FROM MOTION

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Purpose: When two objects are on the same line of sight but are moving in opposite directions, it is often because they are at different distances from the observer. The visual system uses relative motion to derive depth information, a process called structure-from-motion. We used a psychophysical approach to study the mechanisms underlying this process. **Methods:** Randomly positioned dots moved horizontally in the fixation plane in simple harmonic motion. This produced the illusion of a cylinder rotating about its vertical axis. To this we added test dots with binocular disparity corresponding to either the front or back surface of the cylinder. The test moved in the same or opposite direction as the nearest cylinder surface. The test was also slanted so that either its top or bottom was nearer, and the subjects' task was to indicate the nearer half. We measured psychometric functions from four subjects and calculated threshold as the slant disparity required to produce 75% correct responses. **Results:** Thresholds obtained when the test moved in the opposite direction to the nearest cylinder surface were higher than those obtained when the direction matched; the ratio of thresholds averaged 1.55. To understand more about the origin of this threshold elevation, we performed a second experiment in which simple harmonic motion was replaced by translation. In this case subjects perceived two surfaces translating in opposite directions with one nearer than the other. The resulting threshold ratio was 1.45. **Conclusion:** The results suggest suppressive interactions between opposing motions at the same depth, yielding a single perceived direction of motion at each depth. These interactions may evolve from low cortical areas encoding linear velocities.

POS-TUE-085

THE TIME COURSE OF INTEROCULAR SUPPRESSION

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Purpose: For both interocular masking and binocular rivalry, the presentation of a stimulus to one eye reduces the visibility of an incompatible stimulus presented to the other eye. We aimed to measure the time course of the interocular suppression that underlies such effects. **Methods:** Four subjects were tested. The left eye viewed a conditioning stimulus comprising a vertical Gabor patch for 100 ms. This was followed by a blank interstimulus interval varying from 0 to 500 ms. A test stimulus was then presented to the right eye for 14 ms. The test was a Gabor patch tilted 30° either leftward or rightward from vertical. Subjects judged the orientation of the test stimulus, and test contrast was adjusted so that 75% of judgements were correct. In a second experiment, the intraocular condition, both conditioning and test stimuli were presented to the right eye. **Results:** Thresholds were elevated for short interstimulus intervals and fell as the interval lengthened. Initial threshold elevation for the intraocular condition was approximately twice that for the interocular condition. Time constants for intraocular and interocular suppression averaged 22 and 50 ms respectively. In the middle of the time course, the interocular threshold plateaued while the intraocular threshold showed a smoother descent with time. **Conclusion:** There is a two-stage time course for interocular suppression that is not seen with intraocular suppression. This suggests that interocular flash suppression occurs in two stages along the visual pathway: first in the subcortical visual areas, such as the lateral geniculate nucleus, and then in the visual areas of the cerebral cortex.

POS-TUE-086

POST-TRANSLATIONAL MODIFICATIONS OF APOLIPOPROTEIN-E IN THE HUMAN BRAIN

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Purpose: Polymorphism of apolipoprotein-E (apoE) is the greatest genetic risk factor for developing late onset Alzheimer's Disease (AD). We have investigated the influence of polymorphism and disease status on apoE fragmentation and self-association in the human brain. **Methods:** Human brain homogenate samples were prepared from hippocampal, frontal and occipital regions obtained from AD and age- and sex-matched control donors of known apoE genotype. ApoE was examined by western blotting under either reducing or non-reducing conditions. ApoE was also examined in SKNSH neuronal cells and rabbit brain homogenate prepared immediately after death. **Results:** A greater percentage of apoE was present as fragmentation products in the control E3/E3 brain (n=5) when compared to the diseased E4/E4 brain (n=5). This reached statistical significance in all three brain regions examined ($p<0.05$). Analysis of diseased E3/E3 brains (n=3) has so far revealed a non-significant trend for greater fragmentation than diseased E4/E4 but less than control E3/E3. Examination of the brain samples under non-reducing conditions revealed that 15 to 45% of apoE is present as a homo-dimer in E3/E3 brains, regardless of the disease status. This was not observed in E4/E4 brains as E4 lacks a cysteine residue and thus is unable to form disulphide-linked bonds. The contribution of post-mortem delay and contamination by serum and cerebrospinal fluid seems an unlikely cause for this phenomenon as the homo-dimer was also observed in fresh rabbit brain and SKNSH neurons. **Conclusions:** ApoE fragmentation in the brain is associated with the E3 isoform in the absence of AD. ApoE3 exists in the brain as a homo-dimer, thus representing a major difference between apoE3 and apoE4 in terms of conformation and possibly function.

POS-TUE-087

NEURONAL TOXICITY OF β-AMYLOID AND AMYLIN

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In Alzheimer's disease (AD) β-amyloid (Aβ) deposits in the brain and is associated with neuronal dysfunction and death. In type 2 diabetes (DM2) functional impairment and loss of insulin producing β-cells in pancreatic islets correlates with amylin deposition. Both, Aβ and amylin undergo structural changes leading to aggregation and increased toxicity. Therefore, both AD and DM2 are referred to as conformational diseases. Here, we compared the toxic effects of different forms of Aβ and amylin on primary cultured hippocampal mouse neurons. We found that pre-aggregated, but not monomeric Aβ and amylin induced degeneration of primary neurons, with amylin being more toxic than Aβ. The non-amyloidogenic rat amylin failed to induce cell death in contrast to human amylin. Since human amylin and amyloid-beta do not share similar primary sequences, our results highlight that the secondary structure of aggregates is important to induce neuro-toxicity. The dissection of common pathomechamisms between DM2 and AD may prove to be beneficial in their etiologies to find a common cure.

POS-TUE-088

EFFECT OF QUINOLINIC ACID ON HUMAN ASTROCYTES, IN RELATION TO ALZHEIMER'S DISEASE

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Purpose: Inflammation related to activated microglia and astrocytes is found in Alzheimer disease (AD). The kynurenine pathway (KP) of tryptophan degradation is activated and production of the excitotoxin quinolinic acid (QA) by monocytic cells increased. We studied effects of QA in pathophysiological concentrations on the gene expression of IL-1β, IL-6, Glutamate synthetase (GS), as well as protein expression of S100β, vimentin (Vim) and glial fibrillary acidic protein (GFAP) in human astrocytes (HA). **Methods:** primary cultures of human astrocytes were used and methods included real-time PCR (n=3), immunocytochemistry (n=3), indirect ELISA (n=3), cell proliferation (n=6) and MTT assays (n=6). **Results:** IL-6, S100β and GS genes were constitutively expressed in HA and with TNFα, but not QA, IL-6 and S100β expression were increased compared to controls. IL-1β expression was increased by TNFα, TGFα and by QA (50 to 350nM) in HA. Vim mRNA expression was decreased at first by low QA but start to increase by QA from 350nM in HA. GFAP mRNA expression was decreased by QA in a dose dependent manner. There was complementary difference in expression of structural proteins Vim and GFAP by QA in HA and the ELISA results match well with real-time PCR results. At sub-pathological concentrations QA (150 to 350 nM) for 96h led to increased astroglial proliferation and at pathological concentrations (500 to 1200 nM) QA led to a decrease of proliferation. **Conclusion:** these preliminary results suggest that QA plays a role in the astroglial response in inflammatory brain diseases.

POS-TUE-089

NEURONAL VIABILITY AND TAU PATHOLOGY IS NOT AFFECTED IN HUMAN PRIMARY NEURONS CO-CULTURED WITH ASTROCYTES FOLLOWING STIMULATION WITH SOLUBLE Aβ OLIGOMERS

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Purpose: Inflammation correlates with neuronal loss and cognitive decline in Alzheimer's disease (AD). Using human primary astrocytes we have previously shown that soluble Aβ oligomers are potent stimulators of inflammation via the release of IL-8, IL-6 and TNF-α. The present study was designed to determine how this response relates to tau pathology and neuronal death, which are also consistent features of AD. **Methods:** Neuron and astrocyte cultures were obtained from 16-18 week foetuses after miscarriage or therapeutic termination following informed consent. Oligomeric Aβ42 was prepared as described in the literature. Neurons and neuron/astrocyte co-cultures were stimulated in triplicate with 10μM Aβ42 for 16 hours. Conditioned media was assessed for lactate dehydrogenase release as a marker for neuronal death. Total tau, PHF-1 and AT8 were quantified by Western blot analysis. Mann Whitney U tests determined significant differences between groups. **Results:** Neuronal death occurred following culture stimulation with oligomeric Aβ42. Isolated neuronal cultures had significantly more neuronal death following Aβ stimulation (55.8±5.0 % of cells viable, p=0.014) compared with neuron/astrocyte co-cultures (96.8±2.4 %, p=0.02). This was accompanied by a decrease in total tau (p=0.05) and an increase in PHF-1 (p=0.05). No change in AT8 was observed in neurons either cultured alone or with astrocytes. **Conclusion:** These results indicate that astrocytes provide protective factors that change neuronal death and tau phosphorylation under our experimental conditions. We previously showed astrocytes release IL-8, IL-6 and TNF-α under these conditions, implicating these inflammatory mediators in this response. Further studies to determine the intracellular mechanism are warranted.

POS-TUE-090

ATP-BINDING CASSETTE (ABC) TRANSPORTER A7 STIMULATES CHOLESTEROL EFFLUX TO APOE DISCS AND REGULATES APP PROCESSING

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ATP-binding cassette (ABC) transporters are a superfamily of highly conserved multispan membrane proteins that transport specific substrates across cell membranes. ABCA7, like ABCA1, belongs to the ABCA subfamily of "full-size" transporters. ABCA1 is known to promote the efflux of cellular cholesterol to lipid free acceptors as well as decrease the production of neurotoxic amyloid-beta peptides. The physiological role of ABCA7 is still unclear. **Purpose:** Since ABCA7 is the closest homologue to ABCA1, this research aimed to investigate the ability of ABCA7 to regulate cholesterol efflux to lipid free acceptors and to determine if ABCA7 had any impact on amyloid-beta production. **Method:** This was achieved by transiently expressing ABCA7 in HEK and CHO cell lines. The CHO cell lines stably express human wild-type amyloid precursor protein (APPwt) or APP containing the familial AD Swedish mutation (APPsw) that is known to increase amyloid-beta production. Cells expressing the ABCA7 protein were then subjected to cholesterol efflux assays and amyloid-beta production analysis. **Result**: This study revealed that ABCA7 stimulated cholesterol efflux to lipidated apoE discs by two- to four-fold (n=3 experiments), but not to lipid-free apoA-I or apoE. No significant difference was observed among the three common isoforms (E2, E3 and E4) of apoE discs. ABCA7 was also found to inhibit amyloid-beta production. Western blot experiments showed that ABCA7 transfection reduced amyloid-beta production from APPwt cells by 65 ± 8% (mean ± SD; n=3) and from APPsw cells by 42 ± 3% (mean ± SD; n=3) when compared to mock-transfected cells. **Conclusion**: ABCA7 was shown for the first time to stimulate cellular cholesterol efflux to apoE discs and to decrease amyloid-beta production.

POS-TUE-091

PRESENILIN-1 FAMILIAL ALZHEIMER'S DISEASE CASES EXHIBIT ALTERED PATHOLOGY IN COMPARISON TO SPORADIC AD

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Purpose: To analyse the densities of plaques and neurofibrillary tangles (NFTs), and the morphology and neurochemistry of dystrophic neurites (DNs) in hereditary AD cases that have presenilin-1 (PS1) gene mutations compared to sporadic AD and preclinical AD cases. **Methods:** PS1 (n=8), AD (n=5) and preclinical AD (n=5) cases were stained with thioflavine S and/or immunolabelled with antibodies to β-amyloid, phosphorylation-independent-tau, hyperphosphorylated-tau and phosphorylated-neurofilament (NF) triplet protein. **Results:** There was a significantly higher area of neocortical thioflavine S-stained plaques in PS1 cases compared to preclinical AD and AD cases ($p<0.05$), while AD cases exhibited a significantly higher neocortical thioflavine S-stained plaque load than preclinical AD cases ($p<0.05$). Additionally, there were significantly more NFTs in neocortical layer III and V of PS1 cases in comparison to AD cases ($p<0.05$). Subsets of plaques present in PS1 cases were associated with ring- and bulb-like NF-labelled DNs and classical elongated tau-labelled DNs. Preclinical AD cases exhibited ring- and bulb-like NF-labelled DNs but no tau-labelled DNs, whereas AD cases demonstrated bulb-like NF-labelled DNs and abundant tau-labelled DNs. In contrast to AD cases, fine calibre undulating and distorted DNs were present in PS1 cases, particularly in association with cotton wool plaques, which were strikingly similar to axons following stretch injuries. **Conclusions:** While the density of plaque and NFT pathology is significantly elevated in PS1 cases, DNs in PS1 cases exhibit phenotypic characteristics of both preclinical AD and AD cases suggesting that the development of pathology may be altered in rapidly progressing PS1cases compared to sporadic AD.

POS-TUE-092

CEREBROSPINAL FLUID TAU AND Aβ IN MILD COGNITIVE IMPAIRMENT, ALZHEIMER DISEASE AND DEMENTIA WITH LEWY BODIES

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Pre-mortem diagnosis of Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) is primarily based on clinical criteria, with auxiliary investigations used in a supporting capacity; definitive confirmation requires neuropathological examination of the brain. Considerable effort has been given in recent years to identify better surrogate biomarkers of these diseases so that the diagnosis can be established more confidently, and at earlier stages. Elevated concentrations of tau proteins and decreased levels of Aβ42 peptides in the cerebrospinal fluid (CSF) have been used to differentiate clinical AD from healthy controls and from other dementias. **Purpose:** To evaluate the diagnostic utility of total tau, phospho-tau (p-tau), Aβ40 and Aβ42 levels in our dementia patients and correlate individual results and composite profiles with the clinical diagnosis, which includes other paraclinical investigations. **Methods:** Tau, p-tau, Aβ40 and Aβ42 were measured by ELISA using commercial and non-commercial systems. **Results:** In our preliminary study examining small cohorts of patients (4-6 per group), tau and p-tau were increased and Aβ42 decreased in AD patients, with less consistent changes observed in these biomarkers in DLB and mild cognitive impairment (MCI) patients compared to the standard cut-off levels. Interestingly, Aβ40 was increased in MCI and AD patients. **Conclusion:** Our study demonstrates that CSF neurobiochemical marker analysis provides objective support in the clinical differential diagnosis of dementias. Additional study is required on larger patient populations to more confidently delineate the diagnostic utility of this type of analysis.

POS-TUE-093

PARKIN CO-REGULATED GENE (PACRG) OVEREXPRESSION LEADS TO INCREASED AGGRESOME FORMATION FOLLOWING PROTEASOMAL INHIBITION

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The function of PArkin Co-Regulated Gene (PACRG) is not currently known, however the gene is coregulated with the Parkinson's disease associated gene parkin, suggesting a potential role in the pathogenesis of this common neurodegenerative disease. We have previously demonstrated that PACRG is ubiquitinated and is present in Lewy bodies, the pathological hallmark feature of PD (Taylor et al., 2007). In vitro, Lewy body-like cytosolic inclusions termed "aggresomes" can occur when the proteasome is inhibited or when certain proteins, including the PD-associated proteins alpha-synuclein, synphilin-1 and parkin are overexpressed. It is possible that aggresomes represent a protective cellular response to the toxic effects of ubiquitinated misfolded or damaged proteins. Purpose: to characterise the cellular localisation of PACRG and potential role in aggresome formation. Methods: Utilising a specific polyclonal antibody directed against endogenous PACRG, we identified PACRG-immunopositive perinuclear structures in HEK293 and BE(2)-M17 neuroblastoma cells. Subsequently, PACRG was overexpressed and its localisation was determined in cells treated with the proteasomal inhibitor MG-132. Results: The PACRG positive inclusions co-stained with vimentin and tubulin, identifying them as aggresomes (n=3). The overexpression of PACRG resulted in an increase in the proportion of cells containing aggresomes compared to control cells from 37 ± 8% to 70 ± 2% (mean ± SEM, n=3). Conclusion: Endogenous PACRG localises to aggresomes in cultured cells. Overexpression of PACRG results in the increased formation of aggresomes in proteasome inhibited cells supporting the involvement of this protein in the UPS. Further studies are needed to understand the functional role PACRG may play in the formation of aggresomes, Lewy bodies and indeed Parkinson's disease.

POS-TUE-094

NEUROTRANSMITTER RELEASE MECHANISMS IN MIDBRAIN NEURONS IMPLICATED IN PARKINSON'S DISEASE

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Purpose: Medications that treat diseases such as Parkinson's disease work by regulating dopamine (DA) transmission. Surprisingly, little is known about the mechanisms regulating DA release at presynaptic structures. We investigated the regulation of presynaptic function and synaptic vesicle (SV) recycling in DA neurons. **Methods:** We cultured midbrain neurons and used FM 1-43 to label recycling SVs. We then identified presynaptic structures formed by DA neurons by post hoc immunostaining. This technique allowed us to examine the SV pool size and release probability in DA neurons. **Results:** We defined two kinds of presynaptic structures formed by DA neurons: synaptic sites, which were formed in very close proximity to dendrites, and non-synaptic sites, which consisted of a presynaptic varicosity with no associated postsynaptic dendrite. Synaptic and non-synaptic structures contained several proteins important in regulating presynaptic function, including Bassoon, Synapsins and Synaptophysin. Synaptic sites also contained more recycling SVs and a higher rate of SV release (when electrically stimulated at 10 Hz) than non-synaptic sites. Presynaptic structures formed by DA neurons were then compared against hippocampal synapses, which are a classical model of presynaptic function. When synaptic sites formed by these two neuron populations were compared, hippocampal neurons exhibited a much larger recycling pool of SVs and a higher rate of SV release compared to DA neurons. By contrast, non-synaptic sites did not differ significantly between hippocampal and DA neurons. These observations indicated that synapses formed by DA neurons have fewer vesicles, which are also released with a lower probability, compared to hippocampal neurons. **Conclusions:** Our investigations constitute the first examination of the regulation of SV recycling in DA neurons. Our results suggest functional differences exist between synapses and non-synaptic DA release sites. In addition, our work indicates functional differences between presynaptic structures formed by DA neurons and hippocampal neurons.

POS-TUE-095

POTENTIAL REGULATION OF HUMAN ALPHA-SYNUCLEIN GENE EXPRESSION BY LIVER X RECEPTOR ALPHA

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Parkinson's disease (PD) is an idiopathic neurodegenerative disorder that affects approximately 1% of the western population over 65 years of age. Lewy bodies are neuropathological inclusions found in PD brain and are composed of aggregated proteins including alpha-synuclein and other components including lipids. Alpha-synuclein is a 14 kDa intracellular neuronal protein that is found natively in its monomeric form but undergoes aggregation in neurodegenerative diseases. Purpose: While its exact function is unknown, alpha-synuclein binds phospholipids, fatty acids, and cholesterol which has raised the question as to whether alpha-synuclein can be considered as a specialised apoplipoprotein. Previous work has shown that increased cellular alpha-synuclein predisposes towards aggregation, however, very little is known regarding the regulation of alpha-synuclein gene expression in the brain. We have recently identified putative binding sites for the nuclear hormone receptor liver X receptor alpha (LXR-alpha) in the alpha-synuclein gene. Oxysterols regulate gene expression by activating LXR-alpha however it is not known whether oxysterols or other LXR ligands regulate alpha-synuclein expression. Methods and Results: Using western blotting and quantitative PCR analysis we have shown that neuroblastoma cell lines (n=9) and primary human neurons (n=3) treated with LXR ligands TO-901317, GW-3695 or 27-hydroxycholesterol significantly up-regulate their alpha-synuclein expression approximately 2-fold. Conclusion: These data indicate for the first time that alpha-synuclein gene expression may be at least partially regulated by LXRalpha.

POS-TUE-096

ALPHA-SYNUCLEIN UPREGULATION IS ASSOCIATED WITH NEUROPROTECTION IN DOPAMINERGIC NEURONS EXPOSED TO CHRONIC OXIDATIVE STRESS AND A HETEROGENEOUS SUBGROUP OF CORTICAL NEURONS

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Purpose: Previous investigations indicate that upregualtion of alpha-synuclein occurs in a subset of cortical neurons and is associated with neuroprotection in an in vitro model of chronic oxidative stress. We have characterised the phenotype of neurons that exhibit this response and furthermore, have investigated the response of cultured dopaminergic neurons in the same model. **Methods**: Cortical and brainstem neurons were derived from E18 and E14 Hooded Wistar pups, respectively. Cultures (n=5) were maintained in Neurobasal media + B27 supplement until 11 DIV when it was replaced with antioxidant-free media. At relative maturity, 21DIV, cultures were fixed and fluorescently immunolabelled. **Results**: There was no significant increase in the mean proportion of dopaminergic cells that express alpha-synuclein (44.31% ± 1.89) compared with controls (33.29% ± 7.21) after chronic oxidative stress. However, there was a significant increase ($P < 0.05$) in the proportion of these cells that had high levels of alpha-synuclein (2.76% (± 2.86) in controls and 11.17% (± 5.32) in treated cultures). In cultures grown in conditions of oxidative stress, there was a significant increase in the number of neurons with high levels of ubiquitin (27.55% ± 4.67). Of these cells only 2.23% (±1.94) had high levels of alpha-synuclein. Cells expressing high levels of alpha-synuclein were not immunopositive for markers of protein aggregation, Thioflavine S or oligomeric Abeta. **Conclusion**: Oxidative stress has been further implicated in the select degeneration of dopaminergic neurons in PD, as in the current model these cells are shown to have increased susceptibility. Alpha-synuclein is up-regulated in response to chronic oxidative stress by a heterogeneous cell population and this up-regulation is not associated with protein aggregation.

POS-TUE-097

SUSTAINED TYROSINE HYDROXYLASE PHOSPHORYLATION IN PC12 CELLS EXPOSED TO MANGANESE

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Purpose: Manganese (Mn) is an essential trace metal which on chronic exposure produces symptoms of idiopathic Parkinsonism in humans. Mn toxicity was observed in dopaminergic neurons and is related to oxidative stress and toxic catecholamine metabolites production. Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis. Its activity is controlled acutely by protein phosphorylation and chronically by protein synthesis. Mitogen activated protein kinases (MAPKs), including p38, JNK1/2 and ERK1/2, are involved in TH phosphorylation *in situ*. Here, we investigated the effects of Mn on TH phosphorylation at Ser 19, Ser 31 and Ser 40 and on activation of MAPKs. **Methods**: Pheochromocytoma cells (PC12) were incubated in a low serum media (2% Fetal Calf Serum and 1% Horse Serum) with $MnCl_2$ (1-1000 µM) for 24 h. Cell viability was analysed using the MTT reduction assay. For analysis of TH and MAPK phosphorylation, cells were lysed in SDS buffer and proteins were separated by SDS-PAGE. Immunoblotting was performed using appropriate antibodies and the enhanced chemiluminescence (ECL) system. **Results**: Treatment of PC12 cells with $MnCl_2$ for 24 h did not alter cell viability or TH protein content even at 1000 µM (n=2 in duplicate). On the other hand, we observed a concentration dependent increase in TH phosphorylation at Ser40, Ser31 and Ser19 (n=2 in triplicate). In parallel we observed an increase in phosphorylation of MAPKs (n=2 in duplicate). **Conclusion**: Sustained phosphorylation and activation of TH may be a mechanism involved in $MnCl_2$ neurotoxicity.

POS-TUE-098

THE METALLOBIOLOGY OF HUMAN NEUROMELANIN DURING DEVELOPMENT AND AGING REVEALED USING SYNCHROTRON CHEMICAL X-RAY MICROSCOPY

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Purpose: The metal binding properties of neuromelanin represent an endogenous metal-ion regulation system within the human brain, but have not previously been studied in whole neurons. The current work studied the spatial and temporal distribution of seven elements in whole human substantia nigra neurons ex vivo over the human lifespan. **Methods:** Eight micron formalin-fixed paraffin-embedded sections of the substantia nigra were prepared from seven control individuals aged 24 weeks to 94 years. Micro- and nanoprobe imaging of the samples were carried out at the European Synchrotron Radiation Facility, Grenoble, France. Quantitative maps of sulphur, calcium, iron, copper, zinc, manganese and selenium averaged from 15-20 single whole neurons were calculated for each subject. **Results:** The concentration of neuromelanin-associated elements increased with aging, but the timing of this increase varied depending upon the element. High spatial resolution investigations revealed age-dependant iron-rich micro-domains within the pigment that co-localized with other trace elements. Intracellular speciation of sulfur in neuromelanin revealed the presence of reduced sulfur compounds and various forms of oxidized sulfur compounds, which have not previously been reported. **Conclusions:** This work furthers our understanding of the structure and development of neuromelanin in the healthy human brain. Our ongoing work will compare this data from the healthy brain with the proposed malfunction of these metal binding properties in neurodegenerative disease.

POS-TUE-099

METALLOTHIONEIN-3 IS UPREGULATED IN MULTIPLE SYSTEM ATROPHY AND IS ASSOCIATED WITH OLIGODENDROGLIAL CYTOPLASMIC INCLUSIONS

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Metallothioneins (MTs) are a class of low-molecular-weight (6-7 KDa), cysteine-rich metal-binding proteins the expression of which is induced by heavy metals, glucocorticoids, cytokines and oxidative stress. Recent interest has focussed on the brain-specific MT isoform, MT-3, also known as neuronal growth inhibitory factor, due to the down-regulation of this protein in Alzheimer's disease neurons. Multiple system atrophy (MSA) is an adult-onset neurodegenerative disease characterized by Parkinsonian and other symptoms and by widespread intracytoplasmic inclusion bodies in oligodendrocytes. These glial cytoplasmic inclusions (GCIs) comprised of 9-10 nm filaments rich in the protein, alpha-synuclein, which is also found in the neuronal inclusion bodies (Lewy bodies) associated with Parkinson's disease and Lewy body dementia. Here we show that MT-3 immunoreactivity is increased in MSA brain tissue and is associated with GCIs. The morphology of MT-3-positive inclusions is closely similar to alpha-synuclein-positive GCIs in serial brain tissue sections. Immunofluorescence double labelling and laser scanning confocal microscopy reveals the colocalization of MT-3 and alpha-synuclein in GCIs. Immunoisolated GCIs are strongly immunopositive for MT-3 and Western analysis of the detergent-solubilised GCI proteins comfirms the presence of MT-3. Comparison of immunostaining of MSA (n=4) and normal control (n=4) human brains shows that the number of cells immunopositive for MT-3 is increased in MSA (30%), predominantly in oligodendrocytes, whereas, MT-1/2 isoforms showed a similar distribution of immunopositive cells in MSA compared to normal tissue.

POS-TUE-100

APTOGENIC MITOCHONDRIAL SIGNALING AND PROTEASE ACTIVATION IN STRIATAL GABAERGIC NEURONES

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Injury of striatal GABAergic neurons in Huntington's disease, involves excitotoxicity, metabolic and oxidative stress, which recruit aptogenic mitochondrial signalling. **Purpose**: Elucidation of the insult-dependent recruitment of caspase-dependent/-independent "death" signaling in primary striatal cultures. **Methods**: Using primary striatal cultures (E18 C57Bl6 mice), insults targeting various injury processes were analysed by neurochemical and cytochemical procedures (≥3 independent experiments). At 6d drugs (3-nitropropionate (3-NP), 3-morpholinosyndnonimine (SIN-1), N-methyl-D-aspartic acid (NMDA), 3,5-dihydroxyphenylglycine (DHPG) & staurosporine) were added (24 or 48h) in the presence and absence of inhibitors of caspase-9, caspase-3 and calpains. **Results**: Insults produced concentration-dependent injury, which at EC50 concentrations were shown to be apoptotic by patterns of annexin V and propidium iodide (PI) labeling. Fast (24h, staurosporine and SIN-1) and slow (48h, NMDA, DHPG and 3-NP) timecourses of injury were delineated and employed in further analyses of injury. Using double immunocytochemistry of the redistribution of cytochrome c and apoptosis-inducing factor (AIF), the recruitment of caspase-dependent and -independent "death" signaling, respectively, was quantitated. 3-NP and NMDA, unlike STS, produced earlier release of AIF, while SIN-1 displayed an intermediate profile, suggestive of differential profiles of caspase-independent injury. Protease inhibition failed to attenuate patterns of insult-induced PI-labeling indicating the redundancy of death pathways when cells were committed to die. Protease involvement was further examined by immunoblot analysis of α-fodrin cleavage products. NMDA and DHPG, produced prominent calpain cleavage product (145/150kDa), SIN-1 and 3-NP both calpain and caspase (120kDa) products, whilst STS exhibited predominantly caspase activation. **Conclusion**: Apoptosis of striatal GABAergic neurones by pathologic insults involves substantial calpain activation and caspase-independent AIF redistribution.

POS-TUE-101

MRI LONGITUDINAL ASSESSMENT OF NEUROPATHOLOGY IN R6/1 TRANSGENIC MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a fatal neurodegenerative disease that manifests as a triad of motor, cognitive and psychiatric symptoms. The R6/1 model of HD has the strongest construct validity with 115 expanded CAG repeats within the human huntingtin transgene. A key advantage of MRI is the ability to obtain brain images at several time points to study the effects of disease progression. Purpose: The aim of this study was to investigate the morphological and diffusivity changes in vivo in the R6/1 mouse model. Methods: Seven HD and eight control mice were scanned at 11, 18 and 26 weeks in a 4.7T Bruker scanner with a T2-w sequence, 15 slices, 1 mm thickness, 78x78 um voxel size, a diffusion tensor sequence, 1 image at b=5 s/mm2, 6 images at b=610 s/mm2, (the diffusion gradients for b=610 s/mm2 were oriented in six non-collinear directions) and a T1 3D sequence. The final scans were acquired 24 hours post intracerebroventricular (ICV) Mn2+ injection. Results: The HD mouse brains demonstrated significantly smaller brain volumes, which revealed cerebral atrophy and ventriclar enlargement. Reduced fractional anisotropy in the caudate putamen of HD mice suggests that the integrity of white matter has been disrupted. Significantly narrower gyral layers and enlarged fissure of the hippocampus was observed in HD mice. Conclusion: MRI is powerful technique in the longitudinal evaluation of the in vivo pathogenesis for transgenic models of HD.

POS-TUE-102

MICROSTRUCTURAL STRIATAL DEGENERATION IN HUNTINGTON'S DISEASE: A DIFFUSION TENSOR IMAGING STUDY

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Purpose: This study aimed to investigate striatal pathology in Huntington's disease (HD) using diffusion tensor imaging (DTI). The striatum, and targets of striatal projection fibers, are thought to be the earliest sites of HD pathology. We calculated fractional anisotropy (FA) and mean apparent diffusion coefficient (ADC) in caudate, putamen, thalamus and corpus callosum (CC) of early symptomatic HD patients scanned twice one year apart. **Methods:** Diffusion weighted images were acquired (n=8 patients, n=12 controls) in 28 directions. Based on a voxel-wise model, FA and ADC mean values were calculated for caudate, putamen, thalamus and CC. These values were compared between groups and year using un-paired and paired t-tests, respectively. **Results:** Year 1: Patients had significantly higher putamen ADC compared to controls ($P<0.0001$). There was no significant differences in FA between patients and controls. Year 2: patients showed significantly higher FA and ADC ($P<0.005$) in caudate and putamen compared to controls. There was no effect of time, and neither CC or thalamus showed diffusion abnormalities. **Conclusions:** This longitudinal study reports the progressive onset of abnormal diffusion characteristics in the striatum in early HD. Results agree with other findings of increased striatal FA and ADC in early HD, and indicate that DTI is sensitive to progressive microstructural change in HD. Furthermore putamen ADC may be the most sensitive indicator in HD.

POS-TUE-103

PHOTORECEPTOR DYSFUNCTION IN TRANSGENIC HUNTINGTON'S DISEASE MICE

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Purpose: Huntington's disease(HD) is a fatal neurodegenerative disorder caused by a mutation in the huntingtin gene. Altered visual function has been reported, although not well characterized. The aim of this study was to examine in detail retinal structure and function in a R6/1 transgenic mouse model of HD. **Methods:** Retinal function of wild-type(WT) mice (N=9) and R6/1(HD) mice (N=11) at 13-14weeks of age was measured using a twin flash electroretinogram paradigm. Following measurements of retinal function, all eyes were dissected and fixed in either 4% paraformaldehyde for 30minutes or overnight in a fixative containing glutaraldehyde/paraformaldehyde. Tissues were processed for indirect immunofluorescence/resin embedded. Retinal thickness was measured in Toluedene blue stained sections. Presence of rods and cones was determined by immunolabelling with antibodies directed against rhodopsin, S-cone opsin, L/M-cone opsin and peanut agluttinin(PNA). Tissues of 7 weeks old R6/1 mice (N=5) and WT mice (N=5) were also collected and processed as mentioned above. The presence of apoptosis was determined by TUNEL labeling and single-stranded DNA. **Results:** Electrophysiological findings revealed reduced cone and variable rod responses in HD mice. The retinal thickness at 13-14weeks and 7weeks was similar in both HD and WT mice. Immunolabelling for cone opsins, but not PNA was significantly reduced in HD mice at 13-14 weeks, suggesting a defect in the expression of cone opsins. In addition there was some apoptosis in the outer nuclear layer. **Conclusion:** We conclude that retinal function in R6/1 mice is significantly reduced, especially of the cones. Further work is necessary to determine the influence of these findings on cognition and behaviour in this mouse model and its significance in human disease.

POS-TUE-104

COMBINED NEONATAL STRESS AND ADOLESCENT GLUCOCORTICOID STIMULATION IN RATS INDUCES SELECTIVE COGNITIVE DEFICITS AND REDUCED EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE HIPPOCAMPUS

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Purpose: Epidemiological studies have suggested that schizophrenia is caused by an early disruption, such as a genetic deficit or environmental stress, which increases vulnerability to later factors, such as drug abuse or social stress, i.e. the 'two-hit' hypothesis. **Methods:** Male wistar rats were subjected to maternal deprivation (early stress) and chronic peri-adolescent glucocorticoid treatment (late 'stress'). Thus we investigated 4 groups: controls, early stress only, late stress only and combined stresses ('two hit' group), and assessed cognition using the Morris water maze, Y-maze and T-maze delayed alternation (in each group per test, n=8-10). In similarly treated but experimentally naïve animals, we measured BDNF mRNA expression in the hippocampus with *in situ* hybridization (in each group, n=6). **Results:** The 'two-hit' animals exhibited a selective and significant learning delay in the Morris water maze and a deficiency to recognise the novel arm in the Y-maze test. No major changes were seen in the T-maze test. The single stress groups showed no significant cognitive deficiencies. The two-hit animals also showed a significant ~25% reduction of BDNF expression in the dentate gyrus, and approximately 20% reductions in the CA1 and CA3. **Conclusion:** These results demonstrate a persistent effect of two developmental disruptions on cognitive function and BDNF expression in the hippocampus. These results may help to explain the development of cognitive deficiencies in patient with neurodevelopmental mental illnesses, such as schizophrenia.

POS-TUE-105

ADVANCED PATERNAL AGE ALTERS BRAIN DEVELOPMENT AND BEHAVIOUR IN C57BL/6J MICE

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Advanced paternal age (APA) at birth is associated with an increased risk of neurodevelopmental disorders in offspring, including schizophrenia and autism. **Purpose:** There is currently no established animal model of APA. The present study is the first to model the effects of APA in mice. **Methods:** The offspring (n=142) of 12-18 month-old (APA) and 4 month-old (Control) male C57Bl/6J mice were subjected to both a comprehensive behavioural testing battery and an examination of gross brain anatomy at adulthood (10 weeks) using magnetic resonance imaging (MRI). **Results:** The gross behavioural phenotype of APA mice was normal, with no differences seen in locomotion, social behaviour, prepulse-inhibition of acoustic startle or working or spatial memories. The most pronounced behavioural feature of APA was a significant increase in anxiety-related behaviour on the elevated plus-maze (p<0.05). Specific alterations in exploration and mobility were also demonstrated. Brain volume and volumes of all internal structures measured were unchanged by APA, as were the widths of white matter tracts. Compared to control mice however, APA induced a significant reduction in lateral ventricle volume in males, accompanied by a significant increase in cortical width. APA induced no alteration in female brain anatomy. **Conclusion:** These findings show for the first time that APA results in subtle behavioural and neuroanatomical changes in the adult mouse however only limited support was provided for the proposal that APA models either autism or schizophrenia. This study highlights the potential public health implications of APA, considering the aging population and the current trend towards delayed parenting.

POS-TUE-106

THE EFFECT OF SOCIAL ISOLATION ON THE EXPRESSION AND DISTRIBUTION PATTERN OF ENDOCANNABINOID SYSTEM COMPONENT MRNA IN THE RAT BRAIN

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Purpose: Rearing rats in single cages from weaning until adulthood (social isolation) produces a number of behavioural and neurochemical alterations similar to those observed in psychoses such as schizophrenia. Also, a dysregulation of the endocannabinoid system has been implicated in schizophrenia. The aim of this study was to thoroughly examine the effect of social isolation on mRNA expression of various proteins of the endocannabinoid system in brain regions related to the pathogenesis of schizophrenia. This included measuring mRNA expression in socially isolated rats and grouped rats of 1) the cannabinoid CB_1receptor, 2) enzymes responsible for the synthesis of the endocannabinoids anandamide (N-arachidonoyl phosphatidylethanolamine-phospholipase D or NAPE-PLD) and 2-arachidonoyl-glycerol or 2AG (diacylglycerol lipase or DAGL isozymes α and β) and 3) enzymes that degrade endocannabinoids (fatty acid amide hydrolase/ FAAH for anandamide, and monoacylglycerols lipase/ MAGL for 2AG).**Methods:** 21 day post natal rats were randomly housed individually (n=6) or in groups of 6 (n=7) for 8 weeks. CB1 receptor, DAGL-α, DAGL-βMAG-lipase and FAAH mRNA were measured in the brains using in situ hybridisation histochemistry (ISHH). **Results:** CB_1receptor, DAGL-α, DAGL-βMAGL and NAPE-PLD mRNA expression were significantly higher in a number of socially isolated rat brain regions; particularly in the prefrontal regions, cortical layers and a number of thalamic regions. DAGL-βmRNA was significantly higher in the substantia nigra and ventral tegmental area. FAAH mRNA expression was significantly lower in a number of prefrontal regions, the cortical layers and in the caudate putamen and other associated areas of socially isolated rats. **Conclusion:**This study indicates that the brains of socially isolated rats display a number of differences in the endocannabinoid system to that of a normal rat brain. This further implicates the potential importance of the endocannabinoid system in psychotic disease states.

POS-TUE-107

FEMALE AROMATASE KNOCK-OUT MICE SHOW REDUCED AMPHETAMINE-INDUCED HYPERLOCOMOTION

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Purpose: Increasing evidence suggests oestrogen plays a functionally protective role in various psychiatric illnesses, such as schizophrenia. Aromatase knock-out (ArKO) mice have a targeted disruption in the Cyp19 gene, whereby oestrogen is no longer produced. Amphetamine-induced hyperlocomotion is a widely used animal model of psychosis. Here, we investigated if amphetamine-induced hyperlocomotion is different in male and female wild-type and ArKO mice. We hypothesized in ArKO mice, amphetamine-induced hyperlocomotion would be significantly enhanced when compared to wild-type mice, due to the loss of the protective action of oestrogen. **Methods:** At 16 weeks of age, female and male mice (n=13-16) were randomly treated with saline or 5 mg/kg of amphetamine. Locomotion was analysed using the Ethovision tracking system and results analysed using ANOVA. **Results:** Amphetamine-induced hyperlocomotion was markedly reduced in female ArKO compared to wild-type mice. Total 2 hour locomotor activity scores following amphetamine treatment were 73745 ± 7898 cm in female wild-type versus 46446 ± 4650 cm in female ArKO mice (p=0.004). There was no significant genotype difference in male mice. In contrast to the locomotor hyperactivity effect, there was no change in the action of amphetamine in female or male ArKO mice in prepulse inhibition, another behavioural model of aspects of schizophrenia. **Conclusion:** Oestrogen modulates amphetamine-induced hyperlocomotion in female, but not male mice, a finding which could be important for our understanding of a possible role of oestrogen in psychosis. Further investigations are currently underway using oestrogen replacement pellets in these mice to asses whether dopamine transporter or receptor densities are altered.

POS-TUE-108

JL13, A CLOZAPINE-LIKE POTENTIAL ANTIPSYCHOTIC AGENT, REDUCES SYMPATHETIC CUTANEOUS VASOMOTOR ALERTING RESPONSES (SCVARS) IN RATS, BUT THE DRUG IS LESS POTENT THAN CLOZAPINE

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Purpose Clozapine is an atypical antipsychotic drug used to treat schizophrenia. However clozapine has serious side effects and there is a search for new antipyschotics. One such agent is JL13 [5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-b][1,5] benzoxazepine fumarate], structurally related to clozapine but postulated to have less haematological and cardiological side effects because of reduced sensitivity to oxidation. Clozapine reduces SCVARs in rats. We have evaluated JL13 in this model. **Method** Sprague Dawley rats (250-450 g) were instrumented (isoflurane anesthesia) with Doppler blood flow probe at the base of the tail artery and the effect of JL13 (0.0625-5 mg/kg s.c.) on SCVARs (1) was evaluated in the conscious freely moving animal. **Results** JL13 significantly reduced SCVARs, (linear regression between log dose and SCVAR index P<0.001, R^2 =0.60, n=6 rats at each dose). Prior blockade of dopamine D2 receptors with spiperone (25 µg/kg) did not reduce the effect of JL13. **Conclusion** Comparison with previous results (1) indicates that the SCVAR-reducing action of JL13 is only approximately one-fifth as potent as clozapine, and dopamine D2 stimulation does not contribute to the action of JL13. Supported by NHMRC. 1. Blessing, Psychopharmacol., 2005.

POS-TUE-109

RECEPTOR CHANGES IN BRAIN TISSUE OF RATS TREATED AS NEONATES WITH CAPSAICIN- TESTING A POTENTIAL ANIMAL MODEL OF ASPECTS OF SCHIZOPHRENIA

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In previous studies we showed that treatment of neonatal rats with capsaicin, which destroys portion of the somatosensory input to the CNS, resulted in reduced brain weight, reduced hippocampal and cortical thickness and increased neuronal density at 5-7 weeks of age. These changes were maintained into adulthood and are similar to those found in schizophrenia. **Purpose.** The purpose of the present study was to examine the effect of neonatal capsaicin treatment on the density of muscarinic, serotonin and dopamine receptors. **Methods**.Wistar male rats were treated on neonatal day 2 with capsaicin 50mg/kg sc or vehicle (n=4 per group). At 15-16 weeks of age, rats were euthanized, brains were removed, frozen and processed for autoradiography using [^{3}H]pirenzepine, [^{3}H]AFDX384, [^{3}H]ketanserin, [^{3}H] raclopride to target muscarinic M_1, muscarinic M_2, serotonin $5HT_{2A}$ and dopamine D_2 receptors, respectively. **Results**.Capsaicin treated animals displayed significantly increased M2 receptor density in the lateral striatum (+39%, p=0.024), cingulate cortex (+33%,p=0.039), retrosplenial cortex (+36%, p=0.033), hippocampal subregions (+36-41%, p=<0.022), thalamic nuclei (+26-45%, p<0.022), and hypothalamus (+64%, p=0.026). Increased numbers of serotonin 5HT2A receptors were observed in medial part of striatum (+50%, p=0.043) and in primary and frontoparietal motor cortex (+45-53%, p<0.036). Finally, a significant 27% increase of dopamine D2 receptors was observed in the striatum of capsaicin treated rats (+27%, p=0.04). **Conclusion**.These data indicate region-specific increases in acetylcholine, serotonin and dopamine receptors in capsaicin-treated rats. The increase in dopamine D2 receptors observed in the striatum mirrors findings in post-mortem brain of schizophrenia patients.

POS-TUE-110

SUBJECTIVE MEASURES OF TREATMENT OUTCOME FOR PEOPLE WITH SCHIZOPHRENIA ON ANTIPSYCHOTIC MEDICATIONS

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T. Bakas, Dr Tina Hinton Department of Pharmacology, University of Sydney. **Purpose:** To investigate variability in outcomes and treatment response to antipsychotics as mediated by the perceived pharmacological action by the individual diagnosed with schizophrenia. **Methods:** A questionnaire consisting of subjective scales was sent to outpatients diagnosed with schizophrenia. The survey pack assesed the variables; symptom severity, medication side-effects, attitudes to treatment, quality of life (QoL), psychosocial function, neuro-cognitive deficits and coping skills. Clinical measures of symptom severity and functioning were collected and correlational analysis performed. A reliability assessment was used to assess internal consistency. A multivariate analysis of variance (MANOVA) was performed, with factors including antipsychotic induced dysphoria and drug compliance with dependant variables of symptoms, side effects, functioning and QoL. Multiple linear regression (MLR) was used to assess QoL and the contribution of symptoms, side effects, psychosocial functioning and treatment attitudes upon QoL's variance. **Results:** Reliability was upheld across the scales and subscales assessed within this sample population (n=185), with cronbach's alpha from 0.6-0.9. MLR had revealed QoL had 69% of its variance accounted by symptoms, side effects, treatment attitudes and by psychosocial functioning (p<0.01, n=185). The participants were divided based on subjective negativity towards treatment (ie dysphoric or non-dysphoric), where the subjectively negative participant appeared to have more severe symptoms, side-effects and a significantly reduced QoL (p<0.01) using MANOVA, as did the non compliant participant. **Conclusion:** Differences based on the perceived pharmacological action saw differential treatment outcomes for symptoms, side effects and QoL. Self report measures can be quantified reliably and may provide insight into the patient (and possibly empower the patient), such that treatment (including non-drug ones) may be more aptly applied and treatment outcomes improved.

POS-TUE-111

MODELLING SEIZURE HETEROGENEITY USING DIFFERENT MICE STRAINS

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Purpose: Incomplete penetrance is a common feature of familial epilepsy suggesting that genetic background is an important factor in defining epileptic outcome. This heterogeneity can be modelled using distinct mouse strains. We have previously shown that that the absence seizure phenotype seen in a mouse knock-in model of familial epilepsy is worse in the DBA background when compared to C57 strain. Here we investigate differences between these strains in an attempt to identify susceptibility traits for developing absence seizures.**Methods** Miniature postsynaptic currents(mIPSC) were recorded using the whole-cell voltage clamp technique in primary brain regions known to be involved in the genesis of absence seizures. Epidural EEG recordings were made during sleep in both strains. **Results** Regional differences in the kinetics and frequency of mIPSCs were observed between the strains. In particular, an almost 2-fold increase in mIPSC frequency was observed in the thalamic region of the DBA strain (n=22, 24). EEG recordings revealed a significant increase in spectral power at frequencies around 7Hz (n=7, 6). **Conclusion** Increased thalamic inhibition may be responsible for differences in seizure susceptibility and sleep EEGs. Similar traits, especially EEG patterns may allow us to predict seizure susceptibility in humans.

POS-TUE-112

DISCORDANCE BETWEEN VALPROATE PLASMA LEVELS AND SEIZURE SUPPRESSION IN A GENETIC RAT MODEL OF ABSENCE EPILEPSY

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Background and Objective: The anti-seizure mechanisms of Valproate (VPA) are uncertain. We explored the pharmacokinetic-pharmacodynamic relationship of valproate (VPA) in Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Methods: Twelve adult GAERS received a bolus of 25, 50, 100 and 200mg/kg iv. EEG was recorded 1h before till 3h after VPA administration during which blood samples were taken. A second group (n=17) received three days of continuous saline iv followed by two serial treatments: 3days of 42mg/kg/h VPA separated by 2days of wash-out: 1) continuous infusion 2) hourly bolus-injections. Seizure activity was quantified on day 3. Rats were euthanised either 5, 30, 55min post last VPA bolus, or during consistent infusion. Brain, plasma and CSF VPA concentrations were analysed. Results: Bolus-injections of 50, 100, 200mg/kg VPA all decreased time in seizure (77%, 82%, 84% respectively, p<0.01), but not 25mg/kg. Seizures were progressively suppressed, with a peak effect at 45-75min remaining reduced until 180min. In contrast, plasma VPA peaked in 2mins dropping to "sub therapeutic" levels by 45min. Continuous and fluctuating VPA infusions suppressed seizures (77% vs. 57% respectively vs. saline, p<0.05). Brain VPA levels closely reflected CSF and plasma in the fluctuating group while mean levels were found with continuous infusion. Conclusion: Temporal discordance between plasma and brain levels (pharmacokinetics) and seizure efficacy (pharmacodynamics) suggests that VPA is acting via a secondary effect rather than direct receptor interaction. Peak VPA concentrations do not result in greater anti-seizure efficacy, with the maintenance of steady mid-range concentrations being as potent.

POS-TUE-113

VOLUMETRIC MRI ABNORMALITIES CORRELATE WITH BEHAVIOURAL DISTURBANCE IN A RAT MODEL OF GENERALIZED ABSENCE EPILEPSY

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Introduction: Mental disorders, such as major depression and anxiety, frequently occur in patients with epilepsy, but the pathophysiological relationship between epilepsy and these co-morbidities remains uncertain. Genetic Absence Epilepsy Rats from Strasbourg (GAERS), specifically bred for their epilepsy phenotype, also exhibit elevated anxiety-like behaviours suggesting a common causality. We examined whether this behavioural disturbance is related to cerebral morphological alterations. Methods: Volumetric MRI and the Open Field test of anxiety was performed in adult GAERS (n=4) and Non-Epileptic Control rats (NEC; n=6). The volume of selected brain regions was blindly measured on the MRI data using Analyze™, including the ventricles, the frontal, medial and posterior cortex, the subcortical regions, the hippocampi, the amygdala, and the thalami. Results: GAERS had increased amygdala (right: p=0.058; left p=0.003) and ventricular volumes (p=0.01), vs. NEC rats. These changes were not global, since we did not observe morphological changes in the other limbic and extralimbic structures. GAERS also exhibited greater levels of anxiety-like behaviours compared with NEC rats: reduced distance traveled (p<0.001), reduced time in the centre area (p=0.046), and reduced centre entries (p=0.027). The regional brain volume changes significantly correlated with the behavioural measures. Conclusions: Morphometric brain changes in GAERS could be causally linked to their increased anxiety-like behaviours. As increased amygdala volumes have also been observed in affective disorders in epileptic and non-epileptic patients, this model may be useful in illuminating the pathogenesis of affective disorders generally, as well as modeling psychiatric comorbidities of epilepsy.

POS-TUE-0114

A MODEL SYSTEM TO STUDY THE REGULATION OF RECEPTORS FOR ANTI-MIGRAINE DRUGS

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Purpose: Migraine is a recurring and debilitating disorder affecting about 15 % of people. Triptans 5-HT1B/1D receptor agonists are effective anti-migraine drugs but regular or extended use can lead to a loss of effectiveness. Little is known about how agonists regulate 5-HT1B/1D receptors and thus we created a model system to study the effects of 5-HT and anti-migraine drugs on 5-HT1B/1D receptors in a neuronal environment. **Methods:** AtT20 mouse pituitary tumor cells were stably transfected with HA-tagged human 5-HT1B or 5-HT1D receptor cDNA. Expression of receptors and candidate regulatory molecules were examined with PCR. Recombinant 5-HT receptor signalling was assessed using whole cell patch clamp recordings of endogenous calcium channels (I_{Ca}) or G protein-gated inwardly rectifying potassium channels (GIRK). Each data point represents 6 or more cells. **Results:** Wild type AtT20 cells did not express significant amounts of 5-HT1B or 5-HT1D receptor mRNA and I_{Ca} or GIRK were not affected by 5-HT(10μM). In an AtT20 clone expressing 5-HT1B receptors, 5-HT produced a small inhibition of I_{Ca}, but coupled strongly to activation of GIRK, with an EC_{50} of 120nM. In these cells sumatriptan activated GIRK with an EC_{50} of 440nM. AtT20 cells were found to express mRNA for receptor regulatory proteins including arrestin2 and arrestin3, G protein receptor kinases2, 3 and 5 and dynamin1 and 2. **Conclusion:** Our data suggest that the AtT20 cell line is appropriate for studying the activation, desensitization and trafficking of 5-HT1B/D receptors in a neuronal-like environment, as we have previously reported for the μ-opioid receptor. These cells are likely to be a valuable tool for enhancing our understanding of triptan function.

POS-TUE-115

FUNCTIONAL AND STRUCTURAL CONNECTIVITY BASED PARCELLATION OF THE HUMAN THALAMUS

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Purpose: Recent advances in *in-vivo* MR connectivity analysis and stereotaxic surgical treatment of movement disorders such as Parkinson's disease, has renewed interest in the development of individualised atlases of the motor nuclei of the thalamus. This study aimed to parcellate the thalamus based on functional and structural connections to the basal ganglia, cerebellum, and parietal cortex in healthy young adults. **Methods:** Functional MRI (fMRI) and diffusion tensor imaging (DTI) was performed on 15 healthy right handed subjects. Functional tasks included: visually-paced finger tapping with the rate of 1, 3 and 6 Hz; vibration sensation; and visual shape discrimination. These tasks are known to activate the cerebellum, basal ganglia, parietal and thalamus. Diffusion images were acquired in 60 directions. FSL was used for thalamic parcellation and structural connectivity estimation. **Results:** Functional activations were observed in the thalamus, cerebellum, basal ganglia and parietal cortex. Structural connectivity was evident between these regions in all subjects. Functionally and structurally parcellated regions of the thalamus correlated well with histological atlas parcellations. **Conclusion:** We have identified functional and structural connections between the thalamus and the cerebellum, basal ganglia and parietal cortex in humans. This indicates that fMRI and DTI could be useful for creating individualised thalamic maps for patients undergoing surgery in motor areas of the thalamus and basal ganglia.

POS-TUE-116

DETERMINING THE ADEQUATE STIMULUS FOR COLORECTAL DISTENSION-INDUCED ABDOMINAL CONTRACTION

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Purpose: Experimental studies typically utilise reflex contraction of abdominal muscle evoked by noxious colorectal distension as a surrogate measure of visceral pain in rats and mice. Because the intestinal wall is a complex viscoelastic tissue, the adequate stimulus for evoking this reflex may not be accurately described by the distension pressure. We have sought to determine the contributions of the component forces acting on the intestinal wall to the activation of the visceromotor reflex. Methods: Under general anaesthesia, maintained by α-chloralose infusion, the colorectum was cannulated and exposed to allow video monitoring of its diameter. Pressure, diameter and derived wall tension values at the time point at which the visceromotor reflex was initiated during a controlled monotonic sigmoidal colorectal inflation (up to 75 mmHg) were obtained from 8 adult Sprauge Dawley male rats. Reprosil polymer casts of distended colorectum confirmed a curricular cross section, and the difference between internal and external diameter confirmed that wall thickness was less than 10% of the external diameter. Results: At threshold for reflex activation, the intestinal radius was approximately 106% (+3.1SD), of resting value, ensuring wall tension and pressure tightly co-vary at this level of inflation. Multiple regression analysis showed that circumferential length (strain) was a better determinant of reflex activation than stress (wall tension or pressure). Conclusion: In addition to providing accurate stress-strain measurements for the rat colorectum in vivo, these data suggest that the sensory afferents that evoke the visceromotor reflex respond to changes in circumferential length. Because there is an inverse relationship between rate of distension and threshold, the pressure at threshold (the value usually reported in studies of the visceromotor reflex) is not the best descriptor of its threshold or variation.

POS-TUE-117

A NOVEL STIMULUS ARTEFACT REMOVAL TECHNIQUE FOR HIGH-RATE ELECTRICAL STIMULATION

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Purpose: Studies of neural responses to electrical stimulation are often complicated by the presence of stimulus artefact within the recorded signal. This is particularly evident when investigating short latency neural activity in response to high-rate electrical stimulation. As current techniques (i.e. artefact template subtraction and hardware sample-and-hold circuitry) are unsuitable for such tasks, we developed and evaluated a technique for the efficient removal of stimulus artefact from electrophysiological recordings. **Methods:** Pulsatile electrical stimulation was presented at rates of up to 5000 pulses/s via an intra-cochlea electrode array. The response of single auditory nerve fibres were recorded using standard glass micro-electrode recording techniques, digitised and stored for off-line analysis. Stimulus artefact was removed from these recordings using our newly developed sample-and-interpolate artefact rejection technique. **Results:** The sample-and-interpolate artefact rejection technique has been successfully used to remove electrical stimulus artefact from over one hundred auditory nerve fibre recordings (n=134). The new technique out-performs traditional techniques such as filtering, artefact subtraction and sample-and-hold circuitry; thus enabling the analysis of rapid neural responses that were previously inaccessible. **Conclusion:** We have demonstrated that this computationally efficient sample-and-interpolate technique removes the stimulus artefact while causing minimal distortion of the action potential waveform. We suggest that this technique may have potential applications in a range of electrophysiological recording systems.

POS-TUE-118

CAN NECK MUSCLE SPINDLE AFFERENTS ACTIVATE FUSIMOTOR NEURONES OF THE LIMB IN THE RELAXED, SEATED HUMAN?

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Purpose: The current study investigated whether neck muscle spindle input activates fusimotor neurones of lower limb muscles in the relaxed and seated human. We tested for fusimotor activation using the property that altering the length and activation history of limb muscles alters the mechanical sensitivity of muscle spindles and, as a consequence, the amplitude of tendon reflex. We sought evidence of fusimotor activation by applying a vibratory stimulus to the neck after conditioning the triceps surae (TS) muscles to leave their spindles in a desensitised state. **Methods:** Subjects (*n*=10) were seated in a chair with their head and shoulders restrained. The right TS was conditioned to change the spindles' mechanical sensitivity in 2 ways, tight/sensitive (hold-short) and slack/insensitive (hold-long). Vibration was applied to the dorsal neck muscles for 10 seconds. The tendon jerk was recorded from the right soleus muscle just prior to the offset of vibration (During) or 5 seconds after the offset of vibration (Interposed). The same procedure was repeated using left forearm vibration to test for a non-specific stimulation effect. **Results:** Interposed neck vibration did not significantly increase the size of the hold-long reflex. Both hold-short (+76±64%, *p*=.004) and hold-long (+19±19%, *p*=.011) reflexes were larger during neck vibration. Forearm vibration did not increase reflex size significantly from control values. **Conclusion:** This result suggests that neck vibration does not activate fusimotor neurones innervating TS muscles since the interposed hold-long amplitude was not different from control values. The increase in amplitude of the tendon jerk after both forms of conditioning supports the proposal that the neck vibratory stimulus potentiates spinal reflex excitability of the lower limb.

POS-TUE-119

NEUROANATOMICAL STUDIES WITHIN THE SUBSTANTIA NIGRA TO VALIDATE IMMUNOFLUORESCENCE METHODOLOGIES

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Aims: Characterize tyrosine hydroxylase (TH) antigenicity within the Substantia Nigra (SN) whilst varying tissue handling protocols. **Background:** Antibodies require careful characterization prior to their utility in answering specific research questions (1). In adopting methodologies and antibodies for specific studies, the conservation of antigens, cellular structure and specificity of the antibodies need defining. Here we optimize immunofluorescence methodologies, quantified at the cellular level, in the rat SN with relevance to studies on human brain tissues. **Methods:** Adult male Wistar rats (250-300g) were euthanised (i.p. lethabarb), stored at 4ºC and brains harvested at 0, 2, 4, 8, 16 and 24 hours post-mortem (n=4 for each timepoint), then immersion fixed overnight at room temperature in 4% paraformaldehyde. Brains were bisected midline sagitally and one half processed for paraffin, the other for polyethylene glycol embedded sectioning. Four rats were perfusion fixed transcardially and brains processed as above, as controls. Perivascular sympathetic nerves in human tissues were used to test cross species specificity of tyrosine hydroxylase (TH) antibodies (4 antibodies). Cytoplasmic fluorescence intensity in dopaminergic neurons was quantified using a randomized protocol. Cross reactivity of TH antisera with tryptophan hydroxylase (TPH) was tested in rat raphe nucleus. Antibodies against serotonin (5-HT), Girk-2 and calbindin were used to further validate the results. **Conclusions:** Increasing postmortem duration and paraffin embedding reduced TH antigenicity, in a cumulative manner defined by intensity scores. All TH-antibodies showed affinity for rodent and human antigen, with only one exclusive for TH. Here we define findings relevant for human cadaver studies. *1. Rhodes, K.J. and J.S. Trimmer, Antibodies as valuable neuroscience research tools versus reagents of mass distraction. J Neurosci, 26(31): 8017-8020 (2006).*

POS-TUE-120

CLINICAL CHARACTERISATION OF BRAIN TISSUE FOR NEUROSCIENCE RESEARCH: A COMPARISON OF ANTE-MORTEM AND POST-MORTEM DIAGNOSES

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Purpose: The validity of post-mortem human brain research relies upon accurate clinical and psychopathological diagnosis. Current literature reveals few instances where standardised diagnostic assessment tools such as the Diagnostic Instrument for Brain Studies (DIBS) have been utilised. The present study investigates the degree of concordance between predominant ante-mortem psychiatric diagnoses indicated in medical records, and post-mortem diagnoses derived through structured diagnostic instruments such as the DIBS and the Item Group Checklist (IGCL) of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN). **Methods:** Fifty-eight subjects from the New South Wales Tissue Resource Centre with a recorded diagnosis of mental illness during their lifetime, were included in the study. The predominant ante-mortem psychiatric diagnosis of each case was compared to its corresponding post-mortem diagnosis obtained through structured case reviews to which either the DIBS or the IGCL of the SCAN were applied. Demographic variables such as age of illness onset, and alcohol or other drug use were also examined. **Results:** Comparison of diagnoses obtained from these two approaches produced an overall kappa coefficient of 0.66. Kappa coefficients for the schizophrenia cohort were 0.61, 0.35 for the schizoaffective cohort, 0.95 for the major depressive disorder cohort and 0.70 for the bipolar disorder cohort. **Conclusions:** These results indicate moderate to excellent inter-rater reliability for most cohorts in this sample. There is sufficient disagreement however, particularly in the schizoaffective cohort, to suggest the value of applying standardised and structured assessment to enhance both the accuracy of diagnosis and the prospective validity of tissue-based research.

POS-TUE-121

RNA STABILISATION FOR IMMUNO-LASER-MICRODISSECTION OF RAT MIDBRAIN DOPAMINE NEURONS

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Purpose: Gene expression analysis is important for determination of cell phenotype and function. Typically, expression analyses are carried out using RNA extracted from tissue homogenates. However, this practice precludes attainment of gene expression profiles for specific cell types. Therefore, development of protocols that enable cell-specific gene expression to be measured are required. Laser assisted microdissection of immunolabelled cells permits collection of phenotypically identified cells. Unfortunately, RNA readily degrades during standard immunolabelling processes. In the present study, we have developed a method that maintains RNA stability throughout immunolabelling and, furthermore, we demonstrate that gene expression can be measured in a pure population of microdissected dopamine neurons. **Methods:** Fresh frozen (FF) midbrain cryosections were obtained from Sprague Dawley rats (n=3). Total RNA was extracted from FF sections, FF sections fixed in 70% ethanol, and fixed sections subsequently incubated in PBS or PBS plus RNAse inhibitors for varying lengths of time to mimic antibody incubations. RNA integrity was determined by quantifying ribosomal RNA (rRNA) degradation on BioRad Experion generated electropherograms. **Results:** We found increasing rRNA degradation with increasing PBS incubation times. Notably, even short incubations (5-10 mins) showed degradation. In contrast, overnight incubation in PBS with high NaCl concentration, or RNA Later, resulted in rRNA integrity similar to FF controls. The compatibility of NaCl and RNA Later with immunolabelling for tyrosine hydroxylase (TH) was then tested. NaCl concentrations up to 3M were compatible with TH immunolabelling but RNA Later was not. We then laser microdissected midbrain dopamine neurons using the high NaCl TH immunolabelling protocol and were able to amplify dopamine neuron-specific genes. **Conclusions:** High NaCl solutions stabilize RNA during, and are compatible with, immunolabelling making it possible to microdissect identified neuronal populations for gene expression analysis.

POS-TUE-122

USE OF FLUORESCENCE CORRELATION SPECTROSCOPY TO MONITOR DIFFUSION OF SUBSTANCE P – ALEXA488 CONJUGATE IN LIVE CELL ENVIRONMENTS

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Purpose: Fluorescence correlation spectroscopy (FCS) measures movement of single molecules through a small, confocal observation volume. FCS examines numerous parameters (eg. concentration, bound/free ratios, kinetic parameters) in a natural environment at the single molecule level. We aimed to harness the FCS capabilities of our Leica TCS SP5 confocal microscope to study the diffusion and binding of substance P-Alexa488 conjugate (SP-A488) in an environment of CHO cells expressing neurokinin receptor 1 (NK1R). **Methods:** A live cell chamber was set-up using 30mm diameter coverslips, either blank or seeded with CHO cells expressing NK1R or vector controls. The chamber was filled with HEPES buffered salt solution containing 10nM SP-A488. FCS measurements were acquired using avalanche photodiodes in photon counting mode. The excitation laser (488nm) was parked 10 microns above the top surface of the coverslip or at various levels in the immediate environment of cells. Recordings at each site were replicated 4-8 times. Diffusion time (TauD) and number of particles in confocal volume (N) were extracted from the autocorrelation curves fitted and analysed using ISS software. **Results:** Readings in free solution were comparable whether or not cells were present and the autocorrelation curves gave tight fits (blank coverslip: TauD=31.8+/-1.5µs, N=3.2+/-1.0; coverslips with control cells: TauD=42.5+/-1.6µs, N=4.4+/-0.3; coverslips with NK1R cells: TauD=34.0+/-1.4µs, N=3.8+/-1.1). In contrast, readings near the cell surface were highly variable as were corresponding autocorrelation curves. **Conclusion:** Analysis of transmitter diffusion in the region of cells is complex and difficult to model. A step-wise approach is critical when establishing FCS experiments to provide a strong baseline for 3 dimensional live cell environments.

POS-TUE-123

VALIDATION OF A NOVEL METHOD OF DETECTION OF CELL PROLIFERATION

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Neurogenesis occurs in limited areas of the adult mammalian brain such as the hippocampus, sub-ventricular zone and olfactory neuroepithelium, and at low levels in some other regions of cortex. Detection of cells undergoing proliferation is a fundamental method for assessing neurogenesis. The most accurate method utilizes direct detection of DNA synthesised de novo. Traditionally, this has been achieved through the incorporation of tritiated thymidine or by incorporating the nucleoside analogue bromodeoxyuridine (BrdU) into the replicating DNA. Subsequent to the incorporation of these tracers, they are detected using photographic emulsion or using an anti-BrdU antibody, respectively. Although effective, these method can be lengthy and difficult to perform consistently, and can adversely affect the sample. We validate Click-iT™ EdU (5-ethynyl-2´- deoxyuridine; a product from Invitrogen) that eliminates the need to denature DNA, providing a superior alternative to the previous methodologies. We used intraperitoneal injections of the tracer to label proliferative cells in embryonic and post-natal mice. Tissues were harvested at varying time-points post injection. Our results demonstrate that Click-iT™ EdU clearly labelled neurogenic regions of the brain including hippocampus, sub-ventricular zone and olfactory epithelium during embryonic and postnatal stages. Following an extended incorporation period, labelled cells were detected in regions that undergoe regeneration such as the olfactory bulb, and the olfactory neuroepithelium. In the olfactory epithelium, EdU-positive cells were present in different stages of maturation after an extended period of exposure. In conclusion, we introduce a novel method for measuring cell proliferation in vivo, within the brain.

POS-TUE-124

AUTOMATED NEURITE TRACING, COCULTURE AND COLOCALISATION ANALYSIS WITH HCA -VISION

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Automated neurite tracing and other morphological analyses are essential for drug development and biological research to quantify the effect of treatments and candidate drugs on cultured cells. While automated platforms provide powerful high throughput capability, they usually lack the flexibility and detection sensitivity of human operators. HCA-Vision has been developed to combine the high throughput batch processing power with interactive tuning of the image analysis parameters. It also features database and data mining support to formulate and answer specific biological questions. On top of its neurite analysis module, coculture and subcellular analysis modules have been added recently. In this poster, we use HCA-Vision to solve four representative High Content Analysis problems. These include neurite analysis with mixed cell populations, where cell gating is required; coculture analysis to simultaneously analyse multiple cell-type populations and to study how they interact; subcellular analysis to quantitatively measure the translocation of proteins between the cell membrane and other organelles, and to measure colocalization of two different proteins by combining intensity information from different channels. Results from HCA-Vision are more objective, reliable and reproducible than those from manual or semi-automated approaches.

POS-TUE-125

RESEARCH COCHLEAR IMPLANT FOR SMALL LABORATORY ANIMALS

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Studies of the neural changes induced by cochlear implantation depend upon the availability of a flexible and low-cost animal model. A fully implantable cochlear stimulator, suitable for rats and mice, was recently developed in-house to address this need. This design has been extended to include multiple channels, adjustable currents, and remote adjustment of stimulation parameters. Power is provided inductively, with three orthogonal sets of coils surrounding the animal's enclosure coupling to a smaller coil situated on the implant. This arrangement provides for continuous power, irrespective of animal position. Communication with the implant is achieved via a 2.4 GHz radio link. A series of switches generate two channels of current-regulated biphasic stimulation, delivered to the cochlea via a two/three ringed electrode array. The implant utilises inexpensive off-the-shelf parts, and can be fabricated in a modestly equipped workshop. The resulting implant will be used in a variety of physiological, anatomical and behavioural studies, providing valuable insights for the development of next-generation implants and speech processing strategies. Such studies will also guide clinicians, especially with respect to the roles of plasticity and sensitive periods in predicting outcomes.

POS-TUE-126

MANNOSE RECEPTOR MEDIATED ENDOCYTOSIS IN ASTROCYTES

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Purpose: To characterise the astrocytic expression of the mannose receptor in adult rat brain and evaluate its potential use for receptor mediated gene delivery. **Methods:** Immunohistochemical studies were performed in primary cultures of astrocytes prepared from neonatal rat brain and in sections from perfusion fixed brain sections from adult Sprague Dawley rats. Confocal microscopy was used to characterise the internalization of the mannose receptor antibody CD206. Real time analyses were performed in live cultures using fluorescently labelled markers for clathrin, transferrin and lysosomes. In addition, in vivo assessment of receptor expression and internalization was performed after stereotaxic injections of labelled antibody into the lateral ventricle of anesthetized male rats. **Results:** Mannose receptor expression was found in > 90% of all GFAP positive cells in primary astrocytic cultures and in the majority of GFAP positive cells in cortical brain sections. Intraventricular injection of labelled the CD206 antibody resulted in a selective uptake in periventricular GFAP positive astrocytes as assessed at 2 hours post-injection. The internalization of the antibody-receptor complex, as assessed in cell cultures, was rapid and fully completed in individual cells within 5 minutes and in 85% of the cultured astrocytes within 15 minutes. A majority of the astrocytes internalized the antibody through a clathrin-dependent endocytosis followed by rapid receptor recycling and a final lysosomal destination of the antibody. A sub population of the cultured astrocytes exhibited a clathrin-independent process with only a minor co-localization with transferrin and no lysosomal endpoint. **Conclusion:** The mannose receptor is widely expressed in astrocytes in primary cultures and in the brain. It mediates a rapid internalization of ligand-receptor complex both in vitro and in vivo. Characterisation of the internalization process in these cells suggests that the mannose receptor constitutes an attractive candidate for receptor mediated gene delivery to astrocytes.

POS-TUE-127

SCAVENGER RECEPTOR CLASS B, TYPE I; EXPRESSION IN THE ADULT RAT BRAIN AND IMPLICATIONS FOR RECEPTOR MEDIATED GENE DELIVERY

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The scavenger receptor SR-BI is a low-density lipoprotein receptor responsible for cellular uptake of cholesteryl esters and other lipids. The internalization process of SR-BI is believed to involve both clathrin-dependent and independent pathways, making this receptor an attractive target for receptor-mediated gene delivery. In the CNS, SR-BI expression has previously been demonstrated in astrocytes although recent studies have linked SR-BI expression to production of neurostereoids in retinal neurons. As neurosteroidogenesis also occurs in pyramidal neurons and granule cells of the hippocampus, it is possible that neuronal sub-populations in the brain may also express the receptor. **Purpose:** To characterise the expression of SR-BI in adult rat brain and evaluate its potential use for receptor mediated gene delivery. **Methods:** Sections from perfusion fixed brain from adult Sprague Dawley rats were immunohistochemically assessed for cellular expression of SR-BI. Stereotaxic injections of fluorescently labelled antibody raised against the extracellular domain of SR-BI were used for assessing in vivo receptor expression and internalization. **Results:** Immunohistochemistry demonstrated an intense SR-BI expression in the granular cell layer of the hippocampus and throughout most cortical structures. Western blots confirmed expression of SR-BI protein. A majority of the SR-BI expressing cells were co-expressing the neuronal indicator NeuN, but not markers for microglia or oligodendrocytes. A small proportion of cortical SR-BI expressing cells were GFAP positive. Intraventricular injection of a fluorescently labelled SR-BI antibody resulted in a selective uptake in periventricular NeuN positive cells. Preliminary experiments injecting an antibody to SR-BI linked to a DNA construct expressing green fluorescent protein under the control of a CMV promoter resulted in a population of morphologically-identified cortical neurons expressing the transgene. **Conclusion:** SR-BI expression is not confined to astrocytes in the adult rat brain. Furthermore, this receptor has the potential to be used for receptor mediated gene delivery.

POS-TUE-128

AUSTRALIAN NEUROSCIENCE PATENTING TRENDS

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Purpose: Patent information can be used to monitor developments in a particular field, to identify gaps in the existing patent field, to monitor competing businesses or individuals, or to identify the names and addresses of individuals with specific expertise. The value of intelligence available through published patent data, however, is often overlooked. We set out to analyse recent patenting trends in the neurosciences in Australia. Methods: • Neuroscience applications identified by the prefix "neuro" in the title, abstract or claims. • Derwent international patent database searched in respect of all patents filed in Australia since 2002, including local and those entering national phase. Results: Since 2002, 4143 patent applications have been filed in Australia relating to neuroscience fields, half of which (2009) originated from the USA. The major applicants include Merck (190), Hoffman La-Roche (178) and AstraZeneca (148). The most applications in a single IPC class were in the field of Heterocyclic compounds having nitrogen as the only ring hetero atoms, which have applications for Alzheimer's and pain. The most prolific inventors did not come from the big pharma, with Li Li (30) and Kimberly Spytek (29) from CuraGen Corp., which are primarily in the area of Alzheimer's. The top inventors in (1) PAIN are Dan Peters, Elsebet Ostergaard Nielsen, and Gunnar M Olsen; (2) ALZHEIMER'S, Li Li, Meera Patturajan, and Muralidhara Padigaru; (3) ALS, Juergen Wichmann, Vincent Mutel, and Bernd Buettelmann; (4) BRAIN/INJURY, Christopher Walpole, and (5) MORPHOGENS, Thomas Weller. Conclusion: Patent monitoring can be a valuable tool for identifying competitors/collaborators, areas heavily populated by IP protection, patent trends and potential employees.

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