Abstracts

01

AXONAL REGENERATION FROM CNS NEURONS INJURED IN ADULT MAMMALS

A.J. Aguayo
Centre for Research in Neuroscience, McGill University, Montreal, Canada.

Axons cut in the CNS of adult mammals typically fail to regrow and restore connections. It has been shown that components of the neuronal growth cone environment play an important role in the failure of these fibres to regenerate. Indeed, the Nogo gene product, MAG and a number of molecules expressed by oligodendrocytes and other non-neuronal cells can divert or arrest nerve fibre extension in the brain and spinal cord while in the PNS Schwann cells and the extra-cellular matrix facilitate elongation. Changes in the damaged CNS environment induced in laboratory animals by the transplantation of Schwann or olfactory sheath cells and also by blocking inhibitory molecules have demonstrated that some CNS neurons are intrinsically capable to regenerate lengthy axons. Under these experimental conditions cut nerve fibres can grow very long distances and form well differentiated functional synapses with cells in the regions to which they are guided. However, not all CNS neurons have been proven capable of regeneration. Furthermore, there is yet no clear evidence that regenerating axons can retrieve their original targets with the precision that sensory and motor systems may require for complex functions to be restored.

02

MOLECULAR REGULATION OF GABA-A RECEPTORS

TG Smart
Department of Pharmacology, University College London, Gower Street, London

The GABAA receptor is the main inhibitory neurotransmitter receptor in the brain and a recognised target for many drugs. It is increasingly being associated with neurological diseases such as epilepsy and anxiety. These receptors can be modulated by endogenous agents, including $Zn2^{+}$, protons and neurosteroids, as well as by signalling pathways involving protein phosphorylation. GABAA receptors are complex hetero-pentameric structures, with interacting sub-domains. The `core` subunit members are usually selected from the alpha, beta and gamma families, with additional minor populations containing delta, epsilon, theta or pi subunits. To date, deducing the underlying molecular mechanisms by which GABAA receptors operate, in the absence of any crystalline receptor structure, has proved difficult.
To precisely locate ligand binding sites and the domains involved in signal transduction we have utilised two approaches to probe the structure of GABAA receptors: a molecular modelling comparison between the GABAA receptor and the acetylcholine binding protein; and a rationale site-directed mutagenesis programme. These have been applied to completely resolve the molecular determinants involved in Zn^{2+} and proton regulation of the receptor, and have been instrumental in determining how GABA binding may enable ion channel activation.
Supported by the MRC and Wellcome Trust.

03.01

CEREBROSPINAL FLUID IN THE HYDROCEPHALIC BRAIN ARRESTS CORTICAL DEVELOPMENT THROUGH A DIRECT EFFECT ON CORTICAL PROGENITORS

Mohammad Nabiyouni, Mahjuib Zendah, Jaleel Miyan & P.Jane Owen-Lynch*
*Department of Biomolecular Sciences, UMIST, P.O.Box 88, Manchester M60 1QD and *Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster.*

Cerebrospinal fluid (CSF) is produced by the choroid plexus within the ventricles of the brain. Hydrocephalus occurs when the normal flow of CSF is obstructed and fluid accumulates. Cerebrospinal fluid taken from the lateral ventricle of normal and hydrocephalic fetal rat brains was tested on the growth and development of cortical progenitors from normal fetuses. At 10% addition of CSF to the culture medium normal CSF has no effect on proliferation or differentiation. Hydrocephalic CSF, however, produced a profound inhibition of proliferation. Moreover, the effect of hydrocephalic CSF was to arrest proliferating cells in the S phase of the cell cycle. This effect can be observed in tissue sections of the hydrocephalic cortex using a DNA stains indicating that developmental arrest rather than damage is responsible for much of the effects attributable to early-onset hydrocephalus. Cortical progenitors from hydrocephalic brains show a normal ability to proliferate when removed from their in vivo environment. These data indicate an important function for the CSF in the development of the cerebral cortex.

03.02

EFFECT OF BRAIN INJURY UPON SPINAL CORD DEVELOPMENT: MODELLING CEREBRAL PALSY IN THE RAT

Claire Gibson , Ben Davies, Janet Eyre, Philip Bradley, Gavin Clowry
Brain Development, Plasticity and Repair Group,School of Clinical Medical Sciences,University of Newcastle upon Tyne, Sir James Spence Institute, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP.

In humans, perinatal corticospinal (CS) lesions lead to the disordered spinal reflex development characteristic of cerebral palsy. In rat, CS innervation occurs between postnatal days (P) 7-28, coincident with increased activity dependent protein expression and pruning of muscle afferent (MA) boutons in the ventral horn. Following Home Office approved procedures, the forelimb motor cortex was lesioned unilaterally on P7 either by aspiration, or chronically diminishing neuronal activity by local release of muscimol. Controls involved simply exposing the motor cortex or infusing inactive agents. After P28, cervical cord sections were immunostained for parvalbumin (PV), cJun and extensor digitorum muscle afferents transganglionically labelled with cholera toxin B. Significantly fewer PV positive neurons were found contralateral to either a lesion or inhibition. Ablation increased both MA bouton density in the dorsolateral ventral horn and the number of cJun positive motoneurons contralaterally, however inhibition had the opposite effect. Thus PV expression is linked to CS input and cJun to MA input. MA connectivity may be shaped by competing mechanisms; increasing synaptic space (cortical removal) decreases elimination whilst inhibiting cortical input increases elimination of MA boutons, perhaps by preventing reinforcement of functionally appropriate connections.

03.03

INFLUENCE OF CEREBROSPINAL FLUID ON THE DEVELOPMENT OF THE CEREBRAL CORTEX

Mahjuib Zendah, Mohammad Nabiyouni, Carys Bannister, P.Jane Owen-Lynch* and Jaleel Miyan
*Department of Biomolecular Sciences, UMIST, P.O.Box 88, Manchester M60 1QD and *Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster.*

Cerebrospinal fluid (CSF) is present from the formation of the neural tube and constitutes a potentially important environmental influence on neuroepithelial cell proliferation, differentiation and migration. The brain and spinal cord develop around the fluid-filled tube and the cerebral cortex develops between the fluid within the tube and that within the sub-arachnoid spaces around the brain. We have taken CSF from the lateral ventricles and cisterna magna of embryonic and fetal rat brain and exposed neuronal progenitors to this fluid in in vitro cultures. Our data show that fluid extracted at different stages of development contains factors that drive the proliferation of cortical progenitor cells and also their differentiation to different cell types. CSF at these stages contains everything necessary for survival of cortical progenitors as well as signals for proliferation and differentiation. This suggests that the CSF is a vital delivery system for developmental signals.

03.04

THALAMOCORTICAL SYNAPSE REORGANIZATION FROM SUBPLATE TO LAYER IV DURING POSTNATAL DEVELOPMENT IN THE REELER-LIKE MUTANT RAT (SHAKING RAT KAWASAKI)

S Higashi1, K Hioki2 and T Kurotani3 Z Molnár4
1 Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto, 2 Central Institute for Experimental Animals, Kawasaki, Kanagawa 3 Department of Visual Neuroscience, Nagoya University, Japan 4 Department of Human Anatomy and Genetics, University of Oxford, UK.

Transient synapse formation between thalamic axons and subplate neurons is thought to be important in the targeting of thalamocortical connections. The mutant rat, Shaking Rat Kawasaki (SRK), provides an interesting model system to test this idea. In thalamocortical slice preparations from SRK the spatial and temporal pattern of excitation was investigated using optical recording with voltage sensitive dyes during postnatal periods (P0-10). At birth, a strong optical response was elicited within the superplate of the SRK, in the cell layer corresponding to subplate in wild type (WT) rats. This response rapidly decreased during postnatal days, as the activation descended into deep cortical layers comprised of layer IV cells, as identified by birthdating with 5-bromo-2'-deoxyuridine at embryonic day 17. Tracing individual axons in SRK revealed that at P0 a large number of thalamocortical axons reach the superplate, but by P10 only a fraction of them preserve the loop up to the pial surface. The periphery related thalamocortical axon patterning was normal in SRK, but the cytoarchitectonic barrels were not apparent. These results suggest that the general developmental pattern of synapse formation between thalamic axons and subplate (superplate) neurons in WT and SRK is very similar.

03.05

MUTATIONS IN HUMAN ASPM SUGGEST CEREBRAL CORTEX SIZE IS DETERMINED BY CONTROL OF MITOTIC SPINDLE ACTIVITY IN NEURONAL PROGENITOR CELLS

J Bond, E Roberts, J Askham, S Lindsay, C Walsh, E Morrison, CG Woods.
Molecular Medicine Unit, St James's University Hospital, Leeds, UK. Cancer Campaign UK, St James's University Hospital, Leeds UK. Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne, UK. Department of Neurology, Harvard Institute of Medicine, Boston, USA.

Increase in cerebral cortical size is one of the most notable trends in mammalian evolution. Humans with autosomal recessive primary microcephaly (MCPH) show a small but structurally normal cerebral cortex and mild to moderate mental retardation. Potential insights into cerebral cortex development and evolution are therefore offered by the identification of MCPH genes. The commonest cause of MCPH is mutation of ASPM, at the MCPH5 locus on chromosome 1. ASPM is the human orthologue of the Drosophila gene, abnormal spindle (asp), which is required for normal mitotic spindle function in embryonic neuroblasts. Interspecies comparisons of the predicted ASPM proteins show overall conservation and a correlation of increase in protein size to greater brain size. We have identified a spectrum of pathogenic mutations in ASPM and have performed phenotype–genotype comparisons. In situ hybridisation of ASPM to human foetal brain has confirmed ASPM is expressed in the major sites of prenatal cerebral cortical neurogenesis. Using immunostaining we found that ASPM localised to nuclei in interphase HeLa cells while a microtubule-dependent localisation to the spindle poles was observed during mitosis. These findings suggest modulation of mitotic spindle activity in neuronal progenitor cells is a major evolutionary and developmental control of brain size.

03.06

A ROLE FOR PKARIIß SIGNALLING IN WHISKER PATTERNING IN THE SOMATOSENSORY SYSTEM

P.C. Kind, R Abdel-Majid, M. W. Barnett, B.S. Willis, A. Katsnelson, G.S. McKnight and P.E. Neumann
Genes and Development IDG, Hugh Robson Building, George Square, Edinbugh

Glutamate neurotransmission is essential for the formation of barrels, the prominent anatomical features in layer 4 of the somatosensory cortex. Both NMDAR-/- and mGluR5-/- mice fail to develop barrels however little is known of the downstream signalling pathways necessary for barrel formation. One potential candidate is cAMP-dependent protein kinase A, which is known to play a role in plasticity in both the hippocampus and visual cortex. The PKA holoenzyme is comprised of two regulatory and two catalytic subunits. Furthermore 4 regulatory and 2 catalytic subunits have been identified. We examined the barrel pattern in mice with null mutations of these genes (except for R1[alpha] since these mice are embryonic lethal). Only mice with a deletion in the RIIß subunit fail to form barrels despite normal segregation of thalamocortical afferents and cytochrome oxidase intensity, strongly suggesting a postsynaptic locus for the barrel defect. Immunoprecipitation with antibodies to the NR1 subunit of the NMDA receptor shows a clear association of this receptor with PKARIIß during barrel formation and both PKARIIß protein and mRNA levels are high in barrel cortex during the first postnatal week. Together these data suggest that PKARIIß signalling downstream of NMDAR signalling is crucial for normal barrel differentiation.

03.07

A ROLE FOR synGAP SIGNALLING IN WHISKER PATTERNING IN THE SOMATOSENSORY SYSTEM

M. W. Barnett, T. Vitalis, N.H. Komiyama, K. Porter, S.G.N. Grant, P.C. Kind
Genes and Development IDG, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh.

Several laboratories have demonstrated the need for glutamate neurotransmission in the formation of barrels, the prominent cytoarchitectural features of the somatosensory cortex. NMDAR-/- and mGluR5-/- mice fail to develop barrels however little is known of the downstream signalling pathways necessary for barrel formation. One potential candidate is SynGAP, a synaptic Ras-GTPase that regulates ERK-MAPK phosphorylation following NMDAR activation. ERK phosphorylation plays a role in hippocampal and visual cortical plasticity. We now demonstrate that in the barrel cortex and VB of the thalamus, SynGAP protein and mRNA are highly expressed over the first postnatal week and at P7. However, by P14, SynGAP protein expression is dramatically reduced in VB and layer 4 of the cortex. No SynGAP expression is seen in the trigeminal nuclei of the brainstem. Mice with a deletion of SynGAP show normal barrelette formation in the brainstem, reduced but visible segregation of barreloids in the thalamus and no evidence of barrels in cortical layer 4 at P6. 5-HT immunohistochemistry shows a complete lack of TCA segregation in SynGAP-/- mice. At P7, SynGAP+/- showed a significant decrease in barrel neuron segregation, however, 5-HTT immunohistochemistry demonstrated normal segregation of TCAs, indicating a role for the NMDAR-SynGAP-ERK pathway in cortical development.

03.08

DIETARY LOW LINOLENIC ACID COMPARED WITH DHA ALTERS SYNAPTIC PLASMA MEMBRANE FATTY ACID COMPOSITION AND NA, K-ATPase KINETICS IN DEVELOPING RATS

Raffick A.R. Bowen & Michael T. Clandinin
4-10 Ag/For Center, University of Alberta, Edmonton, AB, Canada T6G 2P5.

The objective of this study was to investigate if maternal dietary 20:4n-6 arachidonic acid (AA) and 22:6n-3 compared with adequate or low levels of 18:3n-3 linolenic acid (LNA) increase synaptic plasma membrane (SPM) cholesterol and phospholipid content, phospholipid 20:4n-6 and 22:6n-3 content, and Na, K-ATPase kinetics in rat pups at two and five weeks of age. At parturition, Sprague-Dawley rats were fed semi-purified diets containing either AA + docosahexaenoic acid (DHA), adequate LNA or low LNA. During the first two weeks of life, the rat pups received only their dams' milk. After weaning, pups received the same diet as their respective dams to five weeks of age. No significant difference was observed among rat pups fed the diet treatments for SPM cholesterol or total and individual phospholipid content. Maternal dietary AA + DHA, compared with feeding the dams the control diet or the low LNA diet, increased 20:4n-6 in phosphatidylserine and 22:6n-3 content of SPM phospholipids. Rats fed dietary AA + DHA or the control diet exhibited a significantly increased Vmax for SPM Na,K-ATPase. Diet treatment did not alter the Km of SPM Na,K-ATPase in rat pups at two and five weeks of age. It is concluded that dietary AA + DHA does not alter SPM cholesterol and phospholipid content but increases 22:6n-3 content of SPM phospholipids modulating activity of Na, K-ATPase.

03.09

NOVEL FEATURES OF THALAMOCORTICAL DEVELOPMENT IN THE FETAL PRIMATE CORTEX

Carney, R.S.E.(1,2), Molnár, Z.(1), Giroud, P.(2), Cortay, V.(2), Berland, M.(3), Kennedy, H.(2), Dehay, C(2).
(1) Dept. Human Anatomy & Genetics, University of Oxford, UK. (2) INSERM U371 - Cerveau et Vision, Bron, France. (3) Hopital Lyon-Sud, Pierre Bénite, France.

The thalamocortical projections relay sensory information to the neocortex. Thalamic afferents have been shown to provide a major influence on cortical areal identity and differentiation during embryonic development in the fetal monkey (Dehay et al., 1989, 1996; Rakic et al., 1991). We have examined the interactions between the thalamus and developing cortex of the rhesus monkey at embryonic days 40, 55, 59, 71 and 83. Crystals of DiI (1,1`-dioctadecyl-3,3,3`,3`-tetramethylindocarbocyanine perchlorate) were placed in the dorsal thalamus of fixed brains. After incubation, 100 µm sections were processed, counterstained with bisbenzimide, and examined using epifluorescence and confocal microscopy. The primate exhibits similarities to rodent thalamocortical development, such as fasciculation changes along the fibre trajectories, and the presence of internal capsule cells with early projections to the thalamus. However, novel features with no rodent counterparts are present. Firstly, thalamic afferents reach the dorsal cortex at a surprisingly early age, in absence of a distinct subplate layer, and secondly, short thalamic fibres descend into the region of neuron production, the subventricular zone.
Sponsored by EU FP5 program, HFSP, MRC studentship, Mary Goodger Scholarship (Oxford).

03.10

EFFECTS OF NEUROTROPHIC FACTORS ON NEUROCHEMICAL DIFFERENTIATION OF POSTNATAL RAT MYENTERIC PLEXUS GANGLION CELLS *IN VITRO*

O. Xeniou, I. Romero and M. J. Saffrey
Department of Biological Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK.

The enteric nervous system (ENS) is the largest division of the peripheral nervous system in terms of neuronal number and diversity, and is also considered an excellent model of neuronal plasticity. Recent evidence shows that neurogenesis and neuronal differentiation continue after birth in the ENS. Here, we have examined the possible roles of neurotrophic factors (NTFs) in the regulation of neurochemical differentiation, specifically VIP expression by myenteric neurons, in 7-day postnatal rat ileum in vitro. Dissociated cell cultures were prepared from isolated myenteric ganglia, and treated for 48 hr in vitro with GDNF, NT-3 or BDNF (all at 1ng/ml). GDNF and NT-3 had a more marked effect than BDNF on promoting total neuronal survival, as assessed by counts of β-3 tubulin-immunoreactive neurons. GDNF treatment was also found to increase both the proportion of VIP-immunoreactive neurons in the cultures, and the intensity of VIP-immunolabelling in individual neurons. GDNF, but not NT-3 or BDNF, increased VIP mRNA levels as assessed by semi-quantitative RT-PCR using β-3 tubulin as an internal standard. These results suggest that GDNF may play a role in the differentiation of VIP myenteric neurons that continues during postnatal development.

03.11

PROGRAMMING EFFECTS OF NEONATAL DEXAMETHASONE TREATMENT ON MESENCEPHALIC DOPAMINERGIC NUCLEI IN ADULTHOOD

McArthur, S., John, C. D., Theogaraj, E., Buckingham, J.C. & Gillies, G.E.
Department of Neuroendocrinology, Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London. W12 0NN.

Glucocorticoids (GCs) are essential for normal brain development, but inappropriate exposure at critical developmental stages can have life-long effects on CNS function and may increase the risk of developing CNS disorders later in life. By including the synthetic GC dexamethasone (DEX) in the mother's drinking water (1ug/ml) for the first 5 days after birth, this study aimed to investigate the effects of neonatal GC on tyrosine hydroxylase immunopositive (TH+) cell numbers in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) in adult offspring. In the VTA DEX treatment decreased TH+ cell numbers by approximately 50% in males ($p<0.02$) and females ($p<0.001$), whereas in the SNc levels were unaffected in males but significantly increased in females ($p<0.002$). These studies suggest that the survival or phenotypic expression of VTA and SNc dopaminergic neurones are profoundly and differentially influenced by brief post-natal exposure to low GCs levels via the mother's milk and, in the SNc, responsiveness is sexually dimorphic. These findings have implications for the development of diseases such as schizophrenia and Parkinson's disease.

This work was supported by the Medical Research Council, the Biotechnology and Biological Sciences Research Council, the Wellcome Trust and the Hammersmith Hospitals Trust Research Committee.

03.12

MUSCARINIC RECEPTORS MAY MODULATE BOTH SYNAPTIC CONSOLIDATION AND ELIMINATION THROUGH THEIR ASSOCIATION WITH CALCIUM CHANNELS DURING NEUROMUSCULAR JUNCTION DEVELOPMENT

Neus Garcia, Isabel Salon, Manel M. Santafé, M. Angel Lanuza, Osvaldo D. Uchitel*, Josep Tomàs
*Unitat d'Histologia i Neurobiologia (UHN), Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, carrer St. Llorenç num 21, 43201-Reus, SPAIN. *Laboratorio de Fisiología y Biología Molecular (LFBM), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428-Buenos Aires, Argentina.*

We studied the effect of selective muscarinic antagonists on evoked potentials in singly and dually innervated endplates of the Levator auris longus muscle from 3-6-day-old rats. In dually innervated fibers, a second endplate potential (EPP) may appear after the first one when we increase the stimulation intensity. The lowest and highest EPP amplitudes are designated small-EPP and large-EPP, respectively. The response of the large-EPP in dual junctions to the muscarinic agents, involve an inhibition of ACh release by M1-receptor antagonists pirenzepine and MT-7 and M2-receptor antagonists methoctramine and AF-DX 116. The small-EPP was also inhibited by both M2-receptor antagonists methoctramine and AF-DX 116. However, the small-EPP was enhanced by M1-receptor antagonists pirenzepine and MT-7. The M4-receptor selective antagonists tropicamide and MT-3 can also increase the small-EPP amplitude. We observed a change from a multichannel involvement (P/Q- N- and L-type voltage-dependent calcium channels –VDCC-) of all muscarinic responses in the small-EPP to the single channel (P/Q-type) involvement of the M1 and M2 responses in the singly innervated endplates. In conclusion, presynaptic autoreceptors may modulate both synaptic consolidation (large ending potentiation) and elimination (small ending depression) through their association with the VDCC during development.

04.01

AGE-DEPENDENT BI-DIRECTIONAL PLASTICITY AT MOUSE HIPPOCAMPAL SYNAPSES

A. J. Milner (a), D. M. Cummings (a), J. P. Spencer (b), M. G. Stewart (a), K. P. S. J. Murphy (a)
(a) Department of Biological Sciences, Open University, Walton Hall, Milton Keynes, MK7 6AA(b) Department of Pharmacology, Cambridge University, Tennis Court Road, Cambridge, CB2 1QJ, United Kingdom.

Low-frequency stimulation (LFS) is widely used to induce long-term depression (LTD) and depotentiation at rodent CA3-CA1 hippocampal synapses. The relationship between the efficacy of LFS induction and postnatal age remains to be clearly defined in rat and had been previously unstudied in mouse. The data presented here show that in acute mouse hippocampal slices LFS-induced LTD and depotentiation are: synapse specific; NMDA receptor-dependent; and mGluRI/II receptor-independent. Furthermore LFS-induced LTD is highly age-dependent whilst LTP, depotentiation and paired-pulse LFS are not. In slices from very young mice (P6-9) LFS induced a robust and stable LTD ($-28.97 \pm 5.99\%$ $n = 8$, $P < 0.01$) of CA1 field excitatory post-synaptic potentials (fEPSPs) in all experiments. LFS induces LTD ($-19.8 \pm 2.27\%$ $n = 39$, $P < 0.01$) in 77% of slices from mice aged P10-13, and LTD ($-19.3 \pm 4.3\%$ $n = 17$, $P < 0.01$) in 80% of slices from animals aged P14-P17. In slices from animals aged P18-21 LTD ($-4.585 \pm 4.22\%$ $n = 14$) is induced in 29% of experiments but the group mean is no longer significant ($P > 0.05$) when compared to adult slices (P100+). LFS failed to induce LTD in more than 80% of experiments in slices from animals aged between P22-49, and no LFS-induced LTD was seen in those from animals older than postnatal day 50.

04.02

SERIAL ULTRATHIN SECTIONING AND 3D RECONSTRUCTIONS OF SYNAPSES DURING LONG-TERM POTENTIATION IN THE RAT DENTATE GYRUS *IN VIVO*

V.I. Popov1,2, H.A. Davies1, V.V. Rogachevsky1,2, M. L. Errington3,T.V.P. Bliss3, M.G. Stewart1
1The Open University, Milton Keynes MK7 6AA, UK; 2Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino 142290, Russia; 3National Institute for Medical Research, Mill Hill, London, NW7 1AA, UK.

In male rats, long-term potentiation (LTP) was induced unilaterally in the dentate gyrus following high frequency electrical stimulation. Synapse density was estimated by an unbiased volume sampling procedure. AT 6h post LTP no significant changes in synapse density were found after LTP. However, LTP induced an increase in the proportion of thin dendritic spines, most likely by decreasing the proportion of stubby spines and shaft synapses, whilst the proportion of mushroom spines was unchanged. Quantitative analysis of thin and mushroom spines revealed a significant increase in their volume and area compared to those from unpotentiated control tissue. An increase in volume and area of macular and perforated/segmented postsynaptic densities was demonstrated and a transition in shape from convex/flat to concave, of both thin and mushroom dendritic spines, was shown in 3D reconstructions. 2h following LTP fusion of the outer mitochondrial envelope with dendritic membranes was observed and these fusion sites were located in direct apposition in adjacent dendrites. The data obtained suggest the possibility of remodelling of existing synapses during LTP by means of reversible growth of thin dendritic spines from shaft and stubby synapses while mushroom synapses change only in both shape and volume/area.

V.I.P. was supported by The Leverhulme Trust (grant F00269G) and RFBR02-04-48890.

04.03

ACTIVATION OF DOPAMINE RECEPTORS IS REQUIRED FOR THE INDUCTION OF LONG-TERM DEPRESSION IN THE MOUSE PERIRHINAL CORTEX *IN VITRO*

D. M. Cummings, A. J. Milner, J. P. Spencer*, J. Y. Brown, M. G. Stewart and K. P. S. J. Murphy
*Biological Sciences, Open University, Walton Hall, Milton Keynes, MK7 6AA, UK. *Pharmacology, Cambridge University, Tennis Court Rd, Cambridge CB2 1QT, UK.*

The perirhinal cortex is implicated in discrimination memory. As a novel cue becomes familiar, the firing rate of perirhinal neurones decreases. Whilst the mechanism underlying this decrease is not fully understood, there is growing evidence that a reduction in perirhinal synaptic efficacy may be involved. A robust form of long-term depression (LTD) has recently been shown at rat perirhinal synapses (Cho et al., 2000. Nat. Neurosci. 3, 150-6) and we have previously demonstrated that a similar form of LTD is supported at mouse perirhinal synapses (Cummings et al., 2002. FENS vol. 1, A116.6). We report here that LTD at mouse perirhinal synapses is also modulated by dopamine.Under control conditions, paired-pulse stimulation produces paired-pulse profiles favouring depression (PPD). Application of the D1 antagonist SCH23390 (10[mu]M) reduced the degree of PPD observed. The D2 antagonist, Remoxipride (10[mu]M) had a more overt effect, shifting the profile to facilitation.
Low frequency stimulation, applied under control conditions, induced homosynaptic LTD (-11.7±3.2%; n=19; P<0.01). LTD induction was prevented by both SCH23390 and Remoxipride (6.0±3.2%, n=6, P<0.01 and -0.3±4.3%, n=8, p<0.05, respectively).
Our results demonstrate a role for dopamine in synaptic plasticity in the perirhinal cortex.

04.04

CHRONIC RESTRAINT STRESS AND SPATIAL LEARNING INDUCE CONTRASTING PATTERNS ON PSA-NCAM EXPRESSION IN THE ADULT RAT DENTATE GYRUS AND ENTORHINAL CORTEX

Cordero MI (1), Rodríguez-Arellano JJ (1), Davies HA (1), Peddie CJ (1), Sandi C (2), Stewart MG (1)
(1) Dept of Biological Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK(2)Dept of Psychobiology, Universidad Nacional de Educacion a Distancia, Ciudad Universitaria s/n, 28040 Madrid.

Exposure to chronic restraint stress (CRS) induces a variety of morphological changes in the hippocampus and impairs spatial learning. As polysialylation of neural cellular adhesion molecule (PSA-NCAM) regulates cell interactions and contributes to synaptic remodelling, it is considered a good candidate marker for neuronal plasticity. Here we studied the effects of 21-days exposure to CRS and/or spatial learning on the expression of PSA-NCAM in temporal structures of the rat. CRS induced a decrease in PSA-NCAM immunoreactivity (PSA-NCAM-IR) in the suprapyramidal blade of the dentate gyrus (DG) and in the lateral entorhinal cortex but induced an increase in the infrapyramidal blade of DG, whilst no changes were observed in the piriform cortex. In both naïve and stressed rats, spatial learning increased in PSA-NCAM-IR in the suprapyramidal blade of the DG, contrasting with the effects of CRS. These results support the view that PSA-NCAM regulation may be a potent and rapid mechanism facilitating synaptic reorganisation. They also highlight the importance of differentiating between blades of the DG as these areas show differing sensitivity to PSA-NCAM regulation and give some insights into the reversibility of the morphological effects of chronic restraint stress induced by learning.

04.05

SUBCELLULAR LOCALIZATION OF EAAC1 NEURONAL GLUTAMATE TRANSPORTER IN RAT DENTATE GYRUS: RELATIONSHIP WITH PSA-NCAM NEURAL CELL ADHESION MOLECULE

H.A. Davies, M.G. Stewart and J.J. Rodríguez
Department of Biological Sciences, Open University, Milton Keynes, MK7 6AA. UK.

Synaptic strength and regulation are key features of synaptic plasticity in the adult nervous system. Glutamate metabolism and removal from the synaptic cleft is important for the prevention of neural damage and maintenance of synaptic efficacy. Adhesion molecules are involved in synaptic plasticity and tissue reorganization. We examined the localization of EAAC1 and its interaction with PSA-NCAM in rat hippocampal dentate gyrus. EAAC1-labelling was seen in somata and dendrites and rarely present in unmyelinated axons and axon terminals. In somata, EAAC1 immunoreactivity was associated with endoplasmic reticulum and Golgi apparatus, including portions of the plasma membrane. In small dendrites and dendritic spines, EAAC1 was localized mainly to plasma membranes near contacts from unlabelled axon terminals; in large dendrites, immunoreactivity was associated with saccules of smooth endoplasmic reticulum. PSA-NCAM plasmalemmal immunoreactivity was also present in somatodendritic profiles and dendritic spines but co-localized rarely with EAAC1 (<4% of all labelled dendritic profiles). Our results suggest that within DG granule cells undergoing activity-dependent plasticity there is reduced somatodendritic targeting of EAAC1 indicating a possible downregulation or redistribution directly associated with architectural and synaptic modulation.

Supported by BBSRC grant 108/NEU15416

04.06

TRANSIENT INCREASE IN SYNAPTOGENESIS IN CHICK HIPPOCAMPUS FOLLOWING PASSIVE AVOIDANCE TRAINING

Ageliki M. Nikolakopoulou and Michael G. Stewart
Dept. of Biological Sciences, The Open University, Milton Keynes, MK7 6AA, UK.

Water maze training increases synaptogenesis in the rat hippocampus especially in the dorsal region. Studies from our group (Horner et al., 1996 Brain Res 735(2): 354-9) have shown that after transient ischemia in the domestic chick there is a reduction of synapses in the dorsal part of the hippocampus, a region which morphologically has similarities to rat hippocampus. This study has focused on synaptogenesis in chick hippocampus 6h and 24h after one day training on a passive avoidance task using a bitter tasting aversive substance, methyl anthranilate (MeA).
Three different chick groups were used; controls, water-trained and MeA-trained. The animals were tested 6h and 24h after the initial training experience and were then anesthetized and perfused with acrolein/paraformaldehyde. The brains were cut in a vibratome and the tissue was processed for electron microscopy. The hippocampus was then divided into dorsal and ventral part and each part was examined separately for both hemispheres.
Preliminary results show that after 6 hours of training there is a statistically significant increase in the number of synapses in the MeA-trained chicks in the dorsal part of hippocampus in comparison with controls. No significant differences were found in the ventral part or after 24 h.

04.07

POTENTIATION OF mEPSCs IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES BY DEPOLARIZING VOLTAGE PULSES

A. Baxter & D.J.A. Wyllie
Division of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

A rise in postsynaptic calcium is critical for the induction of NMDA-receptor dependent long-term potentiation (LTP) at excitatory synapses in the CA1 region of the hippocampus. Using conventional LTP-inducing protocols only a small percentage of synapses display potentiation and as a result it is difficult to observe changes in either the amplitude or frequency of miniature postsynaptic currents (mESPCs) occurring at a single CA1 neurone. In an attempt to overcome this problem and to achieve a more 'cell-wide' potentiation of synapses we have used the technique of applying a series of depolarizing voltage pulses to whole-cell voltage-clamped CA1 neurones to evoke large rises in intracellular calcium levels. Thirty minute control recordings of mEPSCs from CA1 neurones in organotypic slice cultures indicated that amplitudes and frequencies were stable and gave mean values of 4.4 +/- 0.5 pA and 1.5 +/- 0.04 Hz respectively (n = 6 recordings). Application of twenty depolarizing voltage pulses (–80 mV to 0 mV for 3 seconds every at 0.2 Hz) resulted in a strong potentiation of mEPSC amplitude (control = 5.6 +/- 0.8 pA, potentiated = 10.3 +/- 0.2 pA) with no apparent change in mEPSC frequency (control = 3.3 +/- 0.6 Hz, potentiated = 2.7 +/- 0.9 Hz).

Supported by a BBSRC Committee Studentship to AB and the Royal Society.

04.08

11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 ACTIVITY AND VESTIBULAR NEURONAL PLASTICITY IN RAT

Clare Guilding, Jonathan Seckl, Mayank Dutia
DBCLS, Hugh Robson Building, George Square, Edinburgh.

The early stages of lesion induced plasticity after unilateral labyrinthectomy (UL) in rats is known to be dependent on glucocorticoid receptor activation. Recently it has become apparent that glucocorticoid access to receptors can be potently modulated by isozymes of 11b-hydrosteroid dehydrogenase (11b-HSD). This study investigates the presence, or change in activity after UL, of 11b-HSD type 1 (11b-HSD1), the isozyme that regenerates active glucocorticoids from their inert 11-keto forms. The following brain regions, which are associated with vestibular plasticity, were investigated: the vestibulocerebellum, flocculus/parafloculus complex and the medial vestibular nucleus (MVN). Thin layer chromatography (TLC) detected 11b-HSD1 activity in all three brain regions of control rats. TLC was then used to assess any changes in 11b-HSD1 activity between control, sham UL and animals that had undergone a UL four hours previously, and between ipsi- and contralesional sides of the UL brains. No change in activity was found in any of these areas between experimental conditions. These data demonstrate the presence of 11b-HSD1 in vestibular related brain areas, and rule out the possibility that differential modulation of glucocorticoid access to MVN or cerebellar neurones by 11b-HSD1 is involved in the early stages of plasticity after UL.

04.09

EXTRASYNAPTIC NR2B-CONTAINING NMDA RECEPTORS ARE REQUIRED FOR LTD IN THE ADULT PERIRHINAL CORTEX IN VITRO

P.V.Massey, B.E.Johnson, M.W.Brown, E. Molnar, Z.I.Bashir.
MRC Centre for Synaptic Plasticity, Dept. of Anatomy, University of Bristol, BS8 1TD, UK.

Induction of NMDAR-dependent LTD using standard low frequency stimulation (LFS; 900 stimuli, 1 Hz) is developmentally downregulated in numerous brain areas (Kemp & Bashir, Prog Neurobiol 65,339-65, 2001) including adult rat perirhinal cortex (Ziakopoulos et al Neurosci 92, 459-72, 1999). The aims of the present study were to investigate in perirhinal cortex mechanisms potentially responsible for this down-regulation of LTD.
Field potentials were recorded in layers II/III in response to stimuli delivered to entorhinal and temporal sides of rat perirhinal cortex slices. In juvenile (14-day) perirhinal cortex, LFS resulted in LTD, whereas this same procedure was ineffective in adult slices. However, bath application of NMDA (20uM) resulted in LTD in adult slices. Furthermore, LFS was able to induce NMDAR-dependent LTD in adult slices in the presence of the glutamate uptake inhibitor L-trans-pyrrolidine-2,4-dicarboxylate (t-PDC; 300uM). The NR2B-selective antagonist Ro-25,6981 (3uM) blocked LFS-induced LTD in the presence of t-PDC.
These results suggest that in adult perirhinal cortex activation of extrasynaptic, NR2B subunit-containing NMDARs may be required for the induction of LTD.

Supported by MRC, Wellcome and BBSRC.

04.10

PRESYNAPTIC GROUP I METABOTROPIC GLUTAMATE RECEPTORS MODULATE SHORT-TERM PLASTICITY IN THE RAT SUPERIOR COLLICULUS

L.A. Mathieson1, S.J. McIntosh1, A.M. White1,2 & B. Platt1
1 Biomedical Sciences, University of Aberdeen, Foresterhill, ABERDEEN, AB25 2ZD, UK. 2 Pharmacology and Neuroscience, University of Dundee, DUNDEE DD1 9SY, UK.

There is a well-established link between group I metabotropic glutamate receptors (mGluRs) and the phospholipase C pathway. However, in the superficial superior colliculus (SC) recent data suggest that presynaptic mGluRs activate an independent pathway that involves 4-aminopyridine (4-AP) sensitive K+ channels. The present study investigated whether these receptors contribute to two types of short-term plasticity, paired-pulse depression (PPD) and response habituation (RH).
Field recordings were performed in SC slices, prepared from 3-4 week old rats (in accordance with Home Office regulations). The group I agonists (RS)-3,5-dihydroxyphenylglycine (DHPG, 10 [mu]M) and (RS)-2-chloro-5-hydroxyphenylglycine (CHPG, 500 [mu]M) depressed evoked excitatory postsynaptic potentials (EPSPs), actions that were strongly reduced by the mGluR1 antagonist LY367385 (100 [mu]M).
Interestingly, DHPG abolished PPD for inter-stimulus intervals of 10 and 40 ms. This was reversed by 4-AP (50[mu]M), but not by the GABAA antagonist bicuculline (5[mu]M). DHPG also reduced RH, but the antagonist LY367385 did not alter RH. It therefore appears that mGluR activation can modulate RH but does not directly cause it.
Overall, our observations suggests that mGluRI agonists can regulate transmitter release and short-term plasticity in the SC, presumably via an autoreceptor-mediated feedback mechanism.

04.11

THE ROLE OF c-JUN N-TERMINAL KINASE (JNK) IN LPS-INDUCED CELL DETERIORATION IN RAT HIPPOCAMPUS

C. E. Barry and M. A. Lynch
Dept of Physiology, Trinity College Dublin, Dublin 2, Ireland.

Lipopolysaccharide(LPS), a component of the cell wall of gram-negative bacteria, exerts manifold effects in the central nervous system. It has been reported that i.p. injection of LPS inhibits long-term potentiation (LTP) in the dentate gyrus. While the mechanism by which this is affected remains unclear, LPS administration is associated with stimulation of stress activated protein kinases such as c-Jun N-terminal protein kinase (JNK). There is evidence linking these protein kinases to cell deterioration, possibly accounting for the LPS induced decrease in synaptic efficacy, however until now, the lack of a specific JNK inhibitor has made determination of the role of JNK difficult. Recently a specific JNK inhibitor (D-JNKI1; Alexis Biochemicals) was engineered and here we report that JNK inhibition attenuated the LPS-induced inhibition of LTP in urethane-anaesthetised rats. It was demonstrated that LPS activated JNK, stimulating downstream signalling cascades, including activation of the transcription factor c-jun. It was also shown that LPS leads to Bcl-2 phosphorylation, and subsequently induces apoptosis, as suggested by increases in cytochrome c in the cytosol, and TUNEL staining in cytospun cells. Pre-treatment with D-JNKI1 reduced these changes. In summary, the evidence is consistent with the idea that LPS mediated effects are dependent at least in part on JNK activity.

04.12

AN INPUT SPECIFIC ROLE OF mGluR5 RECEPTORS IN LONG-TERM DEPRESSION IN THE PERIRHINAL CORTEX DURING DEVELOPMENT

J. Jo(1), S.K. Laycock(1), Z.I. Bashir(2) and K. Cho(1)
(1)Biomedical Science, Univ. of Sheffield, Western Bank, Sheffield, S10 2TN, UK, (2)MRC Centre for Synaptic Plasticity, Univ. of Bristol, Bristol, BS8 1TD, UK.

In the present study, we have investigated the role of mGlu5 receptors in activity-dependent synaptic plasticity in the perirhinal cortex during development. Extracellular field potential recordings were made from layer II/III of the perirhinal cortex from neonatal (7-14 days old) and adult rats (4-6 weeks old). All experiments were conducted in accordance with the UK animals (Scientific Procedures) Act 1986. Application of the group II mGlu receptor agonist (DCG-IV) plus 5Hz stimulation (3000 pulses) induced NMDA-independent LTD in both young and adult rats. This form of LTD was blocked by the mGluR5 antagonist (MPEP) in the entorhinal input but not the temporal input in neonatal rats. However, MPEP did not block this LTD in either input in adult rats. These results suggest that NMDA-independent LTD needs activation of mGlu5 receptors in entorhinal input in the neonatal perirhinal cortex. However, the activation of mGluR5 receptors is not necessary to induce this form of LTD in the temporal input in neonatal rats, nor in either input in the adult perirhinal cortex. Therefore, mGluR5 may have a crucial role in NMDA-independent LTD in an input-dependent and developmental manner.

Supported by: The Royal Society

04.13

GLYCOGEN REGULATION AND ITS ROLE IN SUPPORTING HIPPOCAMPAL SYNAPTIC PLASTICITY

A.M. Brown *‡, R.E. Westenbroek†, S. Baltan Tekkok‡ & B.R. Ransom‡.
MRC Applied Neuroscience Group, Biomedical Sciences, University of Nottingham; Dept of Pharmacology† & Neurology‡, University of Washington, Seattle.*

CNS glycogen is located exclusively in astrocytes but its function as an energy source is just beginning to be understood. In this study we show regulation of hippocampal glycogen via astrocytic insulin receptors, and demonstrate the dependence of hippocampal synaptic plasticity on available energy substrate.
Stimulus-evoked EPSPs from the CA1 region of acutely perfused transverse hippocampal slices were dependent upon the presence of glucose. On withdrawal of glucose viable EPSPs could be evoked for a further 30 minutes before failing, consistent with astrocytic glycogenolysis supplying neurones with energy substrate via a transportable conduit to ensure continued support of synaptic activity. Long term potentiation was induced by high frequency stimulation in 10 mM glucose aCSF. Reducing aCSF glucose to 4 mM had no effect on control EPSPs, but inhibited induction of long term potentiation. We hypothesize that under normoglycaemic conditions astrocytic glycogen acts as an energy buffer to supply neurones with utilizable energy substrate during periods of increased synaptic activity.

04.14

TIME WINDOW FOR PLASTIC REORGANISATION OF THE IPSI-LESIONAL AND CONTRA-LESIONAL CORTICOSPINAL TRACT FOLLOWING STROKE*

M Smith, *F Villagra, +F Cowan, +M Rutherford, +E Mercuri, ~F Kirkham, *J A Eyre
**School of Clinical Medical Sciences, Department of Child Health, University of Newcastle upon Tyne, NE1 4LP, +Department of Child Health, Imperial College London, ~Institute of Child Health, Great Ormond Street.*

Our aim was to investigate the time window for plasticity of the ipsi-lesional and contra-lesional corticospinal tract (CST) in man. The subjects all sustained unilateral stoke, 31 in the perinatal period, 13 in childhood (0.5-13 years), 13 as adults. Surface EMG was recorded from biceps brachii. Transcranial magnetic stimulation (TMS) excited the CST and ipsilateral and contralateral central motor conduction delays (CMCDs) were measured. Topographical mapping of the areas of the cortex evoking responses was performed. Contra-lesional CST Significant shortening of contralateral and ipsilateral CMCDs was observed only in subjects with perinatal stroke (Z scores: contralateral CMCD, mean -0.85; 95% confidence limits -1.38 to -0.30; ipsilateral CMCD, mean -6.1, 95% confidence limits -6.98 to-3.62). Ipsi-lesional CST A significant shift in the site for evoking responses was observed only following perinatal stroke. It was displaced laterally and posteriorly in comparison to that in the non infarcted hemisphere (mean lateral displacement 1cm, 95% confidence limits 0.45 to 1,54 cm; mean posterior displacement 1.17cm, 95% confidence limits 2.11 to 0.24 cm). These data demonstrate significantly greater plasticity of the ipsi-lesional and the contra-lesional CST following perinatal infarcts then following infarcts at all other ages.

04.15

SYNAPTOPHYSIN: A MARKER OF SYNAPTOGENESIS AND CELL DEATH?

A.J. Lawman, C.A.Campbell, J. Roberts, A. Parsons, E.A. Irving
Neurology CEDD, GlaxoSmithKline, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AD. UK.

Synaptophysin is a presynaptic vesicle protein found in the majority of nerve terminals and is routinely used as a marker of synaptogenesis following brain injury.
The aim of this study was to investigate the presence of synaptogenesis using anti-synaptophysin antibodies at one and eight weeks following transient middle cerebral artery occlusion (tMCAO) in the rat.
In histologically normal tissue uniform cytoplasmic staining was detected throughout grey matter regions. One week following tMCAO, synaptophysin staining (detected using two distinct monoclonal antibodies) was markedly increased within the lesion area as delineated by cresyl fast violet (CFV) staining. Eight weeks following tMCAO, staining was decreased in lesioned areas when compared to normal tissue. When areas of increased staining were measured at one week, they were found to closely correlate with the lesion area delineated by CFV ($p < 0.00001$). No evidence for increased staining in areas remote from the lesion site were observed.
This data suggests that synaptophysin antibodies routinely used to assess synaptogenesis, can also detect lesioned tissue and highlights that caution should be exercised when interpreting increased synaptophysin staining in models of brain injury. All experiments were performed in accordance with the Animal (Scientific Procedures) Act 1986.

05.01

DIFFERENTIATION POTENTIAL OF THE OLFACTORY EPITHELIUM IN CULTURE

Lynda Chang and Susan C Barnett
Beatson Labs, Garscube Estate, Depts of Neurology and Medical Oncology, University of Glasgow.

Olfactory ensheathing cells (OECs) have become important candidates for transplant-mediated repair of CNS injury, promoting remyelination as well as axonal regeneration. Transplantation studies using tissue from the olfactory epithelium (OE) have been reported with encouraging results, however the cells responsible for promoting repair have not been identified since OE is comprised of many cell types, namely, olfactory receptor neurons (ORNs), sustentacular cells, horizontal (HBCs) and globose basal cells (GBCs). Initial studies of dissociated OE under various culture conditions identified an increased expression of the OEC marker, p75NTR. It is believed that GBCs differentiate to form the entire OE, however the role of HBCs remains unclear. This study examines the differentiation potential of HBCs, in particular to determine if HBCs can form OECs, since an OEC precursor has not been identified. HBCs were purified by FACS sorting using BS-I lectin and incubated with various growth factors to assess their differentiation potential. BS-I expression in the olfactory epithelium appears developmentally regulated with an increased number of BS-I positive HBCs at E18 and P7 than in adult tissue. Results indicate that a glial-like cell can be generated from HBCs cultured with FGF2/EGF or FGF2, forskolin, Hrgb1 and suggests that HBCs may have the potential to form a glial-like-cell.

05.02

IL-6-TYPE CYTOKINE SIGNALLING AND IMPROVE GROWTH OF SENSORY NEURONS AFTER A CONDITIONING INJURY

J. Qiu, W. Cafferty, S. McMahon, S. Thompson
Sensory Function, Centre for Neuroscience Research, Hodgkin Building, King`s College London.

It has long been documented that sciatic nerve transection largely increases growth capacity of DRG neurons and results in regeneration of subsequently lesioned dorsal column axons. This is, partly at least, due to the immune responses after axotomy, in particular the synthesis of IL-6-type cytokines at the injury site and subsequent activation of the JAK/STAT signalling cascade. In the IL-6 knockout mice, improved dorsal column axonal regeneration after a conditioning lesion is significantly impeded. Here, we ask if the JAK/STAT pathway is activated in DRG neurons after sciatic nerve injury and, if so, whether activation of the pathway is necessary for the conditioning effect on dorsal column axonal regeneration. Immunohistochemistry studies showed that STAT3 is rapidly phosphorylated and, therefore, activated in DRG neurons in response to sciatic nerve transection, and the activated status persists till up to 5 weeks. This can be blocked by intraneural infusion of a JAK2 inhibitor, AG490, immediately after sciatic nerve transection. Importantly, AG490, when applied intraneurally after injury, also impairs improved growth of DRG neurons in vitro and dorsal column axonal regeneration in vivo. This is correlated with a reduced up-regulation of GAP43 expression in DRG neurons. Whether or not IL-6 is sufficient to enhance CNS regeneration is currently being tested.

05.03

CHONDROITINASE ABC INDUCES SPROUTING AND PLASTICITY AFTER SPINAL CORD INJURY

Elizabeth J. Bradbury, Nick D. Jeffery, Ruth A. Hartley, Ping Yip, James W. Fawcett and Stephen B. McMahon
Centre for Neuroscience Research, Kings College London, UK; Centre for Brain Repair, University of Cambridge, UK.

Chondroitin sulphate proteoglycans (CSPGs) are inhibitory extracellular matrix molecules associated with the reactive glial scar that forms at CNS injury sites. Inhibitory CSPG activity can be attenuated by degrading CSPG molecules using the enzyme chondroitinase ABC (ChABC). We have previously shown that ChABC treatment can promote functional recovery in adult spinal injured rats. Although some regeneration of ascending sensory and descending motor axons was observed in these studies the effects of ChABC delivery on adjacent undamaged pathways is not known. Therefore, we looked anatomically at the response of different spinal pathways above and below the lesion level following a cervical spinal cord injury and treatment with ChABC. Abnormal sprouting of CGRP-immunoreactive primary afferent fibres was observed in degenerating dorsal column white matter tracts following ChABC, but not vehicle, treatment at 2 and 4 weeks post-injury. The response of corticospinal tract (CST) axons above the injury site was also investigated. Preliminary findings indicate that ChABC treatment promotes aberrant sprouting of CST axons in the brainstem and induces extensive sprouting of the CST projection in the dorsal columns. These findings demonstrate that degradation of CSPGs at the site of a spinal cord injury can promote increased plasticity in intact spinal systems.

05.04

CHONDROITINASE ABC STIMULATES EXPANSION OF SPINAL CORD PRIMARY AFFERENT TERMINAL FIELDS

William. B. J. Cafferty (1), Elizabeth. J. Bradbury (1), James. W. Fawcett (2) and Stephen. B. McMahon (1).
1)Centre for Neuroscience, King`s College, London, United Kingdom; 2)Brain Repair Centre, Cambridge University, Cambridge, United Kingdom.

Damage to dorsal roots engenders reactive glial activity that ultimately results in the formation of an impenetrable inhibitory barrier at the dorsal root entry zone. Chondroitin sulphate proteoglycans (CSPG's) are inhibitory molecules associated with the extracelluar matrix of glial scars. Recent data has shown that degrading CSPG glycosaminoglycan side chains with Chondroitinase ABC (ChABC) enhances regeneration and promotes functional recovery after spinal cord injury. We have investigated the ability of ChABC to alter the central terminal field pattern of brachial plexus primary afferents. ChABC or vehicle was infused in to the dorsal horn at C6 and C8 of adult rats that had undergone a unilateral sextuple rhizotomy (C4-C6) sparing C7 (C8-T2). CTB was injected into the median nerve or directly into the C7 root 3 days before the end of the recovery period. Animals recovered for 3, 7 or 14 days after rhizotomy. ChABC activity was confirmed by the presence of 2B6-IR around the infusion site. Expansion of terminal fields of large fibres (CTB-IR) and small fibres (CGRP-IR and IB4 binding) was observed in the ChABC treated but not in vehicle treated groups. Therefore degradation of CSPG's within areas of primary afferent degeneration may provide a stimulus for anatomical reorganisation.

05.05

UNUSUAL DENDRITIC MORPHOLOGY ROSTRAL TO A SPINAL LESION

C. F. Meehan, N.P Anissimova, T. W. Ford and P. A. Kirkwood
Sobell Dept. Institute of Neurology (UCL) Queen Square, London, WC1N 3BG.

At various survival times following a lateral hemisection of the adult feline thoracic cord, unaxotomised interneurones in the segment rostral to the lesion were intracellulary labelled with Neurobiotin. All procedures were carried out in accordance with the Animal (Scientific Procedures Act), 1986.
Abnormal dendritic processes were observed on the interneurones, similar to those of axotomised neck motor neurones (Rose & Odlazinski, 1998, J. Comp. Neurol. 1998, 390: 392). At 6-8 weeks post-lesion the most common abnormal structures were "tortuosities" (especially near the grey/white border). At longer survival times post-lesion (16 weeks +) "tortuosities" were less common but processes still appeared abnormal (hairy), many reaching the midline or crossing to the contralateral grey, where some branched. Those cells closer to the lesion showed more abnormalities than those further away (same segment).
Neuronal polarity may be abnormal in that some of the dendrites stained negative for MAP 2a/b (a dendritic protein) and/or positive for GAP-43 (an axonal growth protein), as reported for axotomised motoneurones (Rose et al. 2001, Eur. J. Neurosci. 13: 1166-1176).
The results show that these features can result from stimuli other than axotomy. In fact, similar features were also observed in an un-axotomised motoneurone.

Support: ISRT, MRC.

05.06

DIFFERENTIATED BONE MARROW MESENCHYMAL STEM CELLS EXPRESS GLIAL CELL MARKERS AND STIMULATE NERVE REGENERATION

Tohill M., Mantovani C., Wiberg M., Terenghi G.
Blond McIndoe Research Laboratories, Plastic and Reconstructive Surgery Research, The University of Manchester Stopford Building, Oxford Road, Manchester M13 9PT.

Recent reports have shown that bone marrow mesenchymal stem cells (MSCs) are capable of great plasticity in their differentiation pathways. The potential of these cells to cross oligolineage boundaries in addition to their accessibility raises possibilities for their therapeutic use in a wide range of injuries and diseases. This study examined the differentiation of MSCs in culture and their behaviour in a model of peripheral nerve injury. MSCs were harvested from the long bones of adult rats. The cells were cultured in the presence of glial growth factor (GGF) to stimulate differentiation into a glial cell lineage. Immunohistochemical techniques were employed to identify glial cell markers. Differentiated cells were labelled with GFP and transplanted into a polyhydroxybutyrate nerve conduit that was used to span a 1cm gap in the rat sciatic nerve. The conduits were harvested after 15 days and histologically examined for axonal and Schwann cell regeneration and MSC integration. In culture MSCs exposed to GGF were found to express the glial cell markers S100 and GFAP, and to exhibit distinct Schwann cell-like morphology. Following transplantation MSCs were found to maintain their morphological differentiation and conferred a beneficial effect on nerve regeneration.

05.07

THE ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPKS) IN SENSORY NERVE REGENERATION

M.F.Lockwood, A. Verkhratsky and P. Fernyhough
Division of Neurosciences, 1.124 Stopford Building, Oxford Road, Manchester M13 9PT.

This study determined the role of MAPKs in sensory neurone regeneration in response to neurotrophic factors. Axonal regeneration was quantified in dissociated cultures of adult rat dorsal root ganglion (DRG) neurones. Dissociated DRG neurones were treated for 16 hours with either NGF or GDNF in the presence of protein kinase inhibitors: ERK inhibitor U0126 (10μM), p38 inhibitor SB203580 (2μM) or phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (10μM). ERK inhibition reduced the level of the NGF-induced axonal network (2067±1022μm vs 8389±2132μm, $P<0.001$). The p38 and PI3K inhibitors had no effect. The number of neurones that responded to NGF was not affected by any inhibitor treatment. The PI3K inhibitor reduced the GDNF-induced axonal network (2738±501μm vs 7942±2169μm, $P<0.05$); however p38 or ERK inhibition were without effect. Combined p38 and ERK inhibition reduced the level of the GDNF-induced axon network (1955±592μm vs 6454±2320μm, $P<0.05$); the number of neurones that responded to GDNF was reduced with dual inhibition ($P>0.05$).
The results show that NGF and GDNF signalling through ERK and PI3K, respectively are required for maintenance of regenerative axon growth but not for initiation of axonal outgrowth. GDNF signalling through p38 and ERK is required for maintenance and possibly initiation of regenerative axonal outgrowth.BBSRC and Pfizer

05.08

NEURITE OUTGROWTH IS DEPENDENT ON β1-INTEGRIN MEDIATED INTERACTIONS WITH THE EXTRACELLULAR MATRIX

Nicola Wilson, Jonathan D. Moore, P. Marc Watson, Nancy J. Rothwell and Rosemary M. Gibson.
Room 1.124, School of Biological Sciences, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT.

Regulation of neurite outgrowth is a determining factor in neuronal development and regeneration. Extension of neuronal processes is dependent upon their interaction with the extracellular matrix (ECM). β1 integrins are the major receptor through which neurones communicate with the ECM. In the present study we have examined the expression of β1 integrins by primary cortical neurones (CCNs), isolated from embryonic mice, and their ability to extend neurites on ECM proteins. CCNs express integrin heterodimers composed of the β1 subunit in association with several α subunits. The neurones extend neurites on poly-D-lysine, through a non-integrin mediated interaction, and on merosin and laminin via β1 integrins, since outgrowth is blocked by the anti-β1 antibody Ha2/5. Understanding integrin-mediated interactions between neurones and the ECM, and the downstream signalling pathways involved in neurite outgrowth is critical if we are to develop strategies that improve the regenerative capacity of neurones for therapeutic benefit.

05.09

INTEGRIN EXPRESSION IN ADULT RAT SENSORY NEURONES: REGULATION FOLLOWING NERVE INJURY

N.J. Gardiner, P. Fernyhough, D.R. Tomlinson and C.H. Streuli
School of Biological Sciences, University of Manchester, Manchester, UK.

Integrins are a large family of receptors that mediate cell-cell and cell-matrix interactions. The α and β integrin subunits associate to form active heterodimers. The β1 family of integrins mediates neurite outgrowth in a number of cell types, however little is known about expression of integrins in the adult peripheral nervous system,. This study investigates the role of β1 integrins in sensory nerve regeneration. Anaesthetised adult Wistar rats received a unilateral crush at mid-sciatic nerve or were sham operated. The dorsal root ganglion (DRG) and sciatic nerve were removed 2, 7 or 14 days post-injury, and prepared for immunocytochemistry or immunoblotting. Dissociated sensory neurones from naïve adult rats were grown for 18 hrs on laminin or fibronectin in the presence of NGF, NT-3 or GDNF. Neurotrophin-treated neurones grown on laminin showed robust β1-integrin-dependent neurite outgrowth. Since α1 and α3 are potential receptors for laminin we investigated the distribution of these receptors. Both were detected in neurites and growth cones. In vivo studies revealed that α1 and α3 were mainly localised to satellite cells in the DRG of naïve rats. However, following nerve injury α1 and α3 were expressed in neurones and regenerating axons, indicating that these integrins may mediate successful regeneration.

05.10

CHARACTERISATION OF MATRIX METALLOPROTEINASES IN LONG TERM DENERVATED MUSCLE

M. Demestre, G.M. Wells and N.A. Gregson
Department of Neuroimmunology. King's College. Guy's Campus. London SE1 9RT. British Biotech Pharmaceuticals Limited. Watlington Road. Oxford OX4 5LY. Current address: Sobell Department of Motor Neuroscience and Movement Disorder, Institute of Neurology, London WC1N 3BG.

Following denervation the muscle undergoes degenerative changes, which obstruct the reinnervation process. In the rat, muscles that remain denervated for more than 14 days have a reduced capacity to accept regenerating axons and therefore reinnervation is delayed or reduced. Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade components of the extracellular matrix. We have previously demonstrated that following denervation the systemic administration of a broad-spectrum MMP inhibitor improved reinnervation of far-distal muscles. Over two months, levels of MMP mRNA expression and the localisation and activity of MMP proteins were examined following long term denervation. MMPs and enzyme activity were localised to the neuromuscular junction of normal and denervated muscles. Both enzyme activity and MMP mRNA levels were maximal at the time corresponding to the period of reinnervation. We suggest that in denervated muscles MMPs may be involved in the processes leading to atrophy of denervated muscles.

05.11

THE EFFECTS OF LENS TRAUMA ON NEURITE REGENERATION OF RETINAL GANGLION CELLS (RGC) *IN VITRO*

B. Lorber2, D. Tonge1, A. Baird3, A. Logan2 & M. Berry2.
1.Centre for Neuroscience Research, KCL, London, SE1 1UL, UK2.Department of Medicine, University of Birmingham, B15 2TT, UK3. Sackler Institute of Pharmaceutical Science, KCL, London, SE1 1UL, UK.

It has recently been shown that axonal regeneration in the optic nerve of adult rats can be promoted by lens lesion (Leon et al., 2000; Fischer et al., 2000, 2001). In the present study, the effects of a priming lens lesion on the characteristics of neurite outgrowth of dissociated retinal ganglion cells (RGC) from postnatal (P) 9- adult rats have been investigated in vitro. RGC from intact P9-11 rats showed spontaneous neurite outgrowth on laminin-1, which is in contrast to RGC derived from intact P14-adult rats. Prior lens lesion and coculture with lesioned lenses both promoted neurite outgrowth from P9-14 RGC on laminin-1. However, promotion of neurite outgrowth from adult RGC under the same conditions required the use of laminin-2 as a substrate. The addition of K252a (trk receptor blocker) and mAb228 (blocks effects of leukemia inhibitory factor and ciliary neurotrophic factor) to medium conditioned by lesioned lenses did not inhibit its stimulatory effect on neurite outgrowth. Together, these results suggest the presence of a neuritogenic factor(s) derived from the lesioned lens that does not belong to either the neurotrophin or gp130 cytokine family. Further, there is a clear developmental change in substrate requirements of neurite outgrowth.

05.12

CORTICO-STRIATAL ORGANOTYPIC SLICE CULTURES EXPRESS MARKERS OF REGENERATION FOLLOWING HYPOXIA

M.A.Murfin; A.J.Lawman; I.J.M. Beresford; A.M.Ray
Department of NeuroRegeneration, Neurology & GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Research & Development Limited, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK.

The post-ischaemic brain is known to express increased levels of certain markers characteristic of neuroregeneration. The present study was designed to examine the expression of neurabin I and II/spinophilin in cortico-striatal organotypic slice cultures following hypoxia. Neurabins are highly concentrated in dendritic spines and are enriched in the growth cones during neuronal development. Hence they may be useful markers of regeneration. Cortico-striatal slices prepared from postnatal day 1 rat brain were cultured for 14 days, subjected to 1 hour of hypoxia, and then fixed after 24 hours, 3 days, 1 week and 2 weeks. The slices were paraffin embedded, cut at 4 µm and stained for neurabin I and neurabin II. Neurabin I was upregulated at all time points in the hypoxic slices compared to control, while levels of neurabin II in hypoxic slices were unchanged. Hypoxia-evoked lesion in cortico-striatal organotypic cultures may thus represent a novel in vitro model in which to study neuroregeneration.

05.13

OSTEONECTIN PROMOTES SURVIVAL AND NEURITOGENESIS OF MICE SUPERIOR CERVICAL GANGLIA (SCG)

C.H. Ma and Jeremy S.H. Taylor.
Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, UK.

The ability of the peripheral nerve grafts to induce neural regeneration is due primarily to the trophic support supplied by the Schwann cells. Osteonectin (ON) was purified from Schwann cell conditioned medium and we are now looking at its effects on neuronal survival and regeneration. We are using neonatal mouse sympathetic ganglion cells as test neurons, which are known to be dependent upon nerve growth factor (NGF) for their survival. The present study shows that ON potentiates the effects of NGF on P1 SCG neurons. The effects of ON and NGF at different cell densities (500, 2000 and 5000 cells/well) were also examined. As the cell density is increased, the survival promoting effect of ON became more pronounced. In high-density cultures, the number of surviving neurons was increased significantly ($P<0.05$) by ON when compared to either control or NGF alone. ON also promoted neuritogenesis of NGF treated cultures at all cell densities ($P<0.05$) and increased the total neurite outgrowth of individual SCGs. In low-density cultures the neurite stimulating effect of ON was greatest. Taken together, these data indicate that ON potentiates the survival and neurite outgrowth effects of NGF in SCG cultures.

05.14

MUSCLE AND ENDPLATE MORPHOLOGY AFTER TRANSECTION OF THE SCIATIC NERVE AND REINNERVATION. A STUDY IN ADULT RATS

J. IJkema-Paassen, M.F. Meek, A. Gramsbergen
Department of Medical Physiology, A. Deusinglaan 1, Bldg 3215, 9713 AV Groningen, The Netherlands.

Reinnervation after peripheral nerve lesions generally leads to impaired functional recovery. We studied whether changes in muscle morphology and reinnervation patterns contribute to these results. A segment of the sciatic nerve was resected in one hindleg, reversed, and implanted as a nerve guide. We studied muscle histochemistry, endplate morphology and the occurrence of polyneural innervation in the soleus (SOL), lateral gastrocnemius (LGC), and tibial muscles (TA) in both hindlegs.

Type I muscle fibres in the LGC and TA showed initially an increase but values approached the normal again at 21 weeks. The SOL at the operated side, however, had changed into a muscle predominantly containing type II muscle fibres and this persisted until 15 months after transection.

The majority of endplates was immature at 7 weeks although a few normal endplates could be detected. These percentages increased with time. The incidence of polyneurally innervated endplates at 7 weeks was highest in the SOL but at 21 weeks the percentages in all muscles were around 10%. After 15 months we observed an increase in polyneurally innervated endplates in the SOL (around 20 %) but also at the control side (10 %).

These changes might, in part, explain the poor functional recovery.

05.15

REGULATION OF ATF3 PROTEIN EXPRESSION AFTER DIFFERENT TYPES OF PERIPHERAL NERVE INJURY

Sharon Averill, Ben Johnson, Greg Michael and Peter Shortland
Neuroscience Dept., Bart's & The London School of Medicine & Dentistry, Mile End Road, London E1 4NS.

ATF3 is a member of the ATF/CREB transcription factor super-family. ATF3 is induced in sensory and motor neurons by stimuli such as ischaemia or nerve injury. Recent studies suggest that it may be a unique neuronal marker for nerve injury or neuronal regeneration. Although ATF3 is upregulated in neurons after peripheral nerve injury, the different types of injury, their time-course and the signal(s) that regulate its expression remain largely unexplored. We have examined ATF3 expression in dorsal root ganglion and motoneurons at 1, 14 and 30 days after injury in different models of peripheral nerve injury: sciatic nerve section, crush, L4-6 dorsal or ventral root avulsion and following intrathecal neurotrophin treatment. ATF3 is differentially upregulated in DRG cells and motoneurons within 1 day in all the models examined. In motoneurons, expression appears to be regulated by target contact as expression declined towards zero with peripheral nerve regeneration. Intrathecal NGF and GDNF also regulated ATF3 levels after injury. We suggest that ATF3 expression is modulated by the loss of a target derived factor and its magnitude depends on the type and time of injury. All experiments were in carried out accordance with the Animals (Scientific Procedures) Act 1986.

05.16

NEURITE OUTGROWTH OF MOTONEURONES PROMOTED BY EYE LENS CRYSTALLINE *IN VITRO*

Petra Ahmann, Karla S. Tratsk and Solon Thanos
Department of Experimental Ophthalmology, Eye Hospital Münster, Domagkstraße 15, D-48149 Münster, Germany.

The experiments reported here aimed to answer the question, if eye lens crystallins have an direct effect on neurite outgrowth in organotypic spinal cord cultures, and motoneuron single cell culture. All the crystallins tested increased axonal outgrowth significantly (two sided students t-test). Alpha-and beta H-crystallin was most effective in promoting axonal outgrowth and its effect can be blocked nearly completely by specific antibodies. These results indicate for the first time, that crystallins, promote directly neurite outgrowth of Motoneurones *in vitro.*

06.01

HOMOTOPIC THALAMIC TRANSPLANT ABOLISHES HOST THALAMIC PROJECTIONS TO VISUAL CORTICAL TRANSPLANT IN THE ADULT RAT

A. Gaillard; L. Domballe and F. Gaillard
UMR 6558, CNRS, Poitiers, France.

Several studies have shown that embryonic visual tissue transplanted into the visual cortex of adult rat receives sparse thalamic input from the host thalamus. In an attempt to improve the density of these thalamic projections, we have used a double transplantation paradigm. In short, grafts of neocortical visual and thalamic tissues were removed from E16 rat fetuses. The block of visual tissue was transplanted into the previously lesioned left occipital cortex. The transplant of thalamus was injected to the left thalamus as a cell suspension. Several months after transplantation, the pattern of the thalamic connections toward the transplant was examined by injecting a retrograde tracer into the cortical transplant. Surprisingly, our results show that all connections between the host thalamus and the cortical transplant are abolished. One explanation of this finding could be the presence of inhibitory diffusible factors secreted by the thalamic transplant.

06.02

NITRIC OXIDE CELL DEATH AND NEURAL TRANSPLANTATION

Kasim N, Branton RL, Clarke DJ, Wood MJA
Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.

ABSTRACT WITHDRAWN

07.01

NORMAL PRESSURE HYDROCEPHALUS AND DEMENTIA: RECENT DEVELOPMENTS IN DETERMINING SURGICAL SUCCESS

Balamurali, B.*, Starkey, N.J.**, & Golash, A.*
** Department of Neurosurgery, Royal Preston Hospital, Preston, Lancs; ** Department of Psychology,University of Central Lancashire, Preston, Lancs. PR1 2HE.*

Normal pressure hydrocephalus (NPH), (Hakim & Adams, 1965) is a triad of dementia, gait disturbance and urinary symptoms, which occur in adults over 60 years of age. Diagnosis is difficult and estimates suggest that 10% of patients with dementia (including Alzheimer's and Parkinson's Disease) are actually suffering from NPH. The treatment of choice is insertion of ventriculoperitoneal shunt but complications occur in up to 50% of patients (Larsson, 1991). Therefore, accurate diagnosis and careful patient selection is important. In the long term this study seeks to determine which symptoms predict surgical success in NPH using a variety of measures including; radiological & gait assessment, neuropsychological tests (CANTAB), lumbar puncture (opening pressure) & drainage, CSF outflow resistance (Rcsf), ganglioside/sulfatide levels in CSF and ICP monitoring. In the short term it was of interest to examine the effects of a continuous lumbar drain (100mls CSF per day for 2 days) on neuropsychological test performance. Initial data indicate that patients showing improved gait after lumbar drain also showed an improvement on a range of neuropsychological tests. This indicates that administering these tests after lumbar drain may be a useful aid in assessing suitability for shunting.

07.02

REGULATION OF PARP EXPRESSION IN NEUROBLASTOMA CELL

SJ.Sixsmith*, A.Burkle and R.N.Kalaria
Institute for Ageing and Health and Dept of Gerontology, University of Newcastle, Newcastle upon Tyne, NE4 6BE, UK.

Poly(ADP-ribosyl)ation is an immediate post-translational modification of nuclear proteins by the addition of long chain ADP-ribose polymers, using NAD+ as a substrate. It is now apparent there is a growing family of poly(ADP-ribose)polymerase (PARP) proteins. Earlier studies suggested the existence of a single PARP-1. The localisation of different PARPs, however, has not been mapped in the brain. We used real-time and reverse transcription- polymerase chain reaction (PCR) to quantify PARP mRNAs in neuroblastoma (SHSY5Y) cells in culture and post-mortem brain tissue. We found expression of PARP-1, PARP-2, PARP-3, Tankyrase and Vault PARP mRNAs in hippocampus, white matter, cerebellum, meninges, putamen, basal ganglia, temporal cortex and choroids plexus. In a second series of experiments we investigated PARP mRNA expression in SHSY5Y cells subjected to various insults. Initial results demonstrated the presence of all PARP genes but the levels were unaltered at 24 hours after hypoxic-ischaemic injury. The results should provide important mechanistic insight into the differential regulation of PARPs and cellular vulnerability to oxidative damage in brain neurones. Application of PARP-specific pharmacological inhibitors to prevent over-activation of PARP may prove useful in neurodegenerative disorders.

07.03

LONGITUDINAL BEHAVIOURAL ANALYSIS OF BACKGROUND STRAINS FOR ALZHEIMER'S DISEASE MODELS

P.L.Pugh, S.Ahmed, M.Smith, N.Upton, A.J.Hunter
Department of In Vivo Pharmacology, Neurology & GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Research & Development Limited, New Frontiers Science Park, Harlow, Essex CM19 5AW.

Transgenic animals are of importance in the development of models for neurological diseases. In assessing the behaviour of such transgenics it is imperative to investigate the underlying behavioural patterns of the background strain. The FVB/N strain is popular for the generation of transgenic animals but aspects of its behaviour has not been greatly characterised. Previous studies have indicated this strain has elevated aggression and clear learning deficits (Mineur & Crusio, Brain Res Bull. 57(1) 41-47, 2002). Initially, FVB/N and C57BL/6 mice were compared using the Morris Water Maze and Circadian Wheel Activity. In the water maze, FVB/N mice were less able to learn the position of either a hidden ($p<0.001$) or visible platform ($p<0.001$) during acquisition relative to C57BL/6 mice. During circadian wheel activity tests, FVB/N mice were shown to be unable to entrain to a 12:12 hour light/dark period unlike C57BL/6 mice which were able to adapt to this entrainment easily. In a longitudinal study, FVB/N and C57BL/6 mice were tested at 3, 6 and 12 months and were shown to display specific differences in LMA, SHIRPA, Resident-Intruder Paradigm and the Elevated Plus Maze. These studies indicate the FVB/N strain is unsuitable for behavioural analysis of transgenic animals.

08.01

FUNCTIONAL CHARACTERISATION OF Rac FOR THE INDUCTION OF pip92 AND NEURAL DIFFERENTIATION IN EMBRYONIC PROGENITOR CELLS

J.B. Park 1; J.H. Baik 1; K.C. Chung 2
1. Medical Science and Brain Korea 21 Project for Medical Sciences, Yonsei Univ., Coll. of Med., 2. Biology, Yonsei Univ., Seoul, South Korea.

Pip92 is rapidly and transiently induced by serum, NGF, and phorbol ester during cell growth and differentiation as well as by various toxic stimuli during cell death. Although bFGF and active Raf were shown to activate the expression of pip92 via ERK-independent as well as -dependent signalling pathways, its functional role has not been clarified yet. Rho family GTPases are known to be closely related to cell motility and the formation of cell shape. Among of them, Rac1 and Cdc42 contribute to neurite outgrowth in many types of neuronal cells, and their downstream effector Pak1 is highly enriched in neurons. In the present study, the functional roles of Rho family GTPases have been investigated for the induction of pip92 and neuronal differentiation in embryonic neural progenitor cells. Our data showed that the activation, of Rac1, but not of Rho or Cdc42, appears to be important for pip92 expression in response to bFGF. In addition, transient transfection of kinase-inactive MEKK significantly decreased the Rac1 activation by FGF, whereas ERK inhibitor did not have an affect on Rac1. Furthermore, bFGF-induced active Pak1 directly phosphorylated the transcription factor Elk1. These data suggest that the activation of JNK, but not ERK, is the upstream of Rac1, and the active Pak1 subsequently phosphorylates Elk1, whose activation is critical for pip92 induction and neuronal differentiation.

08.02

ULTRASTRUCTURAL CHARACTERISTICS OF GAD65 IMMUNOREACTIVE BOUTONS IN RAT PREFRONTAL CORTEX

Hardwick C1, French SJ1, Southam E2,Totterdell S
1Department of Pharmacology, Oxford University, Mansfield Road, Oxford, OX1 3QT; (2) Psychiatry CEDD, GlaxoSmithKline, Harlow, Essex CM19 5AW.

Changes in levels of the GABA synthesizing enzyme, glutamate decarboxylase (GAD), are reported in schizophrenia. In patients, the density of the 65 kDalton isoform of GAD (GAD65)-immunoreactive (IR) terminals apposed to neurons (pyramidal and nonpyramidal) and in the neuropil of prefrontal cortical (PFC) layers II-VI was unchanged. However, such light microscopic examinations do not address possible rearrangements of synaptic circuitry. We thus determined, in rat PFC, the normal ultrastructural distribution of GAD65 using a mouse monoclonal antibody. Fixed brain sections were processed using the ABC method with DAB as the chromogen. Following light microscopic examination of IR profiles in PFC (medial and orbital), areas were selected for electron microscopic investigation. Immunostaining was restricted to the most superficial layers of the tissue, in varicose profiles. Digital images of GAD65-IR synaptic boutons, identified by systematically scanning sections, were analysed for area, perimeter and synaptic length. Labelled terminals formed exclusively symmetrical synapses targetting large and small profiles, defined by size as proximal or distal dendrites, or spines but never somata. This suggests that, in rat PFC, GAD65 terminals do not form pericellular fibre networks. These data provide a foundation for examining GAD65 synaptic rearrangement in rat models of schizophrenia.

08.03

GLYCOGEN SYNTHASE KINASE ACTIVITY IS ASSOCIATED WITH HYDROGEN PEROXIDE BUT NOT PROSTACYCLIN-INDUCED APOPTOSIS OF HUMAN ASTROCYTES

Rung-chi Li*#, Tai-ping Fan#, Shaun McNulty+, Lynda Sellers*
**Neurobiology Dept., Babraham Institute, Babraham, Cambridge CB2 4AT,#Dept. of Pharmacology, Tennis Court Road, Cambridge CB2 1PD,+GlaxoSmithKline Pharmaceutical, Harlow, Essex, CM19 5AW.*

Oxidative stress is responsible for much of the delayed damage and apoptotic injury induced following a stroke. In addition, increased production of prostaglandins results in brain tissue within minutes to a few hours after a stroke. Nevertheless, little is known about the relationship between prostaglandin production and oxidative stress-mediated brain damage.
Here we have examined the mechanisms through which hydrogen peroxide (H2O2, 100 microM) or the prostacyclin stable analogue, iloprost (1 microM), induces death in the human astrocytic cell line, T10. Treatment for 24h with either agent caused apoptosis as determined by annexin V binding without loss of membrane integrity. In addition, iloprost or H2O2 reduced cytochrome c and increased active caspase 3 staining after application for 4h. Examination of changes in the activity status of members of the MAP kinase family showed both agents to cause a transient increase in the phosphorylation of ERK1/2 (over 30min) and a sustained increase (4h) in the activity of the SAPK and p38 kinases. H2O2 resulted in enhanced activity of glycogen synthase kinase, whereas iloprost was without effect.
It is hoped that a better understanding of the mechanisms which cause cell death following stroke will enable more selective drugs to be designed.

08.04

SPONTANEOUS SYNAPTIC ACTIVITY IN HIPPOCAMPAL NEURONS REGULATES THE EXPRESSION OF THE INOSITOL 1,4,5 TRISPHOSPHATE RECEPTOR

Paul Cuddon 1, H. Llewelyn Roderick 1, Peter B. Simpson 2, Michael J. Berridge 1 and Martin D. Bootman 1
1 Laboratory of Molecular Signalling, Babraham Institute, Cambridge, UK2 Neuroscience Research Centre, Merck, Sharp and Dohme, Harlow, UK.

Despite the many studies regarding the development of the signalling components present in the plasma membrane of neurons, few have addressed the development of intracellular calcium signalling machinery. We have investigated the effects of spontaneous calcium signals, resulting from synaptogenesis, on the maturation of metabotropic responses. Neurons cultured at `high-density` (35,000/cm2) began to exhibit spontaneous, synchronous oscillations in intracellular calcium concentration from day 11 in culture. This correlated to the development of responses to the metabotropic receptor agonists: carbachol and DHPG. Neurons cultured at `low-density` (5,000/cm2) demonstrated an absence of spontaneous activity and with that a complete lack of response to carbachol and DHPG during any stage of development. By culturing `high-density` neurons in conditions that block spontaneous calcium oscillations it was possible to significantly reduce the amplitude of metabotropic calcium responses. Stimulation of the inositol 1,4,5 trisphosphate (Ins1,4,5P3) receptor, using 30 uM Ins1,4,5P3-ester, elicited a large calcium signal only in `high-density` neurons. We conclude that the oscillations in intracellular calcium concentration result in the production of the Ins1,4,5P3 receptor. The transcriptional machinery may be directly activated by calcium or by calcium-mediated release of growth factors.

08.05

EXPRESSION OF Kv1 FAMILY POTASSIUM CHANNELS IN PC12 CELLS

Snape, A.C. and Jones O.T.
Sch. Biol. Sci., Univ. Manchester, 1.124 Stopford Building, Oxford Rd., M13 9PT.

Surface densities and distributions of ion channels are essential in defining neuronal physiology. Unfortunately, little is understood about the mechanisms responsible for their control. Evidence from non-neuronal cells suggests an important role for pore subunit permutations in determining membrane densities. We hypothesise that subunit permutations determine surface densities in neurones via control of their internalisation mechanisms. To test this, endogenous Kv1 potassium channels, expressed in pseudo-neuronal PC12 cells, were investigated as a model for internalisation studies. Western blotting with undifferentiated cell lysates detected Kv1.1-Kv1.4 and Kv1.6, in forms comparable in size to those reported in cells or brain membranes. Upon differentiation with nerve growth factor (100ng/ml), changes were seen in the levels of specific subunits, along with well-documented changes in cell morphology. These changes are likely to be commensurate with development of electrical activity and synaptogenesis. Thus, PC12 cells may prove a useful neuron-like host for studying Kv1 internalisation. However, to compensate for endogenous channels, transfected Kv1 subunits must incorporate a recognisable epitope tag. Dissection of internalisation mechanisms is a requisite to understanding diseases where ion channels fail to faithfully distribute in cell membranes. Work funded by the BBSRC.

08.06

AN IMMUNOHISTOCHEMICAL STUDY MAPPING THE EXPRESSION OF NudE-L IN PRIMATE BRAIN

J. Manolopoulos & K. R. Oliver
Neuroscience Research Centre, Merck Sharp & Dohme, Terlings Park, Harlow, Essex, CM20 2QR, UK.

NudE-like (NudE-L) is a coiled-coil protein thought to be involved in neuronal migration and development. NudE-L was initially demonstrated to interact with LIS1 (a lissencephaly disease gene) and dynein, and has been identified in neuronal somata, processes and growth cones.
Recent data suggests that NudE-L may interact with DISC1 (Disrupted In SChizophrenia-1), a novel gene spanning a translocation breakpoint associated with schizophrenia. Expression of mutant DISC1 in PC12 cells reduces neurite extension. In light of current considerations of schizophrenia as a neurodevelopmental disease, these data are tantalising.In situ hybridization has shown NudE-L mRNA to be expressed in rat brain. In the present study, we sought to investigate the expression of NudE-L protein in macaque primate brain using immunohistochemistry. Our data demonstrate widespread, but heterogenous expression in the CNS. Moreover, localization was noted in areas associated with psychiatric disorders.
Through the use of such cellular localization methodologies in higher species, and making comparative analysis with disease-related proteins known to interact with NudE-L, such as DISC1 or LIS1, further insights into the pathophysiology of psychiatric disorders, and schizophrenia in particular, may be elucidated.

08.07

EFFECTS OF LONG AND SHORT-TERM ETHANOL ON THE INTRACELLULAR Ca^{2+} SIGNALS EVOKED BY STIMULATION OF ACETYLCHOLINE RECEPTORS IN SH-SY5Y CELLS

Sabrià J(1), Dajas-Bailador F(2), Wonnacott S(2)
(1) Institut de Neurociències and Departament de Bioquímica i Biologia Molecular. UAB. 08193.Cerdanyola del Vallès. Barcelona. SPAIN(2) Department of Biology and Biochemistry. University of Bath. BA2 7AY. UK.

SH-SY5Y cells express muscarinic acetylcholine receptors as well as α7 and α3* nicotinic acetylcholine receptors. Numbers of these receptors are sensitive to ethanol.
In this study we have examined the functional consequences of long-term (4 days) or short-term (15 min) ethanol treatments on nicotine, acetylcholine or KCl-evoked increases in intracellular calcium concentracion in SH-SY5Y cells.Ethanol treatment for 4 days was without effect on nicotine-evoked increases in [Ca2+]i. Acetylcholine-evoked responses were decreased by 45%. Responses to KCl were decreased by 25%.
After a short-term (15 min) treatment with ethanol, nicotine-evoked increases in [Ca2+]i were unchanged while acetylcholine and KCl evoked responses were reduced by 32% and 23 % respectively.
These effects are not attributable to changes in cell viability, but are likely to be ascribed to actions of ethanol on muscarinic receptors.

Work supported by Dirección General de Universidades (PR2002-0073)

08.08

TRPC6 LOCALISES TO THE TGN IN CULTURED RAT NEURONS

Rogers, A.T., Wolstenholme, A.J. and Reaves, B.J.
Department of Biology and Biochemistry, University of Bath, Bath, Somerset, UK.

A hybridoma cell line (6H1C4) secreting an IgG1 monoclonal antibody (MAb) specific for TRPC6 has been established. Overexpressed TRPC6 was detected using immunofluorescence microscopy in HEK 293-T cells transiently transfected with TRPC6 cDNA. Western blot analysis of transfected whole cell lysates demonstrates a double band at approximately 105 KDa, while a faint single band (the lower of the doublet) is seen in untransfected cell lysates. The band/s are apparent in several other cell lysates, and activity can be abolished by pre-incubation of the MAb with peptide immunogen. The antibody is also able to immunoprecipitate recombinant TRPC6. TRPC6 was localised using immunofluorescence microscopy in primary cultures of embryonic rat cortex, hippocampus and spinal cord. Though similar results were seen with each, the best results were obtained in spinal cord cultures of 3 weeks maturity, where intense neuronal staining was observed close to the nucleus which in some cases continued intermittently along a neurite. No immunostaining of glial cells was seen. Within the perinuclear region there was colocalisation with a marker for the TGN, TGN38. Western blot analysis confirmed an increase in TRPC6 levels during culture up to three weeks. These results demonstrate a neuron-specific distribution of TRPC6 and suggest it may be transported down neurites in TGN-related organelles.

08.09

EXTRACELLULAR MATRIX EFFECTS ON ASTROCYTE PHENOTYPE: IMPLICATIONS FOR BRAIN SCARRING

J.E.Holley and N.J. Gutowski.
Institute of Biomedical and Clinical Sciences, Peninsula Medical School (Exeter) and Department of Neurology, Royal Devon and Exeter Hospital.

Astrocytes normally exist in a quiescent state but become activated subsequent to injury and extracellular matrix disturbance. Following various types of brain injury, astrocytes form scar tissue, which inhibits repair. In vivo human cerebral white matter scar astrocytes (SA) express the proteins embryonic neural cell adhesion molecule, epidermal growth factor receptor, and basic fibroblast growth factor whereas quiet astrocytes do not. We wish to define factors that produce a human SA phenotype thereby finding mechanisms that inhibit scarring and aid repair. An in vitro model would be beneficial but demands a baseline quiescent astrocyte phenotype. Human astrocytes in vitro, plated on the extracellular matrix poly-L-lysine, in medium that contains serum display features of activation. Serum-free chemically defined medium however induces only a partially quiescent phenotype. The influence of the culture matrix on astrocyte phenotype was assessed using fibronectin, tenascin C, laminin, vitronectin, and collagen 1V, which are found in the human brain. Changing the extracellular matrix on which astrocytes were cultured produced striking differences in phenotype expression. This has allowed us to closely mimic the phenotype of normal quiescent astrocytes in vivo, therefore establishing a viable model for normal human quiescent astrocytes in vitro.

09.01

WHOLE GENOME ANALYSIS OF REPRESSOR ELEMENT-1 SILENCING TRANSCRIPTION FACTOR (REST) BINDING SITES REVEALS NOVEL NEURONAL TARGET GENES

Alexander W. Bruce1, Ian J. Donaldson1, Ian C. Wood1, Sally A. Yerbury1, Michael I. Sadowski1and Noel J. Buckley 1,2
1School of Biochemistry and Molecular Biology, 2School of Biomedical Sciences, University of Leeds, LS29JT, UK.

Specific patterns of gene expression are executed by a combination of transcriptional programs of gene activation and repression. REST is a Krüppel type zinc finger repressor that has been proposed to silence a battery of neuron-specific genes in non-neural tissue and neuroepithelium. To date, around 30 potential target genes have been identified. In the present study, we have carried out a genome-wide search of human, mouse and Fugu genomes to comprehensively identify REST binding sites and their corresponding target genes. We have compiled this information into a searchable database. Using this database, we have identified potential REST binding sites proximal to annotated putative target genes. Further, we have validated many of these putative targets using in vitro and in vivo DNA / protein interaction assays and have correlated these with functional studies using over-expression of wild type and mutant REST constructs. This study represents the first genome-wide analysis of the neuronal target genes of a transcription factor and forms a basis on which to examine the transcriptional programs executed by REST in different cell types.

09.02

INTERACTION OF RE1 SILENCING TRANSCRIPTION FACTOR (REST) WITH NEURONAL TARGET GENES

Nikolai D. Belyaev1, Ian C. Wood1, Alexander W. Bruce1, Miyoko Street1, Jean-Baptiste Trinh1 and Noel J. Buckley 1,2
1School of Biochemistry and Molecular Biology, 2School of Biomedical Sciences, University of Leeds, LS29JT, UK.

Establishment of neuronal identity requires co-ordinated expression of specific batteries of genes. These programs of gene expression are executed by activation of neuron-specific genes in neuronal cells and their repression in non-neuronal cells. The RE1 silencing transcription factor (REST) is a zinc finger repressor that silences transcription of numerous neuron-specific genes in non-neuronal cells via recruitment of two independent histone deacetylase (HDAC) containing co-repressor complexes. However, in vivo, REST appears to be an obligate silencer for only a minority of RE1-bearing genes. We have examined the interaction of REST, Co-REST, Sin3A, HDAC1 and HDAC2 with two archetypical endogenous target genes, the silent M4 muscarinic receptor and the active sodium type II channel (NaV1.2) genes in JTC19 fibroblasts. Using chromatin immunoprecipitation, we show that REST is absent at the endogenous M4 gene but is present at the endogenous NaV1.2 gene where it recruits Sin3 and Co-REST but not HDAC1/2. In contrast, both M4 and NaV1.2 reporter genes interact with REST, Sin3 and Co-REST but only the M4 gene recruits HDAC1/2. We conclude (1) that REST interacts with only a fraction of its target genes in vivo (2) REST represses active genes (3) REST recruits distinct co-repressor complexes to different target genes.

09.03

GABA-A RECEPTOR ACTIVATION INCREASES INTRACELLULAR Ca^{2+} PRIMARILY VIA L-TYPE VOLTAGE-GATED Ca^{2+} CHANNELS IN CULTURED RAT CEREBELLAR GRANULE NEURONES

Robert B. Liversage, Leigh D. Plant, Hugh A. Pearson, David J. Beech and Alan N. Bateson.
School of Biomedical Sciences, University of Leeds, Leeds, UK, LS2 9JT.

GABA-A receptor gene expression is altered upon long-term receptor activation by an unknown mechanism. Intracellular Ca2+, a regulator of gene expression, is raised in developing neurones when GABA-A receptors are excitatory. Rats were killed by cervical dislocation using Schedule 1 procedures and cerebellar granule neurones cultured for 3 day in vitro. Intracellular Ca^{2+} was measured by fluorescence microscopy with fura-PE3.
Muscimol and GABA caused a rapid, transient increase in intracellular Ca^{2+} that was blocked by picrotoxin and gabazine. The GABA-B receptor agonist baclofen had no effect. Furosemide, an antagonist at α6 subunit-containing GABA-A receptors, had no effect upon the muscimol-induced Ca^{2+} response, while flurazepam potentiated this response. This suggests that the Ca^{2+} increases were due to activation of GABA-A receptors containing α1 and γ2 subunits.
Ca^{2+} influx was primarily via L-type voltage-gated Ca^{2+} channels, as demonstrated by the ability of Ca^{2+}-free or Cd^{3+}-containing bath solutions to abolish this effect. Further, the L-type Ca^{2+} channel blockers nifedipine and D600 also attenuated muscimol-induced Ca^{2+} entry. These results and the established role of Ca^{2+} in regulating gene expression suggest a possible pathway for GABA-A receptor activation to modify its own gene expression in immature neurones.

09.04

METABOTROPIC GLUTAMATE RECEPTORS IN *C.ELEGANS*

Vincent O`Connor, James Dillon, Ben Pilkington, Alan Cook, James Wilson and Lindy Holden-Dye
School of Biological Sciences, Bassett Crescent East, Southampton SO16 7PX.

Glutamate co-ordinates animal behavior by binding to evolutionarily-conserved ionotropic (iGluR) or metabotropic (mGluR) receptors. The C.elegans genome project reveals both classes of glutamate receptor exist in this model organism. The tractability of C.elegans has provided important insight into ionotropic glutamate receptor function. However, less is known about the role of mGluRs in C.elegans (CemGluRs). In silico analysis identifies 3 CemGluR genes and preliminary analysispredicts these receptors display the archetypal mGluR structure, encompassing an extracellular N-terminal domain, 7 transmembranous helices and an intracellular C-terminal tail with distinct splicing variants. Such splicing suggests the C-terminal is important for organisation (scaffolding) of CemGluRs function. Our analysis of existing transgenic worms expressing mGluR-GFP fusion proteins(Ishiharaet al., Worm Breeders Gazette 14. 40)has shown potential scaffolding of these receptors within distinct populations of neurons. Furthermore the reversible dose dependent inhibition of pharyngeal action potential frequency by mGluR agonists (trans-ACPD 100uM) implies glutamate receptors contribute to networks that regulate feeding.We are using a yeast two hybrid method to identify CemGluR interacting proteins and developing assays to provide insight into how interacting proteins contribute to receptor function.

09.05

ADENOVIRAL DELIVERY OF HAMMERHEAD RIBOZYMES AS A TOOL TO STUDY NEURONAL GENE FUNCTION

Ioannis Bantounas*, Colin P.J. Glover*, Leonidas A. Phyalctou+, James B. Uney*
**Molecular Neurobiology Laboratories, MRC Centre for Synaptic Plasticity and University Research Centre for Neuroendocrinology, University of Bristol, Bristol BS2 8HW, UK. & The Cyprus Institute of Neurology and Genetics, 6 International Airport Avenue. P.O. Box 3462. 1683. Nicosia, Cyprus.*

In this study we aimed to assess the utility of ribozyme technology when targeted to both exogenous and endogenous RNA targets within post-mitotic neurons. An anti-luciferase and an anti-Caspase-3 ribozyme were designed and adenoviral (Ad) vectors expressing both the active and an inactive form of each were constructed. The Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) enhancer was also cloned 3' to the luciferase ribozyme transcripts. When primary hippocampal cultures expressing luciferase were transfected with the anti-luciferase ribozyme, a significant, viral titre-dependent reduction of luciferase expression was observed (whereas the inactive ribozyme had no effect). The addition of the WPRE enhancer was found not to alter ribozyme activity. Finally, Ad-mediated expression of the anti-Caspase-3 ribozyme reduced Caspase-3 activity in HeLa cells and neurones. The results of this study suggest that ribozymes are active in neuronal cells when delivered by viral vectors and their use will facilitate studies of neuronal gene function.

09.06

HETEROPHILIC CIS INTERACTIONS REGULATE THE STABILITY OF TRANS BINDING BETWEEN IgLONs IN NEURONS

James Reed, Christine McNamee & Diana Moss
Department of Human Anatomy & Cell Biology, The Sherrington Buildings, University of Liverpool, Ashton Street, Liverpool L69 3GE, UK.

Axons are guided through the developing embryo by a variety of molecular cues that enable them to accurately form synapses with their appropriate targets. The IgLON family proteins, LAMP, OBCAM, CEPU-1/neurotrimin and neurotractin/Kilon, are cell adhesion molecules thought to play a role in this process. Two or more IgLONs can be co-expressed on the same cell, and IgLONs can interact both homo- and heterophilically, forming multimeric complexes in cis (within the membrane of the same cell) and in trans (between separate cells). Antibody staining, cell adhesion and biosensor assays have been used to characterise the relative affinities of the homo- and heterophilic IgLON interactions in trans. This has suggested that IgLONs bind with different affinities, with homophilic LAMP interactions being weakest. Cerebellar granule neurons express all three IgLONs on their surface, yet surprisingly, will not bind to dimeric LAMP-Fc. The differing affinities observed for interactions in trans do not explain this, since LAMP binds with higher affinity to CEPU and OBCAM. We hypothesise that heterophilic IgLON dimers in cis govern the stability of interactions in trans between IgLONs, and we are currently generating doubly-transfected cell lines to test this. A putative hierarchy for these interactions will be presented to explain the differential adhesion of IgLONs to neurons.

09.07

THE PRODUCTION OF SOLUBLE DOMAINS OF GABA-B-R1a N-TERMINUS AS PROBES TO STUDY THE INTERACTION PROPERTIES OF THE GABA-B RECEPTOR

R. L. Ginham*, J. H. White^, R. A. J. McIlhinney*
** MRC Anatomical Neuropharmacology Unit, Oxford, U.K.^ Pathway Discovery, GlaxoSmithKline Research Centre, Stevenage, UK.*

Gamma-aminobutyric acid type B (GABAb) receptors mediate the metabotropic actions of the inhibitory neurotransmitter GABA. These seven-transmembrane receptors are known to signal primarily through activation of G proteins to modulate the action of ion channels or second messengers. The functional receptor is a heterodimer consisting of two subunits, GABAb-R1 and GABAb-R2. There are multiple splice variants of GABAb-R1; these include R1a and R1b that are identical except for an extracellular N-terminal extension in R1a that encodes a tandem pair of CCP modules, which may engage in specific protein:protein interactions. Such interactions may have important roles in GABAb receptor biology, such as receptor clustering, trafficking and pharmacology. Previously, using a yeast-two hybrid screen that used the CCP modules as bait, we identified five variants of Fibulin, an extracellular matrix protein, as interactors. In order to further characterise the interactions of the CCP modules we have investigated the possibility of producing secreted, soluble epitope and reporter tagged domains of the N-terminus of GABAb-R1a. These have included the full N-terminus of GABAb-R1a and the CCP modules alone, and the characterisation of these soluble domains will be described.

09.08

USING THE YEAST-2-HYBRID (Y2H) APPROACH TO BEGIN TO CONSTRUCT PROTEOMIC MAPS OF STARGAZIN- AND GABA-A RECEPTOR-INTERACTING MULTIPROTEIN COMPLEXES

J. H. Ives, S. Fung, C. L. Thompson.
School of Biological and Biomedical Sciences, University of Durham, South Road, Durham, DH1 3LE, UK.

The epileptic and ataxic mutant mouse, stargazer fails to translate the product of the CACNgamma2 gene, ‘stargazin’. Stargazin is directly involved in the cell surface trafficking and synaptic targeting of AMPA receptors, the latter occurs through a putative interaction of its extreme C-terminus (-TTPV) with the PDZ-domain of the MAGUK, PSD-95. Studies conducted in our lab revealed that expression, assembly and/or cell surface trafficking of specific GABA-A receptors (GABARs), especially the extrasynaptic, delta-containing, tonic inhibition-conferring subtypes, were also compromised in stargazer neurones. Using Y2H we aim to construct proteomic maps of stargazin- and GABAR subunit-associating proteins and ultimately elucidate the mechanism(s) by which stargazin mediates neurotransmitter receptor assembly, trafficking and targeting.A construct representing the C-terminal 121 amino acids of stargazin was used as our initial ‘bait’ with which to fish for interacting proteins from a mouse brain cDNA library. Bait interacted with a number of PDZ-domain containing proteins e.g. SAP97 and SAP102 which had been previously identified but also novel interactors such as PIST/GOPC, activin receptor interacting protein 2, as well as MAP1A and MAP1B. We are presently testing which of these interactions occur in vivo and elucidating their functional significance.

Funded by the BBSRC.

09.09

SELECTIVE EXPRESSION AND TRANSPORT OF ADENYLYL CYCLASE ISOTYPES TO AXON TERMINALS IN THE NEUROHYPOPHYSIS

F A Antoni*, G B Makara$, A A. Sosunov*, J Black*,
G Dayanithi_, J Simpson
**Division of Neuroscience, University of Edinburgh, Edinburgh EH8 9JZ, Scotland, U.K., $ Institute of Experimental Medicine, Budapest, Hungary, _ U 432-INSERM; CC 89, Univ. of Montpellier II, Montpellier, France.*

The biological importance of the structural and functional diversity of adenylyl cyclases is still largely unexplored. The present study shows that the cell bodies of magnocellular oxytocinergic neurons in the hypothalamic supraoptic and paraventricular nuclei of rats express a protein kinase C-activable adenylyl cyclase (ACII) as well as a calcium/calcineurin inhibited adenylyl cyclase (ACIX). In contrast, the vasopressinergic neurons in these nuclei contain ACIX as well as the calcium-inhibited adenylyl cyclase ACVI. Axon terminals in the neurohypophysis were strongly immunopositive for ACII and ACVI. In contrast, ACIX was detected in pituicytes, but not in nerve endings. In neurosecretosomes, basal and forskolin-induced production of cyclic AMP could be enhanced by phorbol-di-butyrate ester, indicating activation of ACII. In sum, ACIX is in the somatodendritic compartment, whereas ACII and AC VI are transported along the axons. Thus the data show selective neuronal compartmentalisation of specified adenylyl cyclases that can generate cyclic AMP in the context of distinct physiological stimuli.

09.10

CLONING OF PUTATIVE CHLORIDE CHANNEL TOXIN cDNAs FROM THE INDIAN RED SCORPION

KA Newton, A Armugam, PN Strong and K. Jeyaseelan
Biomedical Sciences, Sheffield Hallam University, Sheffield S1 1WB.

Scorpion toxins have provided valuable tools for the pharmacological dissection of sodium and potassium currents. Toxin specificity and selectivity has helped to distinguish ion flow through many different potassium channel subtypes. In comparison, the identification of chloride currents has been hampered by the lack of suitable neurotoxins. Here we clone cDNAs encoding putative short insectotoxins (BtChl1 and BtChl2), believed to act on chloride channels, from the venom of the Indian Red Scorpion, Mesobuthus tamulus. These are cDNAs of approx 260 nucleotides, with 72.4% and 70.8% homology to the cDNA sequence of Bm12 (a chlorotoxin-like peptide from Buthus martensii). BtChl1 and BtChl2 respectively encode 59 and 62 amino acid precursors containing highly conserved 24 residue signal peptides, and 33-38 amino acid mature peptides. We have purified BtChl1 using ion exchange and reverse phase chromatography. Based on the predicted molecular mass of BtChl1 from the cDNA sequence, we have followed purification using MALDI-TOF spectrometry.

09.11

PROFILING GENE EXPRESSION CHANGES IN ACUTE AND CHRONIC MODELS OF EPILEPSY

1W L Peck, 1J Egerton , 2S Blakemore, 2R J Stephens, 2P S Hegde, 1S C Stratton, 1T Stean, 1A Wilson, 1K Brackenborough, 1L Winyard, 1J B Davis, 1P J Green.
1Neurology & GI Centre of Excellence for Drug Discovery and 2Genetics Research, GlaxoSmithKline Research & Development Ltd., NFSP-N, Third Avenue, Harlow, Essex, CM19 5AW.

We have used microarray analysis and RT-PCR, to profile transcriptional events in acute and chronic models of epilepsy, and identify genes that may underlie processes activated during epileptogenesis. Using an acute epilepsy model, rat electroshock, we examined by microarray, a time course of expression changes in three tissues:- hippocampus, cortex and brainstem. Expression changes were confirmed by real-time RT-PCR, and then profiled in a second chronic model of epilepsy, the EL mouse.
Electroshock caused both up- and down-regulation of a large number of transcripts, with a greater number of significant changes seen in cortex and hippocampus than brainstem. BDNF , c-fos, and vesl were selected from our results for confirmation in both models on the basis that they had been shown previously to be regulated in other models of epilepsy, e.g. kainate. All three genes were regulated in hippocampus and cortex in the electroshock model, but none were shown to have significantly altered expression in the EL mouse model. Although many genes were regulated in an acute model of epilepsy, few also show altered expression in a chronic model. Genes regulated in both may be more robust targets for antiepileptic therapy.

09.12

EXPRESSION OF NEUROPILIN 2 PROTEIN IN ADULT RAT NERVOUS SYSTEM

M. Agudo 1; M. Robinson 2; S.P. Hunt 2; S.B. McMahon 1*
1 Sensory Function, Guy`s Campus, King`s College London, LONDON,. 2. Anatomy and Developmental Biology, Medawar Building, University College London, London.

After a CNS injury the damaged axons are unable to re-grow to reach their targets and to re-establish the lost functionality. One group of proteins potentially involved in this inhibition is the semaphorin/neuropilin family of axonal guidance molecules. Class 3 secreted semaphorins (sema3) potently cause axonal growth-collapse, acting through neuropilin (NP) receptors.
NP2 is a receptor member of this family. In human and mouse 6 different splicing isoforms of NP2 have been described and divided into two types NP2(a) and NP2(b). Thus, NP2(a) type is formed by 4 members which differ in the insertion of 0, 5, 17 or 22 (5+17) aminoacids (aa'),corresponding to the alternative spliced exon 15. NP2(b) form has two members characterized so far, NP2b(0) and NP2b (5) which differ in a 5 aa' insertion codified by the exon 16b.We wondered if these variants were also present in the adult rat nervous system, and if so, which NP2 isoform was expressed in dorsal root ganglia (DRG), spinal cord (SC) and other CNS structures. To answer this question, we performed western blots in tissue collected from adult rats. This previous analysis showed that in spinal cord there is a predominance of NP2a isoforms whereas in DRG both NP2a and 2b are present.
This differential expression of NP2 in SC vs DRG could imply a specific role of each isoform in different regions of the nervous system.

09.13

REGULATION OF VOLTAGE-GATED POTASSIUM CHANNEL INTERNALISATION BY THE CARBOXY TERMINUS

Kennedy, L., Callan, A.C., Jugloff, D.G.M and Jones, O.T.
School of Biological Sciences, University of Manchester, 1.124 Stopford Building, Oxford Road, Manchester, M13 9PT.

The surface densities and distributions of voltage-gated potassium channels (KChs) are primary determinants of the integrative electrophysiological properties of neurones. Unfortunately, the trafficking mechanisms that specify the patterns of KCh surface expression are poorly understood. Growing evidence suggests a key role for internalisation mechanisms. However, the nature and location of the requisite endocytic motifs in KChs are unknown. Most internalisation motifs in membrane proteins lie in structurally divergent, cytoplasmic, domains. Thus, we hypothesize the existence of internalisation motifs within the carboxy terminus of KChs. Using rat Kv1.4 as a model KCh, together with chimera and mutants carrying deletions within the carboxy terminus, we have identified a critical region (residues 608-617) controlling Kv1.4 internalisation. To examine the autonomy of this region, the internalisation and expression levels of a novel reporter protein, PIN-G, expressing varying lengths of Kv1.4 carboxy terminus, was also investigated and these parameters compared and contrasted with corresponding mutations in Kv1.4. Surprisingly, the region we have identified lacks a canonical internalisation signal but contains a sequence homologous to a novel motif implicated in ubiquitination of the growth hormone receptor. We, gratefully, acknowledge support from the BBSRC.

09.14

CONTROL OF VOLTAGE-GATED POTASSIUM CHANNEL INTERNALISATION BY A PUTATIVE UbE MOTIF

Callan, A.C., Kennedy, L., Jugloff, D.G.M. and Jones, O.T.
Sch. Biol. Sci., Univ. Manchester, 1.124 Stopford Building, Oxford Rd., Manchester M13 9PT.

Control of the cell surface residence time of membrane proteins, such as voltage-gated potassium channels (KChs), is implicit in their targeting to discrete compartments within neurones and other polarised cells. Unfortunately, the underlying mechanisms are poorly defined. At least one determinant appears to lie within the carboxy terminus of Kv1.4 KChs. Based on sequence homology with growth hormone receptors, we hypothesize that this region consists of a motif –termed UbE- that controls ubiquitin-mediated internalisation. Using site-directed mutagenesis, and total and cell surface expression assays we now show that the putative UbE motif does indeed affect the surface density of Kv1.4 in transfected HEK293 and COS-7 cells. Mutants lacking the UbE motif internalise more slowly than wild-type Kv1.4 (1st order rate constant k=0.005 vs. 0.008/min, respectively). Parallel studies, including the use of a novel reporter construct, indicate the UbE region also controls surface and total expression levels and highlight a central role for a conserved tyrosine residue within the UbE motif. Together, these data suggest a novel mechanism by which KChs and other membrane proteins may be (de)stabilised and, thereby, concentrated in discrete areas of the nerve cell surface.
We, gratefully, acknowledge support from the BBSRC.

09.15

CALCIUM CHANNEL SUBUNITS IN ADULT RAT CEREBELLAR PURKINJE CELLS: A SINGLE-CELL RT-PCR STUDY

S. Kanumilli, E.W. Tringham, C.E. Payne and M.M. Usowicz.
Dept. Pharmacology, University of Bristol, Bristol BS8 1TD.

Pharmacological studies have demonstrated that P-type channels are the dominant type of calcium channel expressed in cerebellar Purkinje neurons. However, the full compliment of subunits making up these channels is unclear. Here we report the expression of multiple mRNAs encoding different subunits in individual Purkinje cells.
Cerebellar slices (250 microns) were made from young adult rats (>40 days, male Wistar, sacrificed in accordance with the Animals (Scientific Procedures) Act 1986). mRNA harvested from individual Purkinje cells was reverse transcribed into cDNA and subsequently amplified with subunit-specific primers using two rounds of PCR.
Analysis of the PCR products confirmed the expression of the alpha1Aa splice variant of Cav2.1 (88% cells) (Bourinet et al., Nat. Neurosci. 2:407, 1999). It also revealed the expression of other Cav2.1 splice variants: alpha1Ab (28% cells), alpha1Ac (19% cells); and two variants previously described in rat pancreatic islet cells: AF051526 (73% cells) and AF051527 (23% cells) (Ligon et al., J. Biol. Chem. 273:13905, 1998). mRNAs for beta2 (40% cells), beta3 (27% cells), beta4 (50% cells), alpha2delta2 (100% cells), and multiple gamma subunits were also detected.
In summary, individual cerebellar Purkinje cells express several variants of multiple classes of calcium channel subunits.

Funded by the MRC

09.16

SIGNALLING PATHWAYS CONTRIBUTING TO c-fos ACTIVATION IN GLIAL CELLS

Ylva Edling, Anastasia Simi, Magnus Ingelman-Sundberg and Niclas Tindberg
National Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

A great variety of stimuli activate c-fos in both neurons and glia. We have attempted a detailed analysis of the cis-acting elements, transcription factors and upstream kinase pathways involved in the activation of c-fos in rat cortical glial cells following glutamate or lipopolysaccharide (LPS) stimulation. We have used several experimental approaches: (1) evaluating the effect of kinase inhibitors on LPS or glutamate-induced c-fos mRNA levels, (2) testing the inducibility of SRE or CRE-driven luciferase reporters in transient transfection assays, (3) introducing mutations/deletions at the different cis-acting elements of the rat c-fos proximal promoter in luciferase reporters, (4) investigating the activation of Elk-1 and CREB as GAL4-fusion proteins in cells co-transfected with a GAL-4-driven luciferase reporter, (5) assessing the phosphorylation status of Elk-1 and CREB / ATF-1 in glial cell extracts using phospho-specific antibodies, (6) assessing DNA binding of DREAM at the c-fos DRE in gel shift assays. We have found that c-fos activation by LPS requires a co-operative activation of CRE and SRE elements by a mechanism dependent on p38 MAP kinase pathways, whereas glutamate, unlike in neuronal cells, seems to act through de-repression of c-fos transcription at the c-fos DRE element.

09.17

IDENTIFICATION OF THE CALCIUM-ACTIVATED CHLORIDE CHANNEL (mCLCA-3) IN THE ADULT MOUSE BRAIN

Fiona C Shenton, John P Winpenny and Paul L Chazot
Sunderland Pharmacy School, University of Sunderland, Sunderland, SR1 3SD, UK.

We have previously reported the distribution of the mCLCA-3 in many mouse peripheral secretory cell types [1]. However, very little is known about the role of calcium-activated chloride (CLCA) channels in the mammalian brain. Here we provide the first evidence for the existence of the mCLCA-3 channel in the adult mouse brain. RT-PCR (35-cycles), using selective mCLCA-3 oligonucleotide primers, identified a robust mRNA species for the mCLCA-3 in both adult mouse forebrain and cerebellum. Our novel affinity-purified anti-mCLCA-3 (KLETFKNAD) antibody was used to map the cellular distribution of the mCLCA-3 channel protein in the adult murine brain using a standard immunohistochemical technique [2]. Widespread neuronal labelling was detected in many key structures within the brain, including the hippocampal formation, striatum, with modest cortical and cerebellar staining. Interestingly, we observed colocalisation of mCLCA-3 in some, but not all, GFAP-positive cells. These findings suggest that the mCLCA-3 channel may have a role in distinct populations of both neurons and glia in the mammalian CNS.
1. Shenton FC et al. (2002) Pediatr. Pulmon. (Suppl.) 24, 243.
2. Thompson, CL et al. (2002) Molecular Brain Res. 102, 55-61.

We thank the Wellcome Trust (Grant No. 059897, JPW, PLC) for funding these studies.

09.18

EXPRESSION OF HIPPOCALCIN, A CALCIUM BINDING PROTEIN, UNDER ISCHAEMIC CONDITIONS

Chapman,D.M., Crook, B.W., Lawman, A.J., Campbell, C.A., Hadingham, S.J., Vinson, M., Collier, S., Irving, E.A., Burbidge, S.A.
Neurology Centres of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AW.

Hippocalcin is part of the Neuronal Calcium Sensor Family (NCS), members of which are thought to function as calcium buffers thereby protecting neurones from ischaemic conditions and excitotoxic cell death. Hippocalcin is exclusively expressed in the CNS and has been reported to be predominantly found in the pyramidal cells of the CA1 region of the hippocampus. However the exact function of hippocalcin within the CNS still needs to be determined.We have shown that hippocalcin gene and protein expression is decreased in the ischaemic cortex of rats following transient middle cerebral artery occlusion (MCAO). Gene expression shows a four-fold depletion 1-week post-MCAO, whereas hippocalcin protein expression reaches a two-fold depletion 2-weeks post-MCAO. Immunohistochemical studies detected areas of reduced hippocalcin staining predominantly within the cortex, striatum and the substantia nigra. Changes were also detected in the hippocampus of the ipsilateral hemisphere from 24h-8 weeks following MCAO.The loss of hippocalcin following stroke could leave cells susceptible to glutamate toxicity and therefore contribute to death in the ischaemic regions.

09.19

RESIDUES WITHIN THE MYRISTOYLATION MOTIF DETERMINE INTRACELLULAR TARGETS FOR NEURONAL CALCIUM SENSOR PROTEINS (NCS)

O`Callaghan DW, Hasdemir B, Burgoyne RD.
Department of Physiology, University of Liverpool, Crown St., Liverpool, L69 3BX, UK.

Using GFP-variant fusion proteins expressed in HeLa cells, we have explored the differentially targeting of neuronal calcium sensor (NCS) proteins to specific intracellular membranes. KChIP1-EYFP was targeted to punctate structures, identified as endosomal, irrespective of calcium concentration. This was distinct from the localisation of NCS1 and hippocalcin on the trans-Golgi network (TGN) and plasma membrane. The membrane localisation of each protein required myristoylation and minimal myristoylation motifs of hippocalcin or KChIP1 were sufficient for this targeting. Residues at positions 3, 7 and 9 were found to determine this intracellular localisation. In cells co-expressing ECFP-Kv4.2 and KChIP1-EYFP, the two proteins were co-localised, including on the plasma membrane. Expression of KChIP1(1-11)-EYFP resulted in the formation of enlarged endosomes and redirected ECFP-Kv4.2 to these structures suggesting that the Kv4.2 channels traffic through the endosomal pathway. Our findings provide insights into how the same myristoyl anchor can be used by proteins in combination with nearby amino acids to locate to distinct intracellular sites and demonstrate a mechanism by which the NCS proteins may be able to sense distinctly localised calcium signals and act as organelle-specific calcium sensors that could contribute to the control of membrane traffic.

09.20

INVESTIGATIONS INTO THE CYTOPLASMIC INTERACTIONS OF NrCAM

M. Hill, F. Davey & F. Gunn-Moore
School of Biology, University of St Andrews, St Andrews, KY16 9TS.

Neuron glial related cell adhesion molecule (NrCAM) belongs to the L1 receptor family of cell adhesion molecules (L1CAMs). NrCAM is found at the node of Ranvier, initial segments and growth cones. It promotes axonal growth in neurons, mediates neuron-glial interactions and is important in CNS and PNS development. The NrCAM cytoplasmic cell signalling mechanisms underlying these functions are poorly understood. Within the cytoplasmic domain there is a five amino-acid sequence; F-I-G-Q-Y, which is highly conserved in other L1 family members. The tyrosine within this sequence can be phosphorylated. The only known intracellular interaction of NrCAM is the binding of ankyrins which link NrCAM to the cytoskeleton; an interaction which is abolished following phosphorylation of the conserved tyrosine. Other L1CAMs bind FERM (4.1 Ezrin Radixin Moesin) proteins and this suggested potential binding candidates for NrCAM. We used the yeast two hybrid technique to:
1) Show the cytoplasmic terminus of NrCAM does not bind ezrin, radixin or another novel FERM protein.
2) Identify new potential cytoplasmic binding partners of NrCAM.

10.01

NEURAL CONTROL OF GFAP EXPRESSION IN TERMINAL SCHWANN CELLS AT RAT NEUROMUSCULAR JUNCTIONS

A.E.T. Finlayson, K.K. Pang, C.R. Slater
School of Neurology, Neurobiology & Psychiatry, University of Newcastle upon Tyne.

The 'terminal Schwann cells' (TSCs) that cap the unmyelinated motor axon terminal at mammalian neuromuscular junctions (NMJs) respond to complete or partial denervation by extending sprouts away from the denervated NMJs. These sprouts promote reinnervation by supporting the outgrowth of surviving axons. The events that underlie sprout formation by TSCs are poorly understood. In frogs, where TSC sprouting takes several weeks to develop, the TSCs express little of the intermediate filament protein GFAP. However, that expression is rapidly (6hr) upregulated by nerve section or block of neuromuscular transmission (Georgiou et al., 1994, Neuron 12:443-455). We are using immunolabeling to study GFAP expression in rat soleus muscles, where TSC sprouting occurs within a few days of denervation (Reynolds & Woolf, 1993, J. Neurocytol. 21:50-66), to see how it is related to TSC sprouting in mammals.
We observe strong immunolabelling of GFAP in TSCs at normal rat NMJs, in contrast to frogs. However, within 24hr of denervation, this labelling has dispersed at many NMJs, and by 7d, at most of them. This dispersion of labelling may reflect loss of protein, depolymerisation of intermediate filaments, or both. In conclusion, in contrast to frogs, TSC sprouting is preceded by, and may depend on, dispersion of GFAP-positive intermediate filaments.

10.02

ROLE OF c-Jun N-TERMINAL KINASE (JNK) IN MEDIATING ASTROCYTIC DEATH

Emmanouil S. Papadakis and Cathy Tournier
G38 Stopford building, The University of Manchester, Oxford Road, Manchester, M13 9PT

C-Jun N-terminal kinase (JNK) is a mitogen-activated protein kinase (MAPK) that is required for mediating apoptosis following brain injury. Based on this, the aim of this study is to characterise the role of JNK in regulating astrocytic death. Astrocytes constitute the majority of the cells in the brain. Results show that cadmium chloride (CdCl2) and UV radiation are strong activators of JNK and potent inducers of astrocytic death. However, in contrast to UV, CdCl2 induces death via a caspase-3 independent mechanism. To further identify how CdCl2 induces astrocytic death we are currently investigating its effect on cytochrome c and apoptosis-inducing factor (AIF) release from mitochondria. In addition, our results demonstrate that the compound SP600125 (25 μ) selectively inhibits JNK activity by approximately 60%, but has no effect on the activity of p38 MAPK or extracellular signal-regulated protein kinase (ERK) in astrocytes. Future studies will focus on testing the potential of SP600125 to inhibit astrocytic death as well as cytochrome c and/or AIF release following UV and CdCl2 treatment.

10.03

METABOTROPIC GLUTAMATE RECEPTORS ARE DEVELOPMENTALLY EXPRESSED BY CELLS OF THE OLIGODENDROGLIAL LINEAGE

Luyt K, Varadi A#, Molnar E
MRC Centre for Synaptic Plasticity, Departments of Anatomy and Biochemistry#, University of Bristol BS8 1TD, UK.

Oligodendroglial cells are involved in myelination and also targeted in multiple sclerosis and periventricular leukomalacia. We recently reported that cultured rodent oligodendroglial progenitors (OPCs) express functional mGluR3 and mGluR5a isoforms and that mGluR5 is also present in situ in developing rat brain oligodendrocytes [Luyt et al. (2003) J. Neurochem. 84, (in press)]. While the developmental expression pattern of mGluR3 and mGluR5 is relatively well established in neurons and astrocytes, similar information is not available for the oligodendroglial lineage. Our current study focuses on the developmental expression pattern of mGluR3 and mGluR5 in cells of the oligodendroglial lineage. We characterised the developmental changes in mGluR3 and mGluR5 mRNA expression in CG-4 OPCs, oligodendrocytes and type-2 astrocytes by using quantitative PCR with isoform specific primers as well as immunoblots and immunocytochemistry with mGluR2/3 and mGluR5 antibodies. The mGluR3 mRNA expression level increased by 3-5 fold during development from OPCs to differentiated oligodendrocytes. Antibodies to mGluR2/3 and mGluR5 detected the corresponding receptor proteins in immunoblots of OPC, oligodendrocyte and type-2 astrocyte membrane fractions. Functional assessment of mGluR5, by fura-2 microfluorometry in the presence of DHPG revealed calcium oscillations at all differentiation stages.

10.04

THE TRANSCRIPTOME SIGNATURE OF MICROGLIA FOLLOWING STIMULATION WITH INTERFERON-GAMMA

D.C. Duke, L.B. Moran L. R. Banati, F.E. Turkheimer, M.B. Graeber
Department of Neuropathology, Division of Neuroscience and Psychological Medicine, Imperial College Faculty of Medicine, Charing Cross Campus, Fulham Palace Road, London, W6 8RF, UK.

Microglia are resident macrophage precursor cells of the CNS. The normal microglial phenotype changes in response to pathological stimuli, and the activated cells can become full-blown phagocytes. Here we report on the gene expression profile of normal and interferon-gamma (IFN-g) treated microglia in vitro. Affymetrix RG_U34A microarrays were used to compare unstimulated (n=5) and treated (IFN-g 100 U/ml, n=5) rat primary microglia cell cultures of >95% purity derived from newborn Lewis rat brains. More than 7,000 known gene sequences and 1,000 EST clusters were examined. Statistical analysis of differences in gene expression was performed using the Wilcoxon rank-sum test ($P<0.05$), and a rank test on the number of present/absent calls ($p<0.05$) with one additional constraint (expression ratio >2). Normal microglia showed a baseline expression profile comprising 326 genes of those tested (182 known genes and 144 ESTs), which were found in all experiments. 34 genes and 9 ESTs were expressed de novo in stimulated cultures, with the majority being pro-inflammatory in nature. The latter included genes involved in MHC-mediated antigen presentation and cytokine release. 10 Genes were down-regulated following stimulation. These data provide a transcriptomic definition of normal microglia in vitro and a molecular signature of these cells under the influence of IFN-gamma.

10.05

MECHANISMS UNDERLYING OLIGODENDROCYTE CELL DEATH *IN VITRO*

Claudia Rosin, Timothy E. Bates* and Stephen D. Skaper
*Neurology & GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Research & Development Ltd, Harlow CM19 5AW, and *School of Biomedical Sciences, University of Nottingham Medical School, Queen`s Medical Centre, Nottingham NG7 2UH,* UK.

Oligodendroglia are an important cell type of the CNS and myelinate axons. Oxidative toxicity can be induced by glutamate treatment in cells that lack ionotropic glutamate receptors, including oligodendroglia. Differentiated oligodendrocytes from rat were highly vulnerable to glutamate-induced cell death. Competitive inhibition of cystine uptake and increased oxidative stress appeared responsible for this death. Glutamate receptor subtype agonists which inhibit cystine uptake were cytotoxic, but not NMDA itself; moreover, glutamate receptor antagonists were not protective. Oligodendrocytes were vulnerable also to overactivation of glutamate receptors, as kainic acid and AMPA proved to be gliotoxic. AMPA toxicity required the presence of cyclothiazide, indicating a role for AMPA receptor desensitisation. Both glutamate-induced oxidative stress and kainate/AMPA receptors triggered mitogen-activated protein kinase pathway activation, and downstream (transcription) modulators (ELK, ATF-2). However, mitogen-activated protein kinase kinase inhibitors only protected against injury from glutamate-induced oxidative stress. Multiple signalling cascades can thus participate in oligodendrocyte cell death, raising the possibility that glutamate toxicity is operative in neuropathological conditions that disrupt neuronal/oligodendrocyte interactions in axons.

10.06

A NOVEL MECHANISM OF REGIONAL ASTROCYTE AND NEURONAL DEATH FOLLOWING INHIBITION OF GLYCOLYSIS

M Fowler, T Lister, C Nolan, T Bates, D Ray
MRC Applied Neuroscience Group, Biomedical Sciences, University of Nottingham, NG7 2UH.

Intraperitoneal injection of 3-chloropropanediol (140 mg/kg) initially causes astrocyte swelling at 4 hours post-dose in the inferior colliculi of rats. This is followed by selective astrocyte death in the period 4-24 hours post-dose. The active metabolite of 3-chloropropanediol is thought to be chlorolactaldehyde, which inhibits glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) activity. Inferior collicular GAPDH activity at 4 hours post-dose was decreased to 40 % ± 0.2 % (mean ± S.E; n = 4) of control values. However, at 24 hours, GAPDH activity was 80 % ± 3.4 % (mean ± S.E, n = 5) of control values. The initial pathological event in this model is death of astrocytes, where a large percentage of GAPDH activity is likely to be inhibited. Therefore it is probable that the surviving GAPDH activity present at 4 hours is primarily in other cell types such as neurons, and that these cell types are responsible for upregulating GAPDH activity at later time points by synthesis of new GAPDH protein. This proposal is consistent with previous studies, which demonstrate increases in enzyme activity following cellular stresses such as heat shock.

10.07

THE *IN VIVO* RESPONSE OF THE BRAIN VASCULATURE TO LOSS OF ASTROCYTES

A Brown, T Lister, G Mavroudis, C Nolan, M Prior*, D Ray, & C.Willis
*MRC Applied Neuroscience Group, Biomedical Sciences or * Physics, University of Nottingham.*

S-3-chloropropanediol (140 mg/kg i.p.) kills astrocytes in the inferior colliculi and other brainstem nuclei of rats over 2-24 hours. From 2 days none could be detected within damaged areas by glial fibrillary acidic protein or vimentin staining.
Inferior collicular blood flow showed no significant change until 24 hours, but by 48 hours rose to 2.68 ± 0.50 times predose (mean ± S.E., n = 9). Vessels also lost occludin staining and became permeable to 10 KDa dextran, these effects also peaking at 2 days. Extravasation of 0.5KDa gadolinium-DTPA was visualised by magnetic resonance imaging, and at 2 days in the inferior colliculus reached 61.3 ± 6.6 % (n = 10) of that seen in the open barrier pineal. By 9 days damaged areas no longer leaked dextran, and gadolinium enhancement had fallen to 42.5 ± 2.7% of its 2 day value. By 30 days enhancement was no longer significantly increased (n = 10), demonstrating effective barrier restoration in the absence of direct astrocytic contact.

10.08

REMYELINATION CAN BE EXTENSIVE IN MULTIPLE SCLEROSIS DESPITE A LONG DISEASE COURSE

Rickie Patani, Michelle Balaratnam, Dimitrios Papadopoulos, Annabella Polito, Richard Reynolds
Department of Neuroinflammation, Division of Neuroscience, Imperial College London, Charing Cross Campus, Fulham Palace Road, London W6 8RF.

Experimental models of MS indicate that rapid and extensive remyelination of demyelinated lesions is possible. Previous studies suggest that remyelination in MS is extensive in the early stages but eventually fails. Here we use an extensive sampling protocol to investigate the extent of remyelination in MS. Serial sections from a total of 185 tissue blocks from two MS brains (age 51 yrs; PM delay 8-15 hr; disease duration 21 yr) were stained with H&E, LFB/CFV and anti-MOG, HLA-DR, neurofilament and GFAP antibodies. Demyelinated areas were identified in 141 blocks, both white matter (WMLs) and/or grey matter lesions (GMLs). 168 WMLs were identified, 37 of which were shadow plaques (22% of all lesions). A further 123 were partially remyelinated (range 1-89% of lesion area, mean = 32% remyelination) and only 8 lesions were completely demyelinated. Smaller lesion size correlated significantly with more extensive remyelination ($p < 0.001$). The average extent of remyelination for all WMLs was 47%. The extent of inflammation in 51 WMLs was determined and an increased density of HLA-DR+ macrophages and microglia, both at lesion border and centre, was found to correlate significantly with more extensive remyelination ($p < 0.05$). Our results using a thorough sampling protocol suggest that remyelination in MS may be more extensive than previously thought.

10.09

THE RESPONSE OF NG-2 EXPRESSING OLIGODENDROCYTE PROGENITORS TO DEMYELINATION IN MOG-EAE AND MS

Annabella Polito, Dimitrios Papadopoulos, Mary Dawson, Joel Levine, Richard Reynolds
Department of Neuroinflammation, Division of Neuroscience, Imperial College London, Charing Cross Campus, London, W6 8RF.

Remyelination of demyelinated lesions is a common feature of experimental models of multiple sclerosis (MS) and is suggested to be the normal response to demyelination during the early stages of MS. The rapid response of OPCs to repopulate lesions and their differentiation into new oligodendrocytes is the key to rapid remyelination. Antibodies to the NG2 proteoglycan have proved useful in following the response of endogenous OPCs to demyelination. Here we provide new evidence on their response to the chronic inflammatory demyelinating environment seen in MOG-induced EAE in the DA rat. NG2+ OPCs responded to inflammatory demyelination in both white and grey matter by becoming reactive and increasing in number in a very focal manner. Evidence of NG2+ OPCs in lesioned areas beginning to express the oligodendrocyte marker CNP was also seen. The response of OPCs occured following successive relapses but did not always lead to remyelination, with areas of chronic demyelination observed. The presence of OPCs in the adult human CNS is clearly of vital importance for repair in MS. As in rat tissue, the antibody labeled an evenly distributed cell population in both white and grey matter. NG2+ cell numbers were highly variable in chronic lesions. OPCs apparently survived demyelination and exhibited a multi-processed or bipolar morphology in the hypocellular environment of the lesion.

10.10

OLFACTORY ENSHEATHING CELLS, SCHWANN CELLS AND CNS AXONAL INTERACTIONS *IN VITRO*

Annette Sorensen, Christine Thomson* and Susan C Barnett
*Beatson Labs, Depts of Neurology and Medical Oncology University of Glasgow, G61 1BD, *Applied Neurobiology Group, Glasgow University Veterinary School, G61 1QH.*

Olfactory ensheathing cells (OECs) affect transplant- mediated repair of the damaged CNS by remyelinating demyelinated axons as well as promoting axonal regeneration. However, their ability to interact with central axons requires further examination in vitro, and comparison made with that of Schwann cells (SC) or oligodendrocytes. In this study we cultured purified rat OECs or SCs, with embryonic spinal cord slices or spinal ganglia, to assess axon-glial interactions. In spinal cord slices cultured on a monolayer of astrocytes, endogenous oligodendrocytes myelinate axons. This myelination was compared to that seen when slices were cultured on a monolayer of either OECs or SC. OECs and SC promoted the outgrowth of oligodendrocyte precursor cells but were less efficient than astrocytes at supporting myelination by 3 weeks. Longer-term cultures are now in progress. Additionally, axonal outgrowth (number and length) was more vigorous on these cells compared to that observed on astrocytes. The ability of OECs and SC to support oligodendroglial growth is important, as they will encounter oligodendrocytes and their progenitor cells after CNS transplantation. Comparing co-cultures of spinal ganglia with OECs versus SC, identified that OECs appear to align and associate with axons in a similar manner to Schwann cells although there was no evidence of myelination.

11.01

NERVE FIBRES ARE REQUIRED TO EVOKE A CUTANEOUS DELAYED TYPE HYPERSENSITIVITY RESPONSE IN MICE

Lorna Beresford, Eric Bell* & Jaleel Miyan
*Department of Biomolecular Sciences, UMIST, P.O.Box 88, Manchester M60 1QD, UK.and *School of Biological Sciences, Manchester University, Oxford Road, Manchester M13 9PT, UK.*

The epidermis of skin contains abundant nerve fibres closely associated with Langerhans cells. The neurotransmitter in these nerve fibres, calcitonin gene-related peptide (CGRP) has been shown to inhibit antigen presentation by Langerhans cells in vitro. We have investigated whether these nerve endings are necessary for inducing and evoking a contact sensitivity response. Topical application of a general (phenol) or a CGRP-specific (capsaicin) neurotoxin was employed to destroy the nerve fibres at skin sites subsequently used to induce or evoke a cutaneous delayed-type hypersensitivity (DTH) response. Elimination of nerve fibres abolished both induction and effector stages of the specific DTH response. Denervation did not destroy the local Langerhans cells, which were observed in increased numbers, or prevent them from migrating to lymph nodes. The results suggested that nerve fibres are required to elicit a cutaneous DTH response in vivo and may be vital to the normal function of the immune system.

11.02

NEURONAL ACTIVATION IN THE CORTEX INDUCED BY THE CYTOKINE IL-1 IN RATS TREATED WITH THE EXCITOTOXIN AMPA

Patel HC, Heenan LE, Davies RE, Rothwell NJ & Allan SM
School of Biological Sciences, University of Manchester, Manchester, M13 9PT, UK.

Striatal co-injection of AMPA+IL-1 induces 'distant' cortical cell death as well as 'local' striatal death observed with AMPA alone. Co-infusion of AMPA+IL-1 also results in increased seizure activity which is reduced by diazepam, as is the cortical cell death. It remains unclear whether there is enhanced neuronal activation in cortical areas that undergo cell death after intrastriatal AMPA+IL-1 injection. The aim of this study therefore was to investigate the pathways and magnitude of neuronal activation seen in response to intrastriatal injection of AMPA+IL-1 vs AMPA.Male SD rats were anaesthetised and striatal injections of S-AMPA (7.5nmol) and S-AMPA+IL-1β (10ng) performed. Animals were perfused 4h and 8h after and c-fos immunohistochemistry performed on free-floating cryostat sections (30µm).c-fos expression was seen in similar regions in response to AMPA and AMPA+IL-1. The magnitude of c-fos expression was greatest in the neocortex in response to AMPA+IL-1 vs S-AMPA (4h; 69 +/- 19 vs 32 +/- 19 and 8h; 147 +/- 5 vs 44 +/- 2; n=4-6; $P<0.001$). These data suggest that co-infusion of AMPA+IL-1 induces greater neuronal activation in the neocortex than AMPA alone which ultimately leads to cell death.

This work was supported by the MRC.

11.03

EXPRESSION OF NEW PUTATIVE IL-1 RECEPTORS IN THE MOUSE BRAIN

Penglian Wang, Ralph Andre, Elisabet Berglöf, Nancy J. Rothwell and Emmanuel Pinteaux
School of Biological Sciences, University of Manchester, Manchester, UK.

Interleukin-1 (IL-1) is an important pro-inflammatory cytokine that mediates neuroinflammation and acute neurodegeneration by binding to the IL-1 type 1 receptor (IL-1RI) and the IL-1R accessory protein (IL-1RAcP). However the recent characterisation of putative, new IL-1 ligands (IL-1F5 to IL-1F10) and new IL-1 receptor-related molecules (IL-1R1 to IL-1R10) leads to the hypothesis that there might be alternative IL-1 signalling pathway(s) in brain.
The objective of the present study was to investigate expression of the new members of the IL-1 receptor family in cultures glial and neuronal cells from the mouse brain.RT-PCR analysis showed strong (IL-1R6) IL-1Rrp2 mRNA expression in mixed glial and microglia, which decreases significantly in response to bacterial lipopolysaccharide (LPS). IL-1R4 (T1/ST2) expression was observed in astrocytes and microglial cells treated with IL-1a and b. In contrast, constitutive expression of IL-1R4 was detected in O2A progenitor cells, which strongly decreased after LPS. IL-1R9, also known as TIGIRR-1, was also constitutively expressed in glial cells and expression increased after LPS treatment.
These preliminary results show that some of the new orphan IL-1 receptor-related molecules are expressed in the CNS, and their expression is modulated by LPS, suggesting therefore, their involvement in inflammatory processes of the brain.

11.04

DISTRIBUTION OF EXOGENOUSLY ADMINISTERED INTERLEUKIN-1 AND INDUCTION OF ENDOGENOUS INTERLEUKIN-1 IN THE RAT BRAIN

Heenan LE, Davies RE, Patel HC, Rothwell, NJ & Allan SM
School of Biological Sciences, 1.124 Stopford Building, University of Manchester, UK.

Injection of interleukin-1 (IL-1) in the rat brain exacerbates ischaemic and excitotoxic brain damage. IL-1 worsens excitotoxic injury when administered in the striatum or hypothalamus. IL-1 injections in a number of other brain regions can also exacerbate excitotoxic cell death. It is important however to determine the distribution of exogenously administered IL-1 since the site of injection may not reflect the site of action.

The aim of this study was to determine the distribution of human recombinant IL-1β (hIL-1β) in the rat brain after striatal administration. Induction of endogenous rat IL-1β (rIL-1β) was also assessed. Male rats were anaesthetised and hIL-1β (10ng) injected into the striatum. 2h or 6h later animals were sacrificed, blood and CSF samples taken and different brain regions dissected free. IL-1β levels were measured using species-specific ELISA.

The highest levels of hIL-1β were observed locally in the striatum at 2h with reasonable levels still present at 6h. Significant amounts of hIL-1β were also seen in the CSF at 2h with lower levels in the ipsilateral cortex. In contrast the largest increases in endogenous rIL-1β were seen in the striatum, cortex and hypothalamus at 6h.

These data suggest that exogenous IL-1β can enter the CSF after striatal injection and subsequently act to increase endogenous IL-1β expression in different brain regions.

11.05

IL-1 SIGNALLING IN THE BRAIN IN THE ABSENCE OF IL-1R1

Ralph Andre, Emmanuel Pinteaux, Jonathan Moggs, David Moore, Lisa C Parker, Ian Kimber, Nancy J Rothwell
1School of Biological Sciences, University of Manchester, Manchester, M13 9PT, UK2Syngenta, Central Toxicology Laboratory, Alderley Park, Macclesfield, SK10 4TJ, UK

Interleukin-1 (IL-1) is a pro-inflammatory cytokine whose involvement in neuronal damage following acute neurodegeneration is well established. IL-1 binds to a receptor, termed IL-1R1, which upon the recruitment of an accessory protein, designated IL-1AcP, leads to signal transduction. However, recent evidence suggests that some actions of IL-1 in the brain may be independent of IL-1R1, pointing to a new, yet unknown functional receptor for IL-1. The objective of the present study was to compare the signalling mechanisms responsible for the actions of IL-1 in primary mixed glial from wild-type and IL-1R1 deficient (IL-1R1-/-) mice, and to identify IL-1R1-independent IL-1 actions.Our previous results have shown that IL-1β activates nuclear factor-κB and mitogen-activated protein kinases (MAPKs), and induces the release of IL-6 and PGE2, in glia isolated from wild type mice, but not from IL-1R1-/- mice. However, DNA microarray analysis of the expression of 8732 genes, and subsequent semi-quantitative RT-PCR and Northern blot analysis, suggests the existence of IL-1R1-independent IL-1 signalling pathways. The identity and nature of a novel IL-1 receptor in the brain that mediates these effects remains unknown.

11.06

SEIZURES ASSOCIATED WITH ANTIBODIES TO VOLTAGE-GATED POTASSIUM CHANNELS (VGKC) AND GLUTAMIC ACID DECARBOXYLASE (GAD)

McKnight KM, Jiang Y, Hart Y, Palace J, Vincent A, Lang B
Neurosciences Group, Weatherall Institute of Molecular Medicine, The John Radcliffe Site, Headington, Oxford, OX3 9DS.

Epilepsy is a common neurological disorder but the aetiology in the majority of cases is unknown. Genetic defects in ion channels have been associated with a few epilepsy syndromes. Therefore, antibodies directed against central neuronal proteins may lead to seizures.

Sera were obtained from 132 patients with a history of seizures. These patients were categorised into three sub-groups: acute encephalitis (n=7), sub-acute limbic encephalitis-type illness (n=10) or drug-resistant chronic epilepsy (n=115), and sera were screened for antibodies to various neuronal targets.

In the acute encephalitis group, two patients had low positive titres for anti-VGKC antibodies and one patient had a low titre of anti-GAD antibody.

Of those with a sub-acute limbic encephalitis-type illness, nine patients were positive for anti-VGKC antibodies, five having high titres (>200 pM).

In the drug-resistant epilepsy group nine patients were positive for anti-VGKC antibodies, with one having a titre of >200 pM. Eleven patients in this group had raised anti-GAD antibody levels and three patients with focal epilepsy had high titres (>1000 U).

Antibodies to voltage-gated calcium channels and GM1 ganglioside were not detected.These results suggest that antibodies may be involved in certain seizure disorders but further work is required to elucidate their role.

11.07

NEUTROPHILS AND GLIA INTERACT TO INFLUENCE NERVE CELL SURVIVAL

P.J. Kingham and W.G. McLean
Department of Pharmacology, University of Liverpool, The Sherrington Buildings, Liverpool L69 3GE.

Both neutrophil infiltration of the CNS and glial cell activation are associated with ischaemic damage to the brain. We hypothesised that neutrophils might interact with glial cells to modify nerve survival. To investigate this we have used two human cell lines: CHME3 cells with characteristics of microglia and U87 cells, which model astrocytes. To detect direct cell-cell adhesion, neutrophils were isolated from human blood, radiolabelled with 51Cr and incubated with the glial cells. Neutrophils adhered to both cell lines. A blocking antibody against the neutrophil CD18 integrin inhibited adhesion to CHME3 cells but not the U87 cells. This was supported by the fact that CHME3 microglia expressed ICAM-1 a potential cell adhesion molecule partner for the neutrophil CD18 integrin. Interaction between neutrophils and CHME3 microglia led to the formation of reactive oxygen species within the CHME3 cells, as detected by dihydrorhodamine fluorescence staining. No such response was observed in the U87 cells. Neutrophils reduced MTT metabolism (a measure of cytotoxicity) of differentiated IMR32 neuroblastoma cells and this effect was attenuated in the presence of CHME3 microglia. These observations indicate that neutrophils influence glial cell function, which can have consequences for nerve survival.

11.08

REACTIVE OXYGEN SPECIES AND P38 MAP KINASE PLAY A ROLE IN IMMUNE CELL-MEDIATED NEURITE REMODELLING

P.J. Kingham(1), M.T. Walsh(2), R.W. Costello(2) and W.G. McLean(1)
(1) Department of Pharmacology, University of Liverpool, The Sherrington Buildings, Liverpool L69 3GE, UK. (2) Department of Medicine, RCSI, Beaumont Hospital, Dublin 9, Ireland.

Nerves are often exposed to cells of the immune system under both physiological and pathological conditions such as those that occur during gastrointestinal disease. Using an in vitro model we have previously shown that eosinophils adhere to nerves and activate a neuronal NADPH oxidase to produce reactive oxygen species (ROS). In this study, the consequences of these interactions for nerve cell morphology have been examined. Eosinophils induced neurite retraction in differentiated IMR32 neuroblastoma cells, as measured by computerised image analysis. This effect was not a result of cell death and could be reduced by preventing eosinophil adhesion with a blocking antibody against CD18 integrin and also by inhibiting the neuronal NADPH oxidase with DPI. The ROS donor, SIN-1 also induced neurite retraction. Eosinophil adhesion led to rapid tyrosine phosphorylation of a number of nerve proteins and activation of p38 MAP kinase. Genistein, an inhibitor of receptor associated tyrosine kinases, reduced both the production of neuronal ROS and neurite retraction whereas the p38 MAP kinase inhibitor, SB239063, reduced neurite retraction alone with no effect on ROS. These distinct pathways could be responsible for nerve cell remodelling in vivo and may have consequences for long-term nerve cell function.

11.09

IRRADIATION UPREGULATES IL-1β-INDUCED CELL SIGNALLING IN HIPPOCAMPUS: PROTECTIVE EFFECT OF EICOSAPENTANEOIC ACID

Aileen M. Lynch, Michelle Moore, Sarah Craig, Peter E. Lonergan and Marina A. Lynch
Trinity College Institute of Neuroscience, Department of Physiology, Trinity College, Dublin 2, Ireland.

Radiotherapy treatment has been shown to result in deficits in hippocampal-dependent cognitive function. We set out to assess the possibility that whole body gamma-irradiation increases the IL-1b concentration in the hippocampus and investigate the consequences of such a change. We present evidence which reveals that irradiation leads to apoptotic cell death in the hippocampus, and as a consequence, long term potentiation in perforant path-granule cell synapses is markedly impaired. The irradiation-induced increase in IL-1b concentration in hippocampus is accompanied by increased expression of IL-1 type I receptor and IL-1 accessory protein. These changes are coupled with increased activation of JNK, decreased activation of ERK, and with evidence of apoptotic cell death; namely parallel increases in cytochrome c translocation, PARP cleavage and TUNEL staining.
Significantly, the irradiation-induced changes in hippocampus are abrogated by treatment of rats with the polyunsaturated fatty acid, eicosapentaneoic acid. This treatment reverses the irradiation-induced increase in IL-1b concentration, IL-1b-induced signalling and also reduces evidence of apoptosis. In addition, when the irradiation-induced apoptosis was prevented by eicosapentaenoic acid, long term potentiation was sustained in a manner similar to that in the control group.

11.10

CO-STIMULATORY RECEPTOR 4-1BB IN MULTIPLE SCLEROSIS

Andreia C Gomes*1, Guang-zhi Liu*1, Prabhakar Putheti*2, Konstantinos Kostulas1, Rayomand Press1, Peter Hjelmström1, Jan Hillert
11Division of Neurology, NEUROTEC Department, Huddinge University Hospital, SE-141 86 Stockholm, Sweden2 Division of Neuroimmunology, NEUROTEC Department, Karolinska Institute, Alfred Nobels Allé 10, Box 4, SE-141 83 Stockholm, Sweden.

4-1BB (CD137) is expressed on activated CD4+ T cells, CD8+ T cells, natural killer (NK) cells and NK T cells. The ligand of 4-1BB (4-1BBL) is expressed on activated antigen presenting cells (APC). Recent studies have demonstrated that 4-1BB promotes accessory T cell activation, and regulates proliferation and survival of T lymphocytes. We used 3-colour flow cytometry to analyse the expression of 4-1BB on CD4+CD25high T regulatory cells, CD4+, CD8+ T cells and whole peripheral blood (PB) mononuclear cells (MCs). Spontaneous expression of 4-1BB and 4-1BBL mRNA on PBMC was measured by real-time PCR and ELISA was performed in order to quantify plasma soluble 4-1BB levels. Functional analysis of this pathway was done by analysing antigen-specific proliferative responses. CD4+CD25high T cells have shown lower expression of 4-1BB in MS and OND patients compared to healthy controls (HC). 4-1BB mRNA expression was lower in whole PBMC in MS and OND patients compared to HC, and plasma soluble 4-1BB was increased in MS. Incubation with anti-4-1BBL monoclonal antibody did not inhibit proliferation of myelin-reactive cells indicating 4-1BB/4-1BBL pathway blockage alone is not sufficient to inhibit the proliferation of CD4+ T cells. This suggests that 4-1BB may have different immunoregulatory effects on different T cell subsets.

11.11

AXONAL LOSS IS THE PATHOLOGICAL CORRELATE OF CHRONIC DISABILITY IN MOG-INDUCED EAE IN THE RAT

Dimitrios Papadopoulos, Richard Reynolds
Department of Neuroinflammation, Division of Neuroscience, Imperial College London, Charing Cross Campus, London, W6 8RF.

Axonal loss is now considered to be one of the most important contributors to progression of disability in Multiple Sclerosis (MS) and is thought to begin early in the disease course. Here we test the hypothesis that axonal loss determines permanent neurological impairment in inflammatory demyelinating disease. EAE was induced in DA rats by injection of recombinant mouse MOG in IFA. Animals were grouped into those that exhibited a chronic progressive disease course and those that showed a chronic relapsing remitting course. Toluidine blue staining of resin sections and immunohistochemistry for OX-42 were used to quantify demyelination, axonal loss and inflammation at varying time points. In progressive EAE, axon loss, demyelination and inflammation all correlated significantly with clinical severity. In the lateral and dorsal funiculi the decrease in the number of axons reached 64.5% by 40 days after the first clinical symptoms. However, in the late chronic stage of relapsing-remitting EAE only axonal loss correlated significantly with clinical severity. Classification of sampled axons into three groups according to size showed that small caliber axons were preferentially lost in both EAE groups. These results provide evidence that axonal loss can determine irreversible neurological disability in inflammatory demyelinated lesions of the spinal cord, similar to those seen in MS.

11.12

CHARACTERISATION OF GABA-A AND GLYCINE RECEPTORS ON HUMAN LEUKOCYTES

S. Alam, D. Laughton, A. Walding, A. Hibell and A. J. Wolstenholme
University of Bath, Biology and Biochemistry, Claverton Down, Bath BA2 7AY.

Recently evidence for the presence of glycine receptors on rat neutrophils has been reported (Wheeler et.al., 2000, FASEB Journal, 14; 476-484). This includes data suggesting that these receptors downregulate immune cell activation, leading to the inhibition of inflammatory responses. We attempted to determine if such receptors might play a similar role in human neutrophils. To screen for GABAA and glycine receptor subunit mRNAs in human leukocytes, RT-PCR followed by real-time quantitative TaqMan RT-PCR were performed. We could detect many of these subunit mRNAs in PBMC and Jurkat cells, although quantitative studies showed these were expressed at low levels. Functional studies were carried out by studying the effects of GABA and glycine on intracellular calcium and pH levels in human neutrophils. fMLP and LPS were used to induce the release of calcium from intracellular stores. Based on the published rat data, GABA and glycine were expected to inhibit this release. However we found that application of neither GABA nor glycine led to a reduction in the response to either agonist. GABA and glycine also did not lead to a decrease in intracellular pH. These results indicate that human neutrophils unlike those from rats, may not express functional GABAA and glycine receptors.

11.13

EXPRESSION OF ADAMTS -1, -4 AND -5 IN CENTRAL NERVOUS SYSTEM CELL LINES: POSSIBLE ROLE IN CNS PHYSIOLOGY AND PATHOLOGY

AK Cross1,2, G Haddock1,2, J Surr1,2, G.Jones2, MN Vankemmelbeke2,.DJ Buttle2, RAD Bunning1 and MN Woodroofe1
1. Biomedical Research Centre, School of Science and Mathematics, Sheffield Hallam University, Howard Street, Sheffield S1 1WB; 2. Division of Genomic Medicine, University of Sheffield Medical School, Sheffield Childrens' Hospital, Sheffield ,S10 2TH.

ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzymes are a group of metalloproteinases, which degrade large aggregating proteoglycans, suggesting a role in extracellular matrix (ECM) turnover in the CNS. ADAMTS -1 is also known to exhibit anti-angiogenic activity. The involvement of ADAMTSs in CNS pathological conditions, including MS and stroke, may include a role in ECM breakdown, demyelination, prevention of remyelination and in stroke, prevention of angiogenesis. To investigate the potential role of these enzymes, CNS derived cell lines, astroglioma cell lines U373-MG and U87-MG, and a human foetal microglial cell line, CHME3 (from Prof Tardieu, Paris) were assessed for the expression of ADAMTS-1, -4 and -5 at the mRNA and protein level by real-time PCR and western blotting. The cell lines were incubated for varying times to study the time-course of mRNA and protein expression following stimulation with IL-1b and LPS. Preliminary results show a differential expression of ADAMTS-1, -4 and -5 in the microglial and astrocyte cell lines with increased expression following stimulation. This provides the first report of ADAMTS expression by CNS cell lines. Ongoing studies will determine the expression of these enzymes within the CNS in vivo.

This work was funded by The Wellcome Trust.

12.01

CHARACTERISATION OF G-PROTEIN-COUPLED RECEPTORS IN *C.ELEGANS*

N. Kriek, D. Marsden, C. Keating, M. Daniels, J. F. Burke, L. Holden-Dye
University of Southampton;University of Sussex & Genetix Ltd; UK.

Here we describe the classification of C.elegans GPCRs predicted to bind either small molecule neurotransmitters or neuropeptides, their functional analysis using reverse genetics, and putative ligands.We performed RNAi for sixty-five rhodopsin-like GPCRs and report a non-redundant role for seven neuropeptide-like GPCRs in locomotion, and for four other receptors in the regulation of brood size.More than 70 neuropeptide precursor genes have been identified in C.elegans, encoding more that 150 putative neuropeptides (Li et al., 1999, Ann. N. Y. Acad. Sci., 897: 239-252). These represent candidate ligands for GPCRs. To define receptor/ligand pairs we have taken three complementary approaches. First, pharmacological responses to neuropeptides were determined in an isolated pharyngeal preparation taken from worms treated with RNAi for specific GPCRs to test for a reduction in response compared to wildtype. Second, responses to neuropeptides were measured in worms overexpressing GPCRs in the pharynx. Third, we used reporter constructs to determine whether the receptor and ligand are expressed in a physiologically relevant synaptic context.
These experiments enable us to predict receptor:ligand pairs in C.elegans.

Sponsored by the BBSRC.

12.02

A MOTIF IN THE C-TERMINUS OF THE NMDA RECEPTOR NR2B SUBUNIT CONTROLS CELL SURFACE EXPRESSION OF NR1/NR2B RECEPTORS

L.M. Hawkins1, C. Moussan 1, K. Chang 2, F.A.Stephenson 1 and R.J. Wenthold 2
1.The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX. 2. NIDCD/NIH, Building 50, South Drive, Bethesda, Maryland, 20892, USA.

Synaptic N-methyl-D-aspartate (NMDA) receptors play a pivotal role in normal brain function as well as in neurodegenerative disorders. Understanding how NMDA receptors are trafficked to the synapse is thus of fundamental importance. It is well established that formation of functional NMDA receptors requires co-assembly of NR1 and NR2 subunits. Trafficking of NR1 subunits in the absence of NR2 to the cell surface is controlled by an endoplasmic reticulum (ER) retention motif, RXR, found within the NR1 C-terminus. The role of this RXR signal in the trafficking of functional NR1/NR2 receptors is unclear. Here, we describe a motif in the proximal region of the NR2B C-terminus that is essential for cell surface expression of NR1/NR2B receptors. The motif was identified using a series of NR2B truncated and point mutants. Co-expression of NR1-1a with NR2B mutants that either lack the motif or, where the motif has been replaced by alanine residues, did not result in the expression of functional or cell surface NR1/NR2B receptors. Co-immunoprecipitation experiments determined that the motif does not affect co-association of NR1/NR2 subunits. These observations demonstrate that in heteromeric NR1/NR2 receptors, the NR2 subunit regulates cell surface NMDA receptor expression.

12.03

SUBCELLULAR DISTRIBUTION OF NMDA RECEPTOR SUBUNITS IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS FOLLOWING THE INDUCTION OF LONG TERM POTENTIATION (LTP)

C.J. Peddie*, A.J. Milner*, K. P. S. J. Murphy, M.G. Stewart and J.J. Rodríguez. *Both authors have contributed equally to the present work.
Department of Biological Sciences, The Open University, Milton Keynes MK7 6AA. UK.

The N-methyl-D-aspartate (NMDA) glutamate receptor is involved in neurotransmission, neuronal development and synaptic plasticity, including long-term potentiation (LTP). The properties of this receptor are determined by its subunit composition. Hippocampal CA1 neurons in mature animals express NMDA receptors containing both NR1 and NR2 (NR2A and/or NR2B) subunits. To test the hypothesis that the distribution of NR2A and NR2B subunits is altered after LTP induction, we have examined the localisation and subcellular distribution of these subunits in individual CA1 pyramidal neurons that either expressed LTP or were non-potentiated prior to intracellular filling with neurobiotin. In both potentiated and non-potentiated neurons, NR2A/2B immunoreactivity was seen within dendrites, and to a lesser extent in dendritic spines. Interestingly, the subunits were predominantly associated with cytoplasmic elements, but some immunoreactivity was seen at the plasma membrane. NR2A/2B immunoreactivity was also present within neuronal perikarya, axonal profiles and glia. Filled potentiated cells, when compared with filled non-potentiated cells, showed differences in NR2A/2B immunoreactivity within dendrites and dendritic spines. These results indicate that the induction of LTP may be associated with a redistribution of NR2A/2B containing NMDA receptors.

Supported by BBSRC grant 108/NEU15416.

12.04

ULTRASTRUCTURAL LOCALISATION OF SEROTONIN 5-HT2A RECEPTOR WITH GLUTAMATE NMDA AND AMPA RECEPTORS IN THE DENTATE GYRUS OF THE RAT HIPPOCAMPUS

C.J. Peddie, M.G. Stewart and J.J. Rodríguez
Department of Biological Sciences, The Open University, Milton Keynes MK7 6AA. UK.

The serotonergic system extensively innervates many brain regions, including the hippocampus. Serotonin (5-HT) receptor activation can result in improvement of memory and/or recovery of impaired cognitive function. 5-HT receptors are also involved in the modulation of other neurotransmitters, including glutamate. We examined functional sites of 5-HT2A receptor activation and their potential interaction with NR1 and GluR2 containing glutamate receptors in the dentate gyrus (DG) of rat hippocampus. 5-HT2AR labelling was primarily dendritic (24% of labelled profiles), but was also present in small unmyelinated axons and axon terminals (5% of labelled profiles). 7.2% and 4.5% of the total labelled profiles were dendrites containing both 5-HT2AR/NR1, and 5-HT2AR/GluR2 immunoreactivity, respectively. In addition, NR1 and GluR2 immunoreactivity was also observed in smaller dendrites, dendritic spines, and axon terminals which rarely contained 5-HT2AR. These results suggest that somatodendritic 5-HT2AR activation may play an important role in controlling the input and/or output of DG granule cells that are subject to subtle modulation involving NR1 and GluR2 on distal dendrites and dendritic spines. The findings also indicate that 5-HT2AR may have a significant role in cognitive function involving the hippocampal formation, specifically the DG.

Supported by BBSRC grant 108/NEU15416.

12.05

DO ALPHA-2B/C ADRENOCEPTORS HAVE A SUBORDINATE AUTORECEPTOR ROLE IN THE LOCUS COERULEUS OF C3H AND MONOAMINE OXIDASE-A KNOCKOUT MICE?

Catarina A Owesson, Daniel P McLaughlin, *Isabelle Seif & Jonathan A Stamford.
*Neurotransmission Lab, Academic Department of Anaesthesia & Intensive Care, The Royal London Hospital, London E1 1BB and *CNRS UMR 146, Institut Curie, Orsay, France.*

Previous studies have shown that alpha-2A autoreceptors govern locus coeruleus (LC) cell firing. Here we used voltammetry in mouse LC slices to see if stimulated LC noradrenaline (NA) efflux was under similar control. On long stimuli (30 pulses, 10 Hz) NA efflux was increased by the alpha-2A antagonist BRL 44408 ($P<0.001$) but not by the alpha-2B/C antagonist ARC 239 (each 100 nM). The effect of BRL 44408 was significantly greater in MAO-A KO than in C3H mice (308±43% vs 213±31%, $P<0.001$). On short trains (10 pulses, 200 Hz), the non-selective alpha-2 agonist dexmedetomidine (Dex, 10 nM) reduced NA efflux by 78±8% (C3H) and 51±8% (MAO-A KO). In both strains, BRL 44408 and ARC 239 each partially blocked the effect of Dex. In MAO-A KO mice, superfusion of the slices with BRL 44408 increased evoked NA efflux on short trains by 55±12% while ARC 239 had no effect. The two antagonists together increased NA efflux (by 81±34% at maximum, $P<0.001$), an effect greater than that evoked by BRL 44408 alone ($P<0.01$). In C3H mice, the alpha-2 antagonists did not affect LC NA efflux. These data suggest that, in contrast to LC cell firing, NA efflux is controlled by alpha-2A and, albeit to a lesser degree, alpha-2B/C autoreceptors. Moreover, in MAO-A KO mice there appears to be greater tone at the autoreceptors, perhaps due to a lower number of LC NA transporter molecules in this strain.

12.06

LOCALISATION OF VASOPRESSIN V1B RECEPTOR-LIKE IMMUNOREACTIVITY IN RAT BRAIN

K. Fawcett & K. R. Oliver
Neuroscience Research Centre, Merck Sharp and Dohme, Terlings Park, Harlow, Essex. CM20 2QR, UK.

Vasopressin is a nonapeptide neuromodulator that mediates numerous behavioural functions and is synthesised in neurons of the hypothalamic paraventricular, supraoptic and suprachiasmatic nuclei. Whilst they have a profound role in hypophysial function, vasopressingeric neurons innervate limbic structures and other CNS sites, where vasopressin`s activity is largely mediated through V1a and V1b receptor subtypes. Recent studies indicate that V1b receptors may have an important role in anxiety-related behaviours.
To further elucidate the neuroanatomical basis of V1b receptor involvement in such behaviours, we sought to determine their localisation in rat brain using modern immunohistochemical techniques. V1b-like immunoreactive material was widely, yet heterogenously distributed in the brain and was remarkable in its subcellular expression profile. It has been proposed that certain vasopressinergic functions (e.g. anxiety-related behaviours) may be mediated at extrahypothalamic CNS sites. The data presented in this study are intended to further our understanding of the cellular and subcellular distribution of V1b receptors and seek to understand the neuroanatomical basis of their physiological and behavioural functionality. Ultimately, these data may facilitate the future development of anxiolytic and antidepressant agents targeting V1b receptors.

12.07

EXPRESSION PROFILING OF P2X RECEPTOR SUBUNITS IN PRIMARY AUDITORY NEURONES

Denise Greenwood(1), Daniel Jagger(2), LinChien Huang(1) and Gary D. Housley(1)
(*1) Department of Physiology, Faculty of Medicine and Health Science, The University of Auckland, Private Bag 92019, Auckland New, Zealand; (2) UCL Center for Auditory Research and Institute of Laryngology and Otology, University College London, London WCIX 8EE.*

Spiral ganglion neurones (SGN) in the cochlea provide primary afferent innervation of the sensory hair cells. Adenosine 5`-triphosphate (ATP), acting at P2X receptors, has been implicated in auditory neurotransmission. Using RT-PCR, all seven of the P2X receptor subunits were detected in postnatal rat spiral ganglion tissue, with expression of P2X1, P2X3 and P2X5 receptor mRNA diminishing after P4. Three alternatively spliced P2X2 receptor transcripts were also detected in neonatal SGN. Characterisation of P2X receptor subunit expression was undertaken in individual auditory neurones isolated from neonates (P1-P4). Using multiplex single cell RT-PCR, the expression profile was: 100% P2X1, 86% P2X2, 14% P2X3, 72% P2X4, 62% P2X5, 59% P2X6 and 66% P2X7 (n=32 neurones). Splice variants of P2X2 were differentially expressed at 48% P2X2-1, 25% P2X2-2 and 62% P2X2-3 (n=35). Only 5/35 neurones expressed all three P2X2 splice variants. Real-time RT-PCR provided a semi-quantitative analysis of the expression profile in single SGN, with P2X receptor subunit transcripts detected at 1-10 copies per cell. The mRNA expression, was complemented by protein localisation using immunocytochemistry.
Studies approved by the University of Auckland Animal Ethics Committee.

Acknowledgements: Health Research Council of New Zealand, Marsden Fund, University of Auckland Graduate Fund.

12.08

CHARACTERIZATION OF NR1a/NR2D(T692A) NMDA RECEPTOR-MEDIATED CURRENTS BY GLUTAMATE, ASPARTATE AND NMDAA.R

Johnston & D.J.A. Wyllie
Division of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

Previous work on recombinant NR1a/NR2A NMDA receptors has indicated that mutation of a threonine residue to an alanine residue at position 671 in the S2 binding domain reduces glutamate potency and increases the deactivation rate of NMDA receptor-mediated currents following brief application of glutamate (Anson et al. 1998, 2000). In the NR2D subunit the homologous residue is found at position 692. We have investigated the properties of the NR2D(T692A) mutation to understand further the complex nature single-channel activations exhibited by NMDA receptors containing NR2D subunits. To do this we are studying the responses evoked by NMDA and aspartate. Interestingly, the shifts in potency exhibited by NR2D(T692A) containing receptors when activated by aspartate or NMDA are considerably less than that seen for glutamate (EC50asp/wt = 3.3 microM, EC50asp/mut = 191 microM; EC50nmda/wt = 3.7 microM, EC50nmda/mut = 117 microM; EC50glut/wt = 0.45microM, EC50glut/mut = 703 microM; n = 6 – 9). The differences in the length of the carbon-chain backbone of aspartate and NMDA compared to glutamate and the proposed location of the threonine residue may account for these observations. Single-channel recordings and concentration jumps using these three agonists are being made in order to characterize further the properties of this mutation.

Supported by The Wellcome Trust.

12.09

FURTHER INVESTIGATION OF AMINO ACID RESIDUES IN THE NR2A SUBUNIT THAT REDUCE GLUTAMATE POTENCY IN RECOMBINANT NR1a/NR2A NMDA RECEPTORS

P. E. Chen and D. J. A. Wyllie
Division of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

The NMDA subtype of ionotropic glutamate receptor requires both glutamate and glycine for activation and is a hetero-oligomer of two types of subunit, NR1 and NR2A-D. The residues responsible for glycine and glutamate binding are located on the NR1 and NR2 subunits, respectively. We have shown that a NR2A(S670A) mutation, did not affect glutamate potency, in NR1a/NR2A receptors (Anson et al. (1998) JNeurosci. 18: 581-589). However, mutation of an analogous residue in NR2B (S664G), produced a 118-fold reduction in glutamate potency (Laube et al., (1997) Neuron 18: 493-503). Preliminary dose-response curves from receptors containing the same substitution in the NR2A subunit (NR1a/NR2A(S670G)), revealed >100-fold reduction in glutamate potency, compared to wild-type (EC50=421 ± 58 microM, n=6). We found no significant decrease in mean maximal current, Hill slope or glycine potency (EC50=2.3 ± 0.1 microM, n=5). Therefore, a glycine substitution at this position can drastically alter glutamate potency in NMDA receptors. Mutation of a neighbouring residue (NR2A(G669S)), produced a greater reduction in glutamate potency (EC50=3656 ± 649 microM, n=6), as reported previously (Lummis et al., (2002) Neuropharm. 42: 437-443). We are now investigating the single-channel characteristics of these mutants, including their deactivation kinetics to brief applications of glutamate.

Support: BBSRC.

12.10

INSIGHTS INTO THE ASSEMBLY PATHWAY OF NMDA RECEPTORS FROM THE MOLECULAR CHARACTERISATION OF AN EPITOPE-TAGGED NR1 SUBUNIT

M Papadakis, LM Hawkins and FA Stephenson
School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK.

The interaction between NR1 and NR2 subunits is prerequisite for the assembly of NMDA receptors with NR1 1-380 being important for this association (1). Here, further evidence is presented for involvement of the NR1 N–terminal domain in the assembly of functional NMDA receptors. Normally, co-expression of NR1/NR2 receptors results in cell cytotoxicity but introduction of c-Myc into NR1-2a between aa 80-81 does not induce cell death following NR1/NR2 co-expression. NR1-2a80c-Myc81 expressed alone in mammalian cells was comparable to wild-type NR1-2a but co-expression with NR2A resulted in ~ 8-fold reduction in NR1-2a80c-Myc81. [3H] MDL 105, 519 and [3H] MK801 binding to NR1-2a and NR1-2a/NR2A respectively revealed no significant difference in their KDs between wild-type and tagged receptors. Co-immunoprecipitation showed that tagged subunits associate with both NR2A and NR1-4b thus the tag does not interfere with either hetero- or homo-oligomerisation. ELISAs however showed that NR1-2a80c-Myc81/NR2B receptors were not expressed at the cell surface. It is postulated that insertion of the epitope tag disrupts the folding of the NR1 N–terminal domain thus leading to NR1/NR2 lysozomal degradation. Hence, integrity of the NR1 N-terminal domain is essential for proper NMDA receptor oligomerization and cell surface expression.

1. Meddows E et al. (2001) J. Biol. Chem. 276, 18795.

12.11

THE INFLUENCE OF NICOTINIC ALPHA7 AND 5-HT3 RECEPTOR SUBUNIT DOMAINS UPON CELL BIOLOGICAL AND FUNCTIONAL PROPERTIES

Veronica J. Gee, Sandra T. Cooper, Alasdair J. Gibb and Neil S. Millar
Department of Pharmacology, University College London, London, UK.

Both nicotinic acetylcholine receptors (nAChRs) and 5-hydroxytryptamine (5-HT) type 3 receptors are pentameric neurotransmitter-gated cation channels. Whilst most native nAChRs and at least some 5-HT3 receptors are heteromeric complexes, both are able to generate functional homomeric receptors. The nAChR alpha7 subunit and the 5-HT receptor 5-HT3A subunit have been the subject of extensive experimental study due, in part, to their ability to generate homomeric receptors. Previous studies have revealed that homomeric alpha7 and 5-HT3A receptors differ considerably in both their cell biological properties (e.g. efficiency of subunit folding and cell-surface expression) and electrophysiological properties (e,g, single channel conductance and speed of desensitization). A series of subunit chimeras have been constructed containing regions of the alpha7 and 5HT3A subunits with the aim of identifying the influence of subunit domains upon cell biological and functional properties. Heterologous expression studies in mammalian cell lines have revealed the importance of hydrophobic putative transmembrane domains for efficient folding and assembly of functional cell surface receptors. Electrophysiological studies conducted in transfected mammalian cells have revealed the importance of discrete subunit domains upon ion channel properties of nicotinic and 5-HT receptors.

12.12

IDENTIFICATION AND CHARACTERISATION OF AN ER-RETENTION MOTIF WITHIN THE 5-HYDROXYTRYPTAMINE RECEPTOR 5-HT3B SUBUNIT

Anne I. Doward, Gary W. Boyd, Chris N. Connolly, Neil S. Millar
Department of Pharmacology, University College London, London, UK and Department of Pharmacology and Neuroscience, University of Dundee, UK.

Previous studies have revealed that 5-hydroxytryptamine type 3 receptor (5-HT3R) subunits can form both homomeric and heteromeric complexes. In contrast to 5-HT3A (which can generate functional homomeric complexes), the 5-HT3B subunit is retained within the endoplasmic reticulum (ER) when expressed alone. Co-expression of the 5-HT3A and 5-HT3B subunits, however, results in efficient cell-surface expression of co-assembled heteromeric 5-HT3A/5-HT3B subunit complexes. With the aim of identifying sequences influencing cell-surface expression and ER retention, a series of mutated and truncated 5-HT3A and 5-HT3B subunits have been constructed. An ER retention signal (CRAR) has been identified within the short intracellular (M1-M2) loop domain of 5-HT3B which, when introduced into 5-HT3A, causes a substantial reduction in cell-surface expression of homomeric 5-HT3A complexes. Replacement of the CRAR motif in 5-HT3B with the analogous sequence from 5-HT3A (SGER) does not facilitate cell-surface expression of homomeric 5-HT3B subunits, suggesting that additional sequences within 5-HT3B subunit are important in influencing assembly and/or cell-surface expression. Additional heterologous expression studies with a series of truncated 5-HT3B subunits support the conclusion that CRAR acts as an ER retention signal within the 5-HT3B subunit.

12.13

LOCALISATION OF THREE D2-LIKE DOPAMINE RECEPTORS IN RAT BRAIN AND Ntera-2 CELLS USING NEW MONOCLONAL ANTIBODIES

Elizabeth C. Wolstencroft, Ngyen thi Man, Ian Holt, Paul A. Buckland* and Glenn E. Morris
*Biochemistry Group, North East Wales Institute, Mold Road, WREXHAM, LL11 2AW*Dept. of Psychological Medicine, University of Wales College of Medicine, Heath Park, CARDIFF, CF14 4XN.*

Dopamine D2-like receptors belong to the family of G protein-coupled receptors (GPCRs) that interact with inhibitory G proteins and reduce levels of cyclic AMP. We have produced monoclonal antibodies against the three D2-like dopamine receptors, DRD2, DRD3 and DRD4. These antibodies recognise membrane-associated proteins of the expected size in rat/human brain and human Ntera-2 cells.
Immunolocalization studies in rat brain and Ntera-2 cells showed that all three receptors are present at high levels in cytoplasmic vesicles, but are not detectable at the plasma membrane. This distribution pattern was confirmed as authentic for DRD3 using four antibodies against three different epitopes. All three receptors co-localized in specific neuronal cell types in rat brain, including pyramidal layers of the cerebral cortex and hippocampus, cerebellar Purkinje cells and large molecular neurons in the brain stem. This tissue distribution agrees with earlier mRNA and radioligand studies of D2-like dopamine receptors.
Earlier transfection studies of recombinant DRD2 into cultured cells showed localisation in cytoplasmic vesicles, but we have now shown that all three endogenous D2-like dopamine receptors have a similar localisation in both cultured nerve cells and rat brain. Possible origins of the vesicles include agonist-independent internalisation from the plasma membrane.

12.14

CLONING AND CHARACTERISATION OF GPR26, A CNS-RESTRICTED ORPHAN GPCR

Maureen Illand, Fiona Adams, Derek Gatherer, Brian Henry, Morag Grassie.
Organon Laboratories Ltd, Newhouse, Lanarkshire, Ml1 5SH, Scotland.

Herein, we report the characterisation of an orphan G protein-coupled receptor (GPCR) GPR26, identified using a bioinformatics approach. The gene structure and phylogenetic analysis of GPR26 shows this GPCR sits within a family of orphan GPCRs and unusually for a GPCR, has three exons.
In human tissues, GPR26 exhibits a largely CNS-restricted tissue distribution as shown by RT-PCR and multiple tissue slot blot analysis. In situ studies demonstrated that GPR26 was present in forebrain and midbrain regions of the rat brain with a high level of GPR26 expression within cortical and hippocampal regions. Detailed analysis of these in situ studies will be described.
The distribution pattern described suggests that GPR26 may be involved in pharmacological and/or pathological functions associated with dopaminergic pathways and/or cognitive processes such as learning and memory. It is hoped that analysis of mice with a targeted deletion in the gene for GPR26 will yield further clues as to the function of GPR26 in vivo.

12.15

EFFECT OF SODIUM BUTYRATE TREATMENT ON QUINPIROLE-STIMULATED [^{35}S]GTPgS BINDING IN CHO CELLS STABLY EXPRESSING HD2 OR HD3 RECEPTORS

C. Scott, J. L. Heath, M. J. Hill, N. Patel, M. D. Wood and J. Watson
GlaxoSmithKline Pharmaceuticals,Psychiatry Centre of Excellence for Drug Discovery,NFSP-N,Third Avenue, Harlow, Essex, CM19 5AW.

D2/D3 receptors are reported to couple to Gi/Go subtypes of G-proteins (Zaworski et al., 1999) and functional coupling for both receptors has been demonstrated using [35S]GTPgS binding (Zaworski et al., 1999; Gardner et al., 1996). The level of stimulation reported in these assays can lead to variability in the data produced. We have investigated effects of sodium butyrate (Na but.) treatment on functional coupling of hD2 and hD3 receptors stably expressed in CHO cells using [35S]GTPgS binding. Cells were pre-treated with 5mM Na But. (48 hrs). Membranes were prepared and assayed as described by Watson et al. (2000) with minor modifications. Agonist-induced stimulation of [35S]GTPgS binding was determined using 10mM quinpirole. Na but.treatment increased agonist stimulated [35S]GTPgS binding from 39 +/- 5 to 131 +/- 21%(*) basal binding in hD2 cells and 194 +/- 19 to 262 +/- 28% in hD3. (* $P < 0.05$; 1 way ANOVA with post-hoc T-Test (LSD)). In conclusion, our data shows that Na but. has significantly increased the degree of agonist-induced stimulation observed in hD2 [35S]GTPgS binding leading to improvement in the reproducibility of data generated in this assay.

Gardner B. et al., (1996). Br. J. Pharmacol., 118, 1544-1550.
Watson J. et al., (2000). Br. J. Pharmacol., 130, 1108-1114.
Zaworski P. G. et al., (1999). Br. J. Pharmacol., 128, 1181-1188.

13.01

REGULATION OF EARLY CORTICAL DEVELOPMENT BY TRANSCRIPTION FACTORS

David J. Price
Biomedical Sciences, Hugh Robson Building, George Square, Edinburgh EH8 9XD.

The aim of our research is to study how cerebral cortex attains its enormous complexity during embryonic development. A few highly important regulatory genes seem to control this process. One such gene is Pax6, which is essential for developing cortical cells to proliferate normally and for the formation of thalamocortical connections. We have shown that in mutant mice lacking functional Pax6 the cortical cell cycle is shorter than normal early in development and more cells exit the cell cycle than normal. Later in corticogenesis, the cell cycle lengthens. The result of these changes is to produce fewer cells than normal in the cortex. Although the thalamus forms in mice lacking Pax6, thalamocortical axons do not and we have shown that this is due, at least in part, to cell autonomous defects in the thalamus itself. It appears that transcription factors such as Pax6 can exert control over corticogenesis in several different ways at different sites and times during development.

13.02

REMODELLING OF THALAMOCORTICAL ARBORS AND EMERGENCE OF BARREL DOMAINS IN THE PRIMARY SOMATOSENSORY CORTEX: LESSONS LEARNED FROM KO MICE

Patricia Gaspar1*. Alexandra Rebsam1, Isabelle Seif 2
INSERM U106, CHU Pitié-Salpêtrière, Paris, France 2 Faculté de pharmacie, Châtenay Malabry, France.

In the rodent primary somatosensory cortex, the thalamocortical axons (TCAs) are organized into clusters that correspond to functional units in the periphery. Around these axons, neurons in layer IV, aggregate as barrels. To understand how this organization emerges, we analyzed TCA development in mice that do not form barrels, the monoamine oxidase A knockout (MAOA-KO) and in MAOA/5-HT1B receptor double-KO mice, which have a restored barrel field. We found that TCAs already attain cortical layer IV on the day of birth and are uniformly distributed in this layer from P0 to P2. Secondarily TCAs coalesce into barrel domains in layer IV, over a three-day period (P3-P5), with no pre-patterning in the deeper layers. In the MAOA-KO mice, the uniform distribution of the TC projection is maintained and no axon clusters emerge. Individual TCA arbors were traced after carbocyanine injections. At P1, TCAs were poorly branched and covered variable tangential widths, encompassing 1-3 prospective barrel in layer IV. At P7, TCA branches increased tenfold in layer IV and became restricted to one barrel. In MAOA-KO mice, there was a 50% reduction of the TCA terminal branches in layer IV and a 40% increase in their tangential extent. These defects were corrected in the MAOA/5-HT1B DKO mice, indicating an effect of the presynaptic 5-HT1B receptor on axon branching. These results indicate that the barrel deficient phenotype of the MAOA-KO mice results from an altered refinement of the TCA arbors in their target layer IV, involving branch elaboration and collateral retraction during early postnatal life. As shown in parallel investigations (Vitalis et al. J. Neuroscience, 2002), these effects are not mediated by BDNF or TrkB receptors. The MAOA-KO further allowed us to question whether the time for whisker lesion-induced plasticity is related to the time of barrel-emergence. We found it possible to initiate barrel patterning in the MAOA-KO mice by administering the 5-HT synthesising inhibitor, PCPA . at P3. In this context of delayed barrel development, we observed a corresponding delay in the effects of peripheral lesions. Thus, the critical time for the effects of peripheral lesion on axon remodelling appears to depend on the stage of TC development rather than on the absolute age at the time of the lesion.

13.03

EXPERIENCE-DEPENDENT DEVELOPMENT AND PLASTICITY OF THE BARREL CORTEX

Kevin Fox
Cardiff University, Biomedical Bldg, Museum Ave., Cardiff, CF10 3US.

The barrel cortex is most plastic during early development at a stage when the barrels are forming. However, different forms of plasticity can be seen in later development and even into adulthood. For example, removal of whiskers can produce an activity dependent depression of cortical responses up to the first two to three months of life and sparing some whiskers while removing others can produce potentiation of the spared whisker responses throughout life. Depression occurs in the cortex and not in subcortical structures. Depression requires cortical activity and is not due to a passive decay of synaptic strength from lack of use. The molecular mechanisms underlying this form of plasticity are unknown but appear to be related to LTD. Potentiation occurs in adult animals and does not show a critical period in layers II/III of the cortex. Potentiation depends critically on alpha-CaMKII autophosphorylation and partly on the AMPA GluR-A subunit. There are striking similarities between this for of plasticity and LTP, though this is unlikely to be the whole story.

13.04

CORTICAL DEVELOPMENT AND SYNAPTIC PLASTICITY

Gerald Finnerty
Department of Neurology, Institute of Psychiatry, DeCrespigny Park, London, SE5 8AF.

The sensory periphery is represented in the neocortex as a series of topographic maps. These cortical maps can express experience-dependent plasticity during development. In some cases, such as in the supragranular layers of whisker barrel cortex, plasticity can extend through adolescence into adulthood. Cortical map reorganization is a process comprising modifications of synaptic efficacy and structural changes in neurons. The rate and extent of plasticity vary with age, but there are consistent themes in all age groups. This suggests that the mechanisms that underpin cortical map reorganization in earlier stages of development provide a platform to study the more subtle changes seen in adolescence.

14.01

ADRENAL STRESS HORMONE EFFECTS ON BRAIN SYSTEMS REGULATING MEMORY

Benno Roozendaal
Center for the Neurobiology of Learning and Memory and Department of Neurobiology and Behavior, University of California, Irvine CA 92697-3800.

It is well established that adrenocortical hormonesinfluence cognitive performance. Some studies have found glucocorticoid-induced memory enhancement, but others have reportedimpairing effects. I will review recentfindings from our laboratory regarding the acute effects ofglucocorticoids in rats on specific memory functions. The consequences of glucocorticoid activation on cognition depend largely on the different memory functions investigated. Posttraining activation of glucocorticoid-sensitive pathways involving glucocorticoid receptorsdose-dependently enhances memory consolidation. These effects rely onnoradrenergic activation of the basolateral complex of the amygdala (BLA) and interactions with other brain regions. In contrast, memory retrieval and working memory performance are impaired with acutely elevated levels of glucocorticoids. Glucocorticoid-induced modulation of these functions also appears to require the integrity of the BLA and the noradrenergic system. These dual effects of glucocorticoids on memory consolidation versus memory retrieval and working memory appear to be related in terms of function and neurobiological substrate. The BLA is a key structure in a memory modulatory system that regulates, in concert with other brain regions, glucocorticoid effects on these different cognitive processes.
Research supported by MH12526 Grant from NIMH.

14.02

STRESS AND COGNITION: THE INVOLVEMENT OF GLUCOCORTICOIDS AND CELL ADHESION MOLECULES

Carmen Sandi
Departmen of Psychobiology, Universidad Nacional de Educacion a Distancia, Madrid, Spain.

Exposure to stressful situations or to elevated levels of glucocorticoids modulates brain function and cognition. Besides, when solving stressful learning tasks, individuals also differ in their cognitive abilities. In this talk, I will summarize studies aimed to investigate whether individual differences in learning abilities shown by rats in a spatial learning task -the water maze- could be related to behavioural, endocrinological and/or neurobiological factors. Our results indicate that differences in basal and learning-induced corticosterone levels, as well as the hippocampal expression of cell adhesion molecules, are related to differential performance in the learning task. Furthermore, the relevance of psychobiological profiles, such as selecting rats according to the behavioural traits of locomotor reactivity to novelty or anxiety, will be discussed.

14.03

STRESS AND COGNITION: FOCUS ON CORTICOSTEROID ACTION IN LIMBIC BRAIN STRUCTURES

Melly S. Oitzl and E. Ron de Kloet
LACDR/LUMC, Division of Medical Pharmacology, University of Leiden, P.O.Box 9502, 2300 RA Leiden, The Netherlands.

Stress and the effect of the glucocorticoid hormones on the brain have been envisioned as being mainly negative. This view emerged principally from animal and human studies showing that stress or cumulative exposure to high levels of glucocorticoids can have a detrimental impact on hippocampal function: memory impairments as well as atrophy were reported. Why some individuals develop cognitive deficits after stress, while other individuals improve their cognitive performance under similar adverse conditions is still unresolved. We proposed and demonstrated that the action of glucocorticoids and the resulting effects depend on the context as well as the activation of mineralo- and glucocorticoid receptors in the brain (deKloet et al TINS, 1999, 22:422-6). Imbalance of these receptors appears to be more important in producing the effects of stress on hippocampal functioning than the actual levels of circulating glucocorticoids. Interestingly, most recent data from human studies taking into account the conditions critical for positive effects of glucocorticoids in animals, showed positive effects of glucocorticoids on cognitive functions (Lupien et al, J.Clin Endocrinol. Metatab 2002, 87, 3798-3807) indicating that it is time for a re-evaluation of the "dogma" of the predominantly negative influence of stress and its hormones. Finally, a pharmacological approach to counteract glucocorticoid-induced cognitive impairment will be addressed.

14.04

11ß-HYDROXYSTEROID DEHYDROGENASE TYPE 2 (11ß-HSD2), A PROTECTOR OF THE DEVELOPING BRAIN

Megan C. Holmes, Meharpal Sangra and Jonathan R. Seckl
Molecular Endocrinology and Dept of Clinical Neurosciences, Molecular Medicine Centre, Western General Hospital, Edinburgh EH4 2XU.

Glucocorticoids have profound effects in development, altering cell proliferation, differentiation and network formation. The developing fetus is protected from high maternal glucocorticoid levels by the presence of 11ß-HSD2 in the placenta and inhibition of the enzyme programmes adult health (hypertension, hyperglycaemia, insulin resistance). The brain is a sensitive target for glucocorticoids and hence a second barrier to their action is expression of 11ß-HSD2 in proliferating areas. We have used transgenic mice lacking expression of 11ß-HSD2 (11ß-HSD2ko) to determine the consequences of enzyme loss on brain development and behaviour. Consistent with corticosteroid overexposure, the mice have decreased birth weight (10%) and decreased postnatal growth of the cerebellum, an area of the brain still proliferating postnatally, regulated by glucocorticoids and normally protected by expression of 11ß-HSD2. At postnatal day (P)21, the midsagittal area of the cerebellum was reduced by 15% in the 11ß-HSD2ko mice compared to WT controls. Neuronal proliferation is unaltered in 11ß-HSD2ko mice. As adults, these mice develop an anxious phenotype assessed by the elevated plus maze (57% decrease in open arm entries), consistent with glucocorticoid programming. These results confirm the importance of 11ß-HSD2 as a protector from maternal glucocorticoids in the developing brain.

Wellcome Trust supported.

15.01

IDENTIFYING NEW GENES CONTRIBUTING TO ALZHEIMER'S DISEASE

Julie Williams
Department of Psychological Medicine, UWCM, Cardiff, UK.

Four genes have been identified which contribute to the development of Alzheimer's disease (AD), these comprise the Amyloid Precursor Protein Gene, Presenilin 1 & 2, and the Apolipoprotein E gene. Although these discoveries have contributed to our understanding of AD development, there are many questions still remaining. It has been estimated that at least 4 genes of similar or greater magnitude of effect to APOE remain to be identified. We performed a two-stage genome screen to search for novel susceptibility genes for the common form of AD occurring in later life (onset = 65 years). The first stage involved genotyping 292 affected sibling pairs using 237 markers spaced at approximately 20 cM intervals throughout the genome. In the second stage, we genotyped 451 affected sibling pairs (ASPs) with an additional 91 markers, in the 16 regions where the multipoint LOD score was greater than 1 in stage I. Ten regions maintained LOD scores in excess of 1 in stage II, on chromosomes 1 (peak B), 5, 6, 9 (peaks A and B), 10, 12, 19, 21, and X. Our strongest evidence for linkage was on chromosome 10, where we obtained a peak multipoint LOD score (MLS) of 3.9. The linked region on chromosome 10 spans approximately 44 cM from D10S1426 (59 cM) to D10S2327 (103 cM). In the Stage 3 analysis we tested for linkage to complex AD phenotypes using co-variate linkage analysis including factors such as age of onset, sex, linkage status (APOE and chromosome 10 loci), rate of decline, psychosis, agitation, aggression and depression. We observed significant linkage to age of onset in AD cases on chromosome 21 and found other areas which show evidence of linkage to complex AD phenotypes.

15.02

PROMISING BIOMARKERS IN DEMENTIA

Philip Scheltens
VU Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands.

A biomarker in dementia is needed to diagnose the underlying disease earlier and more correctly and to facilitate treatment studies to be carried out in an earlier phase of the disease. Ideally, a biomarker should also be used to monitor the progression of the disease and the treatment effect. The latter becomes more important in the light of disease modifying drugs becoming available in the drug trial arena.
A biomarker should be sensitive and specific, according to a recent consensus statement both need to be >85%, as well as reliable in and between raters and have a small measurement error, especially when needed to detect changes over time.
In dementia many biomarkers have come and gone, but a few remained that deserve attention. These included MR Imaging of the brain, enabling an assessment of focal atrophy such as in the medial temporal lobe as well as global atrophy, and visualizing vascular changes, such as white matter changes and lacunes. On another level CSF biomarkers have come into play, notably assessments of CSF A-beta amyloid, total tau and various forms of phosphorylated tau. Serum and urine markers have been developed also but do not yet reach the required accuracy levels.

15.03

COGNITIVE APPROACHES TO THE EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE AND THE FRONTOTEMPORAL DEMENTIAS

John Hodges
MRC Cognition and Brain Science Unit, 15 Chaucer Road, Cambridge,CB2 2EF.

The past decade has seen an explosion in knowledge concerning the cognitive deficits that characterise the early stages of neurodegenerative diseases. Modern neuropsychological techniques draw on the findings from neuropathology and are complemented by structural and functional neuroimaging methods. In Alzheimer's disease, I will focus on measures of medial temporal lobe dysfunction, particularly tests of episodic memory which involve cross-modal association. Fronto-temporal dementia (FTD), previously encompassed under the rubric of Pick's disease, is a common cause of dementia in younger patients. It is characterised by progressive atrophy of the ventro-medial frontal lobe and/or anterior temporal lobe, particularly the polar, perirhinal and fusiform cortex. The frontal and temporal lobe variants of FTD produce alterations in social cognition and progressive breakdown in semantic memory (semantic dementia), respectively. The talk will outline advances in characterising the neuropsychological deficits in FTD and the relationship to neuropathological changes.

15.04

NICOTINIC ACETYLCHOLINE RECEPTOR DISTRIBUTION STUDY IN NORMAL AND DISEASE HUMAN BRAIN TISSUE USING 5-[^{125}I]-A-85380

S. Pimlott, M. Piggott, J. Owens, L.E. Greally, J.A Court, E.K. Perry, D. Wyper
West of Scotland Radionuclide Dispensary, North Glasgow University Hospitals NHS Trust, Western Infirmary, Dumbarton Rd, Glasgow, G11 6NT, UK.

5-I-A85380 (5-IA) is a novel nicotinic acetylcholine receptor (nAChR) marker, binding predominantly to $\alpha4\beta2$ subtype. This in vitro autoradiography study describes the distribution of 5-[^{125}I]IA binding in post mortem brain tissue from normal elderly and cases with age-associated dementias of both neurodegenerative and vascular type. 5-[^{125}I]IA binding in normal brain tissue was found to be consistent with the reported distribution of other high affinity nicotinic ligands. Moderate 5-[^{125}I]IA binding was also seen in white matter tracts, indicating the presence of nAChRs along nerve fibre tracts. In Parkinson's disease (PD) loss of striatal 5-[^{125}I]IA binding closely parallels the loss of nigrostriatal dopaminergic markers previously observed. In dementia with Lewy bodies (DLB) reduced striatal 5-[^{125}I]IA binding density, comparable to that in PD, maybe a marker of early degeneration in nigrostriatal inputs, while in Alzheimer's disease (AD) reduced striatal 5-[^{125}I]IA binding could be related to reduced cortical inputs. The reductions of nAChRs seen in AD, DLB and PD were not apparent in vascular dementia (VaD). In conclusion 5-IA is clearly a useful ligand for both in vitro and in vivo single photon emission tomography human studies investigating disease symptoms and progression and in differentiating primary degenerative dementia from VaD.

15.05

CHOLINERGIC NEURONES AND MICROVASCULAR PATHOLOGY IN DEMENTIA

JS Keverne, JA Court, M Piggott, PG Ince, RN Kalaria
Institute for the Health of the Elderly and School of Neurology, Neurobiology and Psychiatry, University of Newcastle and Newcastle General Hospital, Newcastle upon Tyne NE4 6BE.

Clinical evidence has led to the hypothesis that cholinergic deficits underlie the cognitive decline seen in patients with Alzheimer's disease (AD). Such deficits have been attributed to decreased cholinergic neuronal markers and dysfunction of the nucleus basalis of Meynert (nBM). Cholinergic neurones from the nBM may also influence local blood flow via cerebral microvessels in the neocortex directly or through nitric oxide synthase interneurones. These links, together with the overlapping clinicopathological features of vascular dementia (VaD) and AD, support the possibility of a cholinergic deficit in VaD. We recently focused on the status of nbM neurones and cholinergic markers in a series of postmortem brains from subjects of 55 to 102 years of age diagnosed with different dementias including VaD and AD-VaD mixed types. Preliminary evidence suggests choline acetyltransferase activity to be significantly decreased not only in AD but also in VaD compared to age-matched controls. These observations are in accord with cerebral ischaemic injury models that exhibit region-specific decreases in cholinergic neuronal markers with greater susceptibility in older than in younger animals. Using confocal microscopy techniques we are currently comparing the neuritic arborisation of the nbM in relation to the microvascular pathology in demented subjects from the CFAS series.

Supported by grants from MRC (UK), Alzheimer's Association (USA) and Alzheimer's Research Trust (UK).

16.01

PROAPOPTOTIC SIGNALLING MODULES IN PRIMARY NEURONS AND ASTROCYTES

HK Wong, HW Yung, A Wyttenbach, E Morrison, A M Tolkovsky
Dept Biochemistry, Tennis Court Road, Cambridge, CB2 1QW.

In sympathetic neurons, there are two distinct modules that mediate apoptosis: one, induced by NGF-deprivation, is mediated by a JNK-dependent pathway whose function is suppressed by PI-3K/Akt signalling. The other, induced in response to cytosine arabinoside and other "DNA-damaging" agents is mediated by p53 and is suppressed in part by the ERK MAPK pathway. Recently, we have been investigating to what extent these pathways are used more generally by CNS neurons and astrocytes in response to environmental toxins, and whether different members of the pro-apoptotic Bcl-2 family 'streamline' or sort these different signals. Arsenite is an environmental pro-oxidant toxin for cortical neurons. Our studies show that it activates at least 3 pathways in cortical neurons: a JNK-dependent pathway, a p53-dependent pathway, and a p53-independent pathway, possibly mediated by p73/63. Each pathway induces a different set of pro-apoptotic members of the Bcl-2 family which together function to induce apoptosis. Though astrocytes are relatively resistant to pro-oxidants, their function too is compromised by oxidative damage. Some of the mediators of oxidative damage in astrocytes will be presented.

16.02

TRANSCRIPTIONAL CONTROL OF Bcl-2 FAMILY MEMBERS AND THE REGULATION OF PROGRAMMED CELL DEATH IN NEURONS

Jonathan Gilley, Paul J. Coffer and Jonathan Ham
Molecular Haematology and Cancer Biology Unit, Camelia Botnar Laboratories, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH.

Apoptosis occurs extensively during the development of the mammalian nervous system and is required for the establishment of neuronal populations of the correct size and for the formation of appropriate connections between neurons and their targets. In addition there is increasing evidence that neurons die by apoptosis following injury and during neurodegenerative diseases. To study the molecular mechanisms of neuronal apoptosis we have used developing sympathetic neurons, which require nerve growth factor (NGF) for survival. In the absence of NGF, sympathetic neurons die by apoptosis in a transcription-dependent manner. Expression of BIM, a proapoptotic member of the BCL-2 family, is induced by NGF deprivation in sympathetic neurons and BIM activity is required for NGF withdrawal-induced death. Recently we have investigated the role of FOXO transcription factors in the regulation of BIM expression by NGF. We find that overexpression of FOXO transcription factors induces BIM expression and promotes death of sympathetic neurons in a BIM-dependent manner. Additionally we find that FKHRL1 (FOXO3a) directly activates the bim promoter via two conserved FOXO binding sites, which are also essential for bim promoter activation following NGF withdrawal. Finally, we show that FOXO activity is required for the NGF deprivation-induced death of sympathetic neurons.

16.03

THE NEUROPROTECTIVE AGENT CHLOMETHIAZOLE INHIBITS p38 MAP KINASE AND INFLAMMATORY RESPONSES IN GLIAL CELLS

Anastasia Simi, Magnus Ingelman-Sundberg, and Niclas Tindberg
National Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

The neuroprotective properties of chlomethiazole have been acknowledged in several models of focal and global ischaemia in rodents. Chlomethiazole was previously shown to potentiate chloride currents at the GABAA receptor complex. We have found that chlomethiazole could also inhibit the c-fos/c-jun/AP-1 activation by lipopolysaccharide (LPS) and interleukin-1beta (IL-1β) in primary cultures of rat cortical glial cells. The inhibition of c-fos/c-jun/AP-1 was found to be due to inhibition of p38 MAP kinase (p38MAPK) activity. Given the important role of inflammatory mediators in cerebral ischaemia, we have also investigated the expression of putative p38MAPK-response genes such as IL-1β and inducible NOS (iNOS). We found that the IL-1β-induced expression of iNOS and NO production by glial cells were inhibited by CMZ, whereas IL-1β biosynthesis or release was unaffected. Similar results were obtained by using the p38MAPK inhibitor SB203580, suggesting that the effect of CMZ on iNOS is due to inhibition of p38MAPK. Since glial iNOS is implicated in neuronal death in the post-ischaemic brain, our data suggest that interfering with p38MAPK signalling and IL-1β effects in glial cells, is an important property of CMZ, which might be of relevance for the neuroprotective actions of the drug seen in vivo.

16.04

INTEGRIN ACTIVATION INHIBITS NEURONAL APOPTOSIS VIA Akt

Rosemary M Gibson, Susan E Craig, Laura Heenan, Cathy Tournier and Martin J Humphries
School of Biological Sciences, University of Manchester, Manchester, M13 9PT

Survival of many cell types depends on adhesion to the extracellular matrix (ECM). We are investigating the role of integrin-mediated adhesion to the ECM in the regulation of neuronal survival and function. The neuronal cell line Ntera2 spreads on fibronectin via the integrin α5β1. Detachment from fibronectin, addition of blocking β1 antibodies or peptides containing the RGD cell binding motif, accelerate apoptosis in the absence of serum-derived growth factors. In contrast, the antibody 12G10 which modulates the β1 integrin conformation in favour of the active form, significantly delays neuronal apoptosis. We have now characterised the signalling cascades initiated by adhesion of the neuronal cells to fibronectin and targeted by 12G10. Whilst adhesion to fibronectin activates multiple signalling pathways that may contribute to neuronal survival, the reduction of apoptosis mediated by 12G10 occurs specifically through an effect on the protein kinase AKT and its substrate GSK-3β. Improved understanding of these hierarchies of adhesion-dependent signalling and their effects on cellular survival and function in the central nervous system should help us design strategies to enhance neuronal survival during neurodegenerative disease.

17.01

REGULATION AND SPECIFICITY OF GABAA/5HT3 RECEPTOR ASSEMBLY AND TRANSPORT TO THE CELL SURFACE

Karen Bollan, Haiyan Tang, Laura Robertson & Christopher Connolly
Department of Pharmacology & Neuroscience, Ninewells Medical School, University of Dundee, Dundee, DD19SY.

Mammalian g-aminobutyric acid type A (GABAA) receptors are constructed from a large repertoire of subunits (α1-6, β1-3, γ1-3, δ, ε, θ and π) into a pentameric ion channel, creating an enormous potential for diversity. However, receptor assembly occurs via defined pathways, which serves to limit the diversity of GABAA receptors. Accurate quality control mechanisms are required to police receptor assembly, detaining inappropriate and partial complexes and ultimately destroying all the 'failures'. Two distinct quality control mechanisms operate on opposing sides of the membrane, ensuring that the correct tertiary and quaternary structures are achieved, prior to release onto the plasma membrane where inappropriate behaviour may be dangerous to the cell.Both mechanisms appear to rely on a requirement to mask undesirable exposed elements that function to retain proteins within the endoplasmic reticulum (ER). Within the lumenal domain, ER chaperones patrol newly synthesized proteins, binding to hydrophobic regions and incomplete N-linked glycosylation sites that identify partially folded/assembled subunits. On the cytoplasmic side, ER-retention signals within the primary sequence of newly synthesized proteins operate. All these signals need to be masked by subunit interactions before receptors can escape onto the surface.

17.02

DIFFERENTIAL CLUSTERING OF DYSTROPHIN AND GABAA RECEPTORS AT POSTSYNAPTIC SITES

Jean-Marc Fritschy, Ina Brünig, Barbara Studler
University of Zurich Institute of Pharmacology and Toxicology, Winterthurerstrasse 190, CH-8057 Zurich.

The mechanisms of postsynaptic clustering of GABAA receptors were investigated in primary hippocampal cultures that contain only few GABAergic neurons. Under these conditions, both GABAA receptors and gephyrin accumulated postsynaptically to glutamatergic terminals, forming mismatched synapses. The occurrence of mismatched synapses was highest in young cultures and decreased with development of GABAergic innervation. In contrast, dystrophin and the dystrophin-associated protein complex (DPC), which are extensively co-localized with GABAA receptors and gephyrin in hippocampus in vivo, were selectively found postsynaptic to GABAergic terminals only in vitro. In cultures derived from gamma2 subunit-deficient mice, no clustering of GABAA receptors and gephyrin was observed. However, the clustering of dystrophin and the DPC was not affected, indicating that it is independent of the presence of either GABAA receptors or gephyrin. These results suggest that signals specific for GABAergic synapses are necessary for the clustering of dystrophin, whereas non-specific signals present also in glutamatergic synapses are sufficient for the clustering of GABAA receptors and gephyrin. During development in vitro, these non-specific signals are superceded by specific signals to ensure appropriate match of neurotransmitter and their corresponding receptors in mature synapses.

17.03

REGULATING THE STABILITY OF GABA-A RECEPTORS IN NEURONAL MEMBRANES

Stephen J. Moss
MRC-LMCB, University College, Gower Street, London WC1E6BT.

A critical determinant for the efficacy of fast phasic inhibitory synaptic neurotransmission is the number of GABAA receptors at inhibitory synapses. It is becoming evident that synaptic GABAA receptors are not static entities, but are undergoing constitutive clathrin-dependent endocytosis, with short cell surface half-lives. Key to regulating this process, is the recruitment of GABAA receptors into clathrin-coated pits, which is facilitated by the direct interaction of individual receptor subunits with the adaptin AP-2 complex. Here, the mechanisms that control these protein-protein interactions will be described and the consequences of these highly regulated protein-protein interactions for synaptic inhibition will be discussed. In addition, the role that in receptor recycling plays in controlling GABAA receptor cell surface levels will also be highlighted with particular emphasis on a novel role for Huntington's associated protein.

17.04

REGULATION OF GEPHYRIN CLUSTERING BY FUNCTIONAL DOMAINS OF COLLYBISTIN

Kirsten Harvey1, Ian C. Duguid2, Mark I. Rees3, Trevor G. Smart2 and Robert J. Harvey1
1Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London, 2Department of Pharmacology, University College London, Gower Street, London, and 3Department of Molecular Medicine, Faculty of Medical and Health Sciences, University of Auckland, New Zealand.

Inhibitory glycine receptors (GlyRs) are clustered at synapses by the multi-domain protein gephyrin and the guanine nucleotide exchange factor (GEF) collybistin, which activates the small GTPase Cdc42. We have characterised novel variants of collybistin, which are created by alternative splicing of exons encoding an N-terminal src homology 3 (SH3) domain and three alternate C-termini. RT-PCR analysis, together with an examination of the rat, mouse and human collybistin genes, suggests that two of these variants (CB1 and CB2) are not present in man, whereas the CB3 isoform is expressed in all species. Interestingly, the presence of the SH3 domain in the CB1, CB2 or CB3 isoforms negatively regulates the ability of collybistin to translocate gephyrin into submembrane microaggregates in transfected mammalian cells. This finding is curious, since the majority of collybistin isoforms in vivo appear to harbour the SH3 domain. We have also mapped the binding sites for collybistin and the GlyR β subunit on gephyrin. Multimerisation of the C-terminal MoeA homology domain of gephyrin is required for robust binding to both collybistin and GlyR β. However, the putative collybistin binding motif is located within the `linker` region of gephyrin. Our findings suggest that gephyrin multimerisation is a requirement for interactions with certain accessory proteins.

18.01

GLIAL-DERIVED EXTRACELLULAR MATRIX IN NEURAL DEVELOPMENT AND REGENERATION

Andreas Faissner
Department of Cell Morphology and Molecular Neurobiology, Ruhr-University, Bochum, Germany.

Glycoproteins and proteoglycans of the extracellular matrix (ECM) mediate astroglial functions during neural development and regeneration. Tenascin-C glycoproteins (TN-C) are transiently expressed by astrocytes during CNS development and comprise EGF-type repeats, fibronectin-type-III (FNIII) modules and a carboxyterminus homologous to fibrinogen-β. A large number of isoforms are generated on the mRNA-level by combinatorial re-arrangement of FNIII domains at a unique splice site. The FNIII-modules of TN-C contain binding sites for various receptors and are involved in neuron binding, neuron migration and axon growth and guidance. In order to assess the functional relevance of isoform variants, distinct pairs of domains were expressed as Fc-chimeras and analysed for their effects on embryonic hippocampal neurons. One variant could be identified which promotes neurite outgrowth via interaction with the Ig-superfamily member F3/contactin. In some regions, TN-C co-localizes with the chondroitin sulfate proteoglycan DSD-1-PG/phosphacan. Phosphacan represents a splice variant of receptor phosphotyrosine phosphatase (RPTP)-β, a receptor of various Ig-CAMs and of TN-C. Phosphacan carries a particular carbohydrate modification named DSD-1-epitope which is specifically recognized by MAb 473HD. RPTP-isoforms and tenascin-C display a complex regulatory pattern in CNS lesions.

18.02

PROTEOGLYCANS AND REGENERATION AND PLASTICITY IN THE CNS

James Fawcett, Elizabeth Bradbury, Stephen McMahon, Tomasso Pizzorusso, Paolo Medini, Lamberto Maffei, Sabrina Chierzi, Richard Asher, Francesca Properzi
Cambridge University Centre for Brain Repair, Robinson Way, Cambridge CB2 2PY, King`s College London and Scuola Normale, Pisa.

The glial scar acts as a barrier to the regeneration of damaged axons and the main inhibitory molecules in it are inhibitory chondrointin sulphate proteoglycans (CSPGs). All CSPGs possess GAG chains of similar structure, and removal of GAG chains removes much of the inhibition from CSPGs in vitro. We therefore tested to see whether GAG digestion by chondroitinase would promote axon regeneration in vivo. We first treated mechanical lesions of the nigrostriatal tract, and saw regeneration of about 4% of axons back to their target. Next dorsal column lesions of the spinal cord at C4 were treated. Both sensory and corticospinal axons regenerated in treated cords, and there was rapid return of function. We hypothesised that some of the recovery might be due to enhanced plasticity. We therefore tested the effects of chondroitinase treatment in a plasticity model, ocular dominance shift in the visual cortex. Monocular deprivation in adult animals normally produces no ocular dominance shift. However in adult animals in which the cortex was treated with chondroitinase there was a large shift in response to monocular deprivation. CSPGs are therefore barrier-forming molecules that can block both axon growth and plasticity.

18.03

TENASCIN-R IN THE VERTEBRATE CNS - A PHYLOGENETIC VIEW TO GLIAL CELL FUNCTION

P. Pesheva
Neuro- and Tumor Cell Biology Group, Department of Nuclear Medicine, University of Bonn, Sigmund Freud Str. 25, 53105 Bonn, Germany. Email: penka.pesheva@ukb.uni-bonn.de

Glia-derived extracellular matrix (ECM) molecules are involved in the control of biological processes as essential as axonal outgrowth and myelination during CNS development or repair and supposed to contribute to the poor regenerative capacity of the adult mammalian CNS. However, much less is known about the expression and functional implication of CNS-associated inhibitory ECM proteins, such as tenascin-R (TN-R), in low vertebrates capable of CNS regeneration. Questions of particular interest in this context concern the (co-)evolution of (a) ligand-receptor pairs in the vertebrate CNS (at the phenomenological level) and (b) cellular response mechanisms associated with axonal growth and oligodendrocyte differentiation (at the functional level). By generating a panel of interspecies-specific monoclonal antibodies to TN-R, we address these questions in a series of comparative analyses of the expression pattern and functional implication of TN-R homologues in the CNS of different vertebrates (from fish to mammals). A special emphasis has been placed on some protein (i.e. F3/F11) and glycolipid cellular receptors for TN-R (i.e. disialogangliosides and sulfatides) identified in our group and shown to mediate a TN-R-dependent inhibition of neural cell adhesion and axonal outgrowth or oligodendrocyte differentiation.

18.04

OCCLUDIN AND ZONULA OCCLUDENS-1 DISRUPTION IN A NON-INVASIVE METHOD OF RAT BLOOD-BRAIN BARRIER DAMAGE.

C.L. Willis, L.A. Apandi, G. Clarke, C.C. Nolan, D.E. Ray
MRC Applied Neuroscience Group, Biomedical Sciences, Queens Medical Centre, University of Nottingham NG7 2UH.

Tight junctions between cerebral vascular endothelial cells are important in the formation of the blood-brain barrier (BBB). BBB breakdown is a feature of many CNS pathologies including cerebral ischaemia and multiple sclerosis. Systemic 3-chloropropanediol induces focal changes in vascular endothelium permeability with astroglial and neuronal loss. Confocal microscopy was used to follow in vivo changes in expression of the tight junction proteins, occludin and zonula occludens-1 (ZO-1). In control tissue, occludin and ZO-1 immunoreactivity appeared as a continuous network on laminin labeled blood vessels. Fibrinogen immunoreactivity, used as a marker of vessel damage, was confined to the lumen. However, 24h after dosing, occludin and ZO-1 immunoreactivity become discontinuous within lesioned areas. This coincided with the appearance of extravascular fibrinogen and loss of GFAP or vimentin positive astrocytes. By 6d, occludin and ZO-1 immunoreactivity was still discontinuous, although fibrinogen was again confined within the vasculature. There was some vascular remodeling, as visualized by increased laminin immunoreactivity, although in the absence of direct astrocytic contact. After 28d some occludin and ZO-1 staining was restored but remained incomplete. These studies enable us to investigate the role of VEGF and other factors which may regulate BBB permeability.

18.05

MATRIX METALLOPROTEASES IN REGENERATING OPTIC NERVES

Zubair Ahmed, Wendy E. Clarke, Russell G. Dent, Martin Berry and Ann Logan
Department of Medicine, University of Birmingham, Birmingham B15 2TT, UK.

We have shown that reduced levels of TGFβ a potent fibrogenic factor, which has a role in CNS scar formation, are not associated with attenuated scarring that is seen in a regenerating optic nerve model. Matrix metalloproteases (MMPs) are capable of degrading a wide variety of extracellular matrix (ECM) components and may facilitate remodeling of the ECM required to allow axons to regenerate. We investigated whether in vivo MMP levels were modulated in a scarring versus a non-scarring model of optic nerve injury using Western blotting, immunohistochemistry and zymography. Western blotting showed that MMP-1, -2 and -9 levels were higher in the regenerating optic nerves and retina compared to that in the non-regenerating model. Immunostaining revealed that MMP-1 and -9 were expressed at a similar intensity in the optic nerve and the retina in both models while MMP-2, MMP-3, TIMP-1 and TIMP-2 were lower in regenerating tissues. MMPs and TIMPs were co-localized with GFAP and CAII+ cells, while zymography showed that active MMP-2 and -9 were upregulated in both models. The results demonstrate that upregulation of MMPs and subsequent downregulation of TIMPs may be responsible for the attenuated scarring thus allowing a pathway for axons to regenerate.

19.01

CONTROL OF NEUROMUSCULAR JUNCTION ASSEMBLY AND MAINTENANCE IN THE MOUSE

P. Caroni, S. Pun, and A.F. Santos
Friedrich Miescher Institut, Basel, Switzerland.

Neuromuscular junctions (NMJ) assemble through two distinct patterns during mouse embryonic development. In FaSyn muscles, focal arrangements of Acetylcholine receptor (AChR) clusters, terminal Schwann cells (tSC) and motor nerves form within 0.5-1 day of the arrival of nerves at their target muscles. In contrast, focal NMJ assembly in DeSyn muscles is preceded by a 4-day phase of dispersed AChR microclusters, occasional apposition between these clusters and nerves, and extensive nerve sprouting. These distinct patterns of NMJ assembly reflect intrinsically distinct patterns of focal AChR cluster assembly in FaSyn and DeSyn muscles. In young adult mice, NMJs on DeSyn muscles respond to a blockade of transmitter release with a robust plasticity response, consisting of ultraterminal nerve sprouting, followed by AChR cluster disassembly, extensive collateral sprouting of nerves and formation of ectopic NMJs. This plasticity is inhibited by factors promoting AChR clustering, is lost progressively between 2 and 6 months postnatally, and is absent in FaSyn muscles. Non-plastic NMJs on FaSyn muscles are lost selectively and early on in a mouse model of motoneuron disease. Our results provide evidence for the existence of a plasticity pathway at this model synapse, controlled at the level of the postsynaptic receptor complex.

19.02

MULTI-DIRECTIONAL NEUROTROPHIN SIGNALLING DURING NEUROMUSCULAR SYNAPTOGENESIS, MAINTENANCE AND REPAIR

Rita Balice-Gordon
University of Pennsylvania Sch. of Medicine, Dept. of Neuroscience, Philadelphia, PA 19104-6074, USA.

Neurotrophins and the Trk family of tyrosine kinase receptors [TrkA (NGF), TrkB (BDNF, NT4/5), TrkC (NT3)] play many roles during development, but it remains unclear what role neurotrophin and Trk signaling plays at the level of the synapse. We are studying this question at neuromuscular synapses in vivo using recombinant adenoviruses to manipulate Trk signaling in different cell types. We found that TrkB and TrkC are localized to different cellular compartments. TrkB signaling in the postsynaptic membrane is required for AChR cluster maintenance. TrkC signaling modulates perisynaptic Schwann cell process extension that is important for successful reinnervation after nerve injury. Recent work focuses on identifying genes involved in synapse formation and maintenance in zebrafish. With Drs. M. Mullins and M. Granato, we have identified several mutants with defects in synapse number, size, maintenance, location and motility. We are analyzing synaptic structure and function in these mutants and using mapping techniques to determine the underlying genetic defects. Taken together, this work suggests that multidirectional signaling is required for the maturation and maintenance of pre- and postsynaptic specializations at neuromuscular synapses, and in modulating interactions with perisynaptic glia.

Supported by grants from the NIH (NS/AR40763, NS04519) and NSF (0130822).

19.03

SYNAPTIC GROWTH AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

Aaron DiAntonio
Dept of Molecular Biology, Washington University, School of Medicine, Campus Box 8103, 660 S Euclid,St.Louis, MO 63110 USA.

Synaptic morphology is dynamic; once formed, synapses evolve by expanding and contracting throughout life. This plasticity underlies the refinement of neuronal circuits during development and may be critical for learning and memory. To initiate a molecular analysis of synaptic growth, we have exploited the genetically tractable Drosophila neuromuscular junction as a model system. Results from our genetic screens identify the ubiquitin system as a potent regulator of synaptic growth and function. Genetic techniques that antagonize ubiquitination profoundly disrupt synaptic growth control. We have identified highwire, a synaptic protein that is a putative ubiquitin ligase, as a key negative regulator of synaptic growth. We are employing genetic techniques in the fly to identify molecular pathways that functionally interact with highwire. We are also investigating the potential roles of the vertebrate highwire homolog in mammalian CNS development.

19.04

SURVIVAL OF AXONS AND SYNAPSES IN INJURY AND DISEASE: THE WldS GENE

Thomas H. Gillingwater*, Bogdan Beirowski**, Till G.A. Mack**, Weiqian Mi**, Richard R. Ribchester**, Michael P. Coleman**
Department of Neuroscience, University of EdinburghZMMK and Institute for Genetics, University of Cologne.*

A chimeric gene, WldS, protects transected axons for several weeks and also offers partial protection of neuromuscular and other synapses. Synapses in 4-5 week old WldS mice are structurally and functionally preserved five or more days after axotomy, while in young adult mice the pattern and rate of synapse loss reverts to wild-type. The WldS gene is likely to work through altering neuronal ubiquitination, although whether such alterations occur in the axon, synapse, or even the cell body is as yet unclear. Structural preservation of the axon by WldS, especially in the distal regions, is stronger than that of the nerve terminal, indicating the existence of compartmentalised mechanisms controlling the survival of axons and their terminals. Further support for independent terminal and axonal degeneration comes from the asynchronous withdrawal or degeneration of nerve terminals after axotomy within a single motor unit. Weakening of synapse preservation with age appears to be a limiting factor in alleviation of diverse neurodegenerative diseases by WldS. This highlights the importance of identifying similar genes that control synapse preservation and of understanding how the WldS protein protects both axons and synapses.

20.01

CHANGES IN EXCITATORY SYNAPTIC INPUTS TO VENTRAL TEGMENTAL AREA (VTA) DOPAMINE CELLS IN RESPONSE TO AMPHETAMINE

Susan Jones(1), Johanna L. Gutlerner(2), Lavina Faleiro(2), and Julie A. Kauer(2)
1 Department of Anatomy, University of Cambridge, UK2 Department of Molecular Pharmacology, Brown University, USA.

Glutamate receptor antagonists administered into VTA block initiation of behavioural and neurochemical sensitization in response to repeated doses of amphetamine. One hypothesis is that amphetamine modifies glutamatergic input to VTA dopamine cells, changing VTA dopamine cell firing and dopamine release in the forebrain, and initiating behavioural sensitization. We have used rat (P15-23) midbrain slices to determine the effect of amphetamine on excitatory postsynaptic currents (EPSCs) in VTA dopamine cells. Low frequency stimulation of glutamatergic afferents to VTA dopamine cells induced long-lasting depression (LTD) of EPSCs. Amphetamine (1 uM, bath application) blocked the induction of LTD, suggesting that a single exposure to amphetamine increases excitatory drive to dopamine cells. In midbrain slices prepared 24h after one systemic dose of amphetamine (2.5 mg/kg/ip), the AMPA receptor component of the EPSC was increased relative to the NMDA receptor component. Cocaine also increases AMPA receptor EPSCs in VTA dopamine cells for 1-5 days after a single dose (Ungless et al., 2000), suggesting that different addictive drugs have a common effect on excitatory input to VTA dopamine cells. Glutamatergic inputs to VTA control dopamine cell firing; therefore, potentiation of glutamtergic EPSCs could facilitate dopamine cell firing and dopamine release in forebrain regions.

20.02

THE ROLE OF DOPAMINE RELEASE IN BEHAVIOUR

Michael H. Joseph
Behavioural Neuroscience Group, School of Psychology, University of Leicester, LE1 7RH, UK.

Dopamine release in the ventral striatum has often been suggested to be rewarding, or at least to underlie the behavioural effects of reward: reinforcement and incentive motivation. The DA releasing effects of drugs of abuse have been suggested to underlie their addictive potential. Many studies, more recently using in vivo methodology, have shown that rewards are indeed associated with DA release in this area, but it is striking that the magnitude of the increases appears to be remarkably consistent across different experiments, across different types and levels of reward, and across different levels of motivation. Furthermore, well established and predicted (or predictable) rewards are no longer associated with increased DA release, even though they are still behaviourally rewarding. These observations seem to make any simple link between DA and reward less likely. However, another striking, and by now many times replicated, finding is that aversive stimuli also increase DA release. Moreover neutral stimuli entering into associations with rewarding or aversive stimuli also acquire the ability to release DA.

20.03

DISCRETE CODING OF REWARD PROBABILITY AND UNCERTAINTY BY DOPAMINE NEURONS

C.D. Fiorillo, P.N. Tobler, W. Schultz
Department of Anatomy, Downing St., Cambridge CB2 3DY.

Dopamine-containing neurons of the ventral midbrain are known to play a critical role in reward and reinforcement. Prior electrophysiological studies have shown that these neurons respond with brief, phasic activation following errors in reward prediction. Prediction is concerned with the probability that a particular event will occur, and necessarily has a measure of uncertainty associated with it, which is maximal when probability equals 0.5 and is absent at probabilities of 0 or 1. We conditioned distinct stimuli in a Pavlovian manner to predict the probability of juice reward. The phasic activation of dopamine neurons varied monotonically with probability across the full range from 0 to 1. In addition, a new type of response was observed which consisted of a gradual increase in activity that reached its maximal level at the time of potential reward. This relatively sustained activation was entirely dependent on uncertainty, as it was maximal at a probability of 0.5 and entirely absent at probabilities of 0 and 1. Thus dopamine neurons separately code the two fundamental statistical parameters of reward probability and uncertainty. The coding of reward uncertainty suggests a possible role for dopamine signals in attention-based learning and risk-taking behavior.

20.04

METHAMPHETAMINE ACTIVATES REWARD CIRCUITRY IN DRUG NAÏVE HUMAN SUBJECTS: AN fMRI STUDY

Völlm B. 1), de Araujo I.E.T. 2), Kringelbach M.L. 2), Rolls E.T. 2) & Cowen P. 1), Paul M Matthews 3)
1) Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford OX3 7JX2) Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford, OX1 3UD 3) Centre for Functional Magnetic Resonance Imaging of the Brain, John Radcliffe Hospital Oxford, OX3 9DU.

Background: – Amphetamine has a number of effects on the mind, including hedonic effects and "mind-racing". The aim of the study was to use fMRI to investigate the areas of the brain activated by the dopaminergic stimulant m-amphetamine.
Methods: – Seven right-handed healthy subjects were studied. Saline was injected i.v. followed by m-amphetamine (0.15 mg per kg) in a single-blind design. Subjective ratings of "mind-racing" (0 to 4) were obtained every 60 seconds using a button box. SPM99 was used to investigate the brain activations modulated by amphetamine in two different models: an on-off model exploring the main effects of amphetamine, and a correlation model correlating the "mind-racing" ratings with changes in the BOLD signal.
Results: – The correlation model showed activations in areas of the medial orbitofrontal cortex, bilateral striatum, medio-dorsal thalamus and the anterior cingulate cortex. The on-off model also showed a large activation in the medial orbitofrontal cortex as well as activations in other parts of the brain including the anterior cingulate cortex, the ventral striatum, and the cerebellum.
Conclusions: – Our results show that iv amphetamine induces activations in brain brain areas that have been implicated with rewarding and locomotor-stimulant effects, and that these brain areas are in agreement with the distributions of D1 receptors.

20.05

FROM BRADYKINESIA TO STEREOTYPY: SIMULATED DOPAMINE REGULATION OF ACTION SELECTION IN A ROBOT MODEL OF THE BASAL GANGLIA

Prescott, T.J., Gurney, K., Gonzalez, F.M., Humphries, M., Redgrave, P.
Adaptive Behaviour Research Group,Department of Psychology, University of Sheffield, Western Bank, Sheffield, S10 2TN.

Manipulations of tonic dopamine levels in the basal ganglia are known to affect action selection (or behaviour switching). For instance, haloperidol (DA antagonist) can induce akinesia or bradykinesia, whilst apomorphine (DA agonist) can lead to behavioural stereotypy. To further the understanding of this aspect of basal ganglia function we have successfully embedded a high-level computational model of basal ganglia circuitry within the control architecture of a mobile robot engaged in a simulated foraging task. In this robot model we have observed similar outcomes from changes in simulated dopamine (simDA) to those seen in animal studies. First, lowering simDA below the normal tonic level causes bradykinesia and difficulty in initiating behaviours. Second, an increase in simDA can cause simultaneous selection of two behaviours resulting in an inappropriate mixture of two activity patterns; at the same time other candidate behaviours are excluded by a property of the basal ganglia model we term `selection limiting`. Where the behavioural `distortion` caused by dual selection prevents one of the active behaviours from completing, the robot can become stuck in a `behavioural trap`. This result suggests that one source of stereotypy could be the failure to deselect or interrupt the activity of two simultaneously selected behaviours.

21.01

PERINATAL BRAIN INJURY: HYPOXIA-ISCHAEMIA AND INFLAMMATION

Hagberg H, Hedtjärn M, Bry K, Lappalainen U, Gustafson-Brywe K, Eklind S, Arvidsson P, Mallard C
Depts of Physiology & Pharmacology and 2Obstetrics &Gynecology, Paediatrics, Göteborg University, Sweden.

Recent clinical and experimental evidence indicates that inflammatory mediators play an important role in the pathogenesis of perinatal hypoxia-ischaemia (HI). The inflammatory gene response was investigated using Affymetrix GeneChip (MG-U74Av2) and we found that 150 genes related to inflammation and adhesion were significantly upregulated at different time points after HI. The upregulation of several already documented proteins was confirmed and a number of other genes were identified e.g. minopontin (65x), mac-2 (21x), MIP-1 gamma (25x), MCP-5 (12x), MCP-3 (12x) and CD72 (8x). In order to study inflammatory lung-brain interaction, transgenic mice in which human IL-1 was expressed in the lungs of fetal and postnatal mice were generated with a doxycycline-inducible system controlled by Clara cell secretory protein (CCSP) promoter. Overexpression of IL-1 produced a state of chronic inflammation in the lung and the gene expression pattern in the CNS was affected (mRNA for neutrophil lipocalin, calgranulin S100A8, Complement component C1qB were all increased) but no brain lesions were detected.These data show that many genes related to the immune-inflammatory system are upregulated after HI in the immature brain and transgenic overexpression of IL-1 during late gestation in the lung affects the gene expression pattern also in the brain, which may affect its vulnerability.

21.02

DISRUPTION TO DEVELOPMENT BY INJURY

Pierre Gressens
INSERM E 9935 & Service de Neurologie Pediatrique, Hôpital Robert Debre, Paris, France.

The pathophysiology of perinatal brain lesions seems to be complex and multifactorial, involving not only hypoxic-ischemic insult but also other pre or perinatal factors including chorioamnionitis and excess production of inflammatory mediators, hormone and growth factor deficiencies, oxidative stress and genetic factors. The development and characterization of separate and complementary animal models should permit to tease out the cellular and molecular mechanisms underlying perinatal brain lesions. This paper deals with the role of glutamatergic receptors and the excitotoxic cascade in brain lesions occurring at different stages of brain development and with the impact of pro-inflammatory cytokines, oxidative stress, loss of maternal growth factors and maternal stress on these lesions which strikingly mimic some of the lesions associated with human cerebral palsy. This type of experimental approach has permitted to describe some of the cellular and molecular mechanisms involved in the pathophysiology of these perinatal brain lesions and to show that these mechanisms are partly dependent upon the glutamatergic receptors involved. Also, novel neuroprotective strategies aiming at modulating endogenous BDNF levels have been tested with success in these models. Finally, long-term electrophysiological, molecular and behavioural consequences of perinatal insults can be observed in adult survivors.

21.03

DAMAGE AND ABNORMAL DEVELOPMENT IN THE NEWDORN BRAIN: NEUROINFORMATIC APPROACHES

Huppi, P
Child Development Unit, Children's Hospital, University of Geneva, Switzerland.

During the last two trimesters of gestation the human cortex changes from a lissencephalic structure of one that is highly convoluted. Advanced Magnetic Resonance Imaging (MRI) techniques have recently provided us with new modalities to study human cortical development in vivo. Volumetric analysis of MRI data sets are achieved by segmentation of the imaged volume into tissue types. Applying these techniques to the study of the premature newborn, new insights into the modification of cortical development have been gained. Preterm infants with perinatal white matter injury were found to have not only reduced myelination but also modification of cortical development at term. Preterm infants exposed to postnatal corticosteroid treatment were found to have a 30% reduction in cortex. A similar reduction in cortex was found in preterm infants after intrauterine growth restriction. Elaboration of white matter connectivity is most likely the driving force for the folding of the cerebral cortex during development. Diffusion tensor imaging, another advanced MRI technique is the first method to allow in vivo evaluation of some of these events. Current advanced MR imaging techniques allow non-invasive methodologies to provide a diversity of information on variations in cortical development and underlying connectivity and hence functional integrity of the brain.

21.04

CONGENITAL HEMIPLEGIA: A POTENTIALLY TREATABLE DISORDER?

*Smith M R, *Dabydeen L, *Villagra F, +Rutherford M, +Mercuri E, +Cowan F, *Eyre J A
**Developmental Neuroscience, Sir James Spence Institute of Child Health, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP +Department of Neonatal Paediatrics, Hammersmith Hospital, London.*

Following perinatal damage to one motor cortex, fast conducting ipsilateral corticospinal (CS) projections from the undamaged hemisphere are present in adulthood. Our hypothesis is that during development ipsilateral CS projections from the undamaged hemisphere, which would normally be withdrawn, competitively displace surviving contralateral CS projections from the damaged hemisphere. Subjects with unilateral perinatal stroke involving the motor cortex were studied: (A) 12 longitudinally from birth and (B) 31 when aged between 3 - 5 years. EMG was recorded from biceps brachii. Transcranial magnetic stimulation (TMS) estimated central motor conduction delays (CMCD). (A) Initially TMS of the infarcted hemisphere evoked responses in contralateral biceps in all subjects. By 2 years responses could not be evoked in 6. (B) There were significant +ve correlations between severity of hemiplegia and absent or prolonged contralateral CMCDs from the infarcted hemisphere and abnormally fast ipsilateral CMCDs from the undamaged hemisphere. Withdrawal of surviving contralateral CS projections from the damaged hemisphere and persistence of fast ipsilateral CS projections from the undamaged hemisphere is associated with poor outcome. By analogy with amblyopia, interventions to improve the competitiveness of CS projections from the infarcted hemisphere may improve outcome.

21.05

THE CONTRIBUTION OF THE ACUTE PHASE RESPONSE TO CNS INJURY IS DEPENDENT ON AGE AND SITE OF LESION

Sandra J Campbell1, Angus Butchart1, Martine Bernardes-Silva2, Navara Anjum1, V.H. Perry2 and D.C. Anthony1
1Molecular Neuropathology Laboratory and 2CNS Inflammation Group, School of Biological Sciences, University of Southampton, Biomedical Sciences Building, Southampton, S016 7PX, UK.

Patterns of neutrophil recruitment that accompany pro-inflammatory challenges in the rodent CNS are dependent on site of injection and on stage of development. In part, this is due to local differences in chemokine production. But it may also reflect variation in the peripheral inflammatory response to acute CNS injury. We examined mRNA and protein expression of rat cytokine-induced neutrophil chemoattractants (CINCs) in the liver and serum of adult or juvenile rats after the focal microinjection of interleukin-1b into brain or spinal cord. 2h after the focal microinjection of interleukin-1b, elevated hepatic CINC mRNA and protein were observed. Also at 2h, elevated CINC protein was detected in serum, which was associated with increased numbers of neutrophils in both blood and, surprisingly, liver. Immunohistochemistry revealed the early expression of the CINC chemokines in the CNS microvasculature 2h after the challenge on luminal and abluminal endothelial surfaces. The hepatic chemokine response following an injury to the CNS was more pronounced after challenges to the spinal cord than to the brain and was more rapid in the juvenile animals than in the adult animals. Thus the characteristics of the peripheral inflammatory response are dependent on the site of injury and the stage of development.

22.01

ASSEMBLY OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS

R.A.Jeffrey McIlhinney#, Elisabeth Philipps$, Beatrice Le Bourdelles$, Sarah Grimwood$, Keith Wafford$, Satpal Sandhu$, and Paul Whiting$.
#MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford, OX1 3TH, UK. $Merck Sharp & Dohme Neuroscience Research Centre, Terling's Park, Eastwick Road, Harlow, Essex, CM20 2QR, UK.

The N-methyl-D aspartate receptor (NMDAR) requires both NR1 and NR2 subunits to form a functional ion channel. Despite the recent advances in our understanding of the contributions of these different subunits to both the function and pharmacology of the NMDAR, the precise subunit stoichiometry of the receptor and the regions of the subunits governing subunit interactions remain unclear. Previously we have shown that NR2 subunits are not transported to the cell surface unless they associate with NR1 subunits and, as a consequence, surface expression of NR2A can be used to monitor the association of the subunits in cells transfected with N- and C-terminally truncated NR1 subunits. By combining measurements of cell surface expression of NR2A with other biochemical methods, we have shown that the N-terminus of NR1 is critical for subunit association, whereas truncation of the C-terminus of NR1 before the last transmembrane region has no effect on the association of the subunits. Using a cell line, which can be induced to express the NMDA receptor we have found that functional NMDA receptors are present within 5 hours of subunit induction, and that there is a delay between the first appearance of the subunits and their stable association. These findings will be discussed in view of our current understanding of the assembly of the glutamate receptors.

22.02

GENETIC DISSECTION APPROACH TO STUDYING SIGNALLING PATHWAYS CONTROLLED BY NMDA RECEPTOR INTERACTING PROTEINS

Noboru Komiyama
Dept of Neuroscience, 1 George Square, Edinburgh, EH18 9JZ.

Patterns of neural activity mediated by NMDA receptors are known to play important roles on plasticity and development of central nervous system. However, signalling pathways downstream from NMDA receptors which are critical for those events are not yet clearly understood. In recent years a number of studies have shown that NMDA receptors can interact with a wide variety of molecules through cytoplasmic domains. These include signalling and scaffold proteins such as Synaptic GTPase-Activating Protein (SynGAP) and PSD-95. We report a genetic dissection approach to studying the NMDA receptor mediated signalling pathways, using mice carrying a single or compound mutations in these genes. Phenotypic comparison suggests that SynGAP signaling can be uncoupled from PSD-95, revealing multiple PSD-95 signaling pathways to synaptic plasticity. Study also shows differential regulation of special and temporal expression patterns of those genes and distinct function on neuronal development.

22.03

TRAFFICKING OF NMDA RECEPTORS

Robert J. Wenthold, Nathalie Sans, Kate Prybylowski, Kai Chang, Ya-Xian Wang, Claudia Racca, Steve Standley, Stefano Vicini, Rana Al-Hallaq, Ronald S. Petralia
NIH, Bethesda, MD and Georgetown University, Washington, DC.

In mammalian neurons, NMDA receptors (NMDARs) are selectively and dynamically targeted to dendrites and anchored at the postsynaptic density through interactions with PDZ proteins. However, little is known about how these receptors are sorted from the endoplasmic reticulum (ER) and Golgi apparatus to the synaptic membrane. Our recent data indicate that the ER plays a critical role in regulating the assembly of the NMDA receptor complex. In neurons, the ER extends into dendrites as well as into spines and could serve as a local reservoir for receptors awaiting synaptic insertion. We found that the synapse-associated protein 102 (SAP102) interacts with the PDZ binding domain of Sec8, a member of the exocyst complex implicated in targeting of membrane vesicles. This interaction begins in the ER and plays a role in the delivery of NMDARs to the cell surface in heterologous cells and neurons. A dominant/negative form of Sec8 blocks the surface delivery of NMDARs in heterologous cells and in neurons. Therefore, an exocyst-SAP102-NMDAR complex is an important component of NMDAR trafficking in the CNS.

23

WHAT IS 'WHAT': CAN THE CONCEPT BE TRANSPOSED FROM THE VISUAL TO THE AUDITORY DOMAIN?

Griffiths, T
Newcatle University Medical School, School of Neurosciences, Framlington Place, Newcastle, NE2 4HH.

The concept of distinct processing mechanisms for the analysis of spatial properties 'where' and object identification 'what' is widely accepted in the visual system. A similar dual mechanism has been proposed in the auditory system, supported by recording experiments in the macaque showing relative specificity for object features in the anterior and spatial features in the posterior part of the superior temporal lobe [1]. Human functional imaging supports a specialisation for spatial sound processing in the planum temporale in the posterior temporal lobe [2]. However, the processing of object properties such as pitch pattern shows less rigid demarcation between the anterior and posterior temporal lobes [3]. This, and other recent data from our group suggests a need for refinement of the model, and a refinement of the concept of 'what'.

1. Tian, B., Reser, D., Durham, A., Kustov, A. & Rauschecker, J.P. Science 292, 290-3 (2001).
2. Warren, J.D., Zielinski, B.A., Green, G.G.R., Rauschecker, J.P. & Griffiths, T.D. Neuron 34, 139-148 (2002).
3. Patterson, R.D., Uppenkamp, S., Johnsrude, I. & Griffiths, T.D. Neuron 36, 767-776 (2002).

24

THE ROLE OF TAU IN ALZHEIMER'S DISEASE – IT'S NOT HOW YOU START BUT HOW YOU FINISH!

Michael Hutton
Mayo Clinic, Jacksonville, Fl32224, USA.

Alzheimer's disease (AD) is the major cause of dementia in the elderly and is characterized by progressive cognitive decline, particularly of short-term memory with relative sparing of motor and sensory functions. Two major pathological lesions occur in AD; extracellular amyloid deposits termed senile plaques (SP) and intraneuronal inclusions called neurofibrillary tangles (NFT). SP are largely composed of Ab, a 40-43 residue peptide, that is generated by sequential proteolytic cleavage of the amyloid precursor protein (APP) by aspartyl protease activities. In contrast, NFT contain a mass of helical filaments, termed paired helical filaments, composed of hyperphosphorylated MAPt (tau) protein. In healthy neurons, tau is a soluble, microtubule associated protein that is thought to regulate the stability of the microtubule network and intracellular transport. However, during the pathogenesis of AD, tau becomes detatched from microtubules, is hyperphosphorylated and aggregates into neurofibrillary inclusions.
Recent consensus has focused on Ab as the primary etiological agent in AD, the so-called "amyloid cascade" hypothesis. This conclusion, is based mainly on genetic evidence specifically the observation that all AD-causing gene mutations, in the APP, PS1 and PS2 genes, alter APP processing or the structural characteristics of Ab such that amyloid deposition is increased. Given the conclusion that Ab likely starts the pathogenic cascade in AD what is the significance of tau to the disease process and is it a viable therapeutic target?
It has long been recognized that the progression of neurofibrillary pathology is exquisitely correlated with memory decline. In addition, the recent observation that mutations in tau cause Fronto-Temporal Dementia with Parkinsonism linked to chromosome 17 demonstrated that tau dysfunction is sufficient to cause neurodegeneration. Finally, the development of transgenic models that develop NFT has further demonstrated the close association between the appearance of these lesions and neurodegeneration. Together these observations suggest that while tau dysfunction is probably not the primary cause of AD it is a crucial event in the process of neurodegeneration.

25.01

DISTURBANCES OF BRAIN DEVELOPMENT AND BEHAVIOUR OF RATS AFTER PRENATAL HYPOXIA

Zhuravin I.A., Dubrovskaya N.M., Tumanova N.L., *Nalivaeva N.N., Plesneva S.A., Bahvalova O.V., Fedoseeva K.N., Kochkina E.G., *Fisk L., *Turner A.J.
*Institute of Evolutionary Physiology & Biochemistry, RAS, 44 M.Thorez av, 194223, St. Petersburg, Russia;*School of Biochemistry and Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK.*

This study reports the effect of prenatal hypoxia (3h, 7% oxygen, 13th day of gestation) on the development of the sensorimotor cortex (Cx), striatum (Str) and hippocampus (Hip) and rat behaviour during postnatal ontogenesis. The rats subjected to prenatal hypoxia have demonstrated compared to controls: 1) a delay in physiological development and later completion of behavioural reactions during early ontogenesis revealed by various tests; 2) reduced total density of neurones and necrotic areas in the Cx, increased degeneration of neurones in the Cx, Str and Hip observed by Nissl and Golgi methods; 3) changed pattern of AChE activity analysed by Ellman's procedure; 4) changes in the expression and processing of amyloid precursor protein (APP) revealed by immunoblotting; 5) decreased learning abilities. These data suggest that prenatal hypoxia leads to a significant neuronal loss and pathology of the development of the Cx, Str and Hip, which results in developmental deficit of new-born animals with further deterioration of their behaviour and learning. Hypoxia also affected cholinergic functions of these brain structures at the level of AChE and expression of APP, which might have implications in the development and learning deficit. All experiments were performed in accordance with the Animals (Scientific Procedures) Act 1986. Supported by RBRF (02-04-49385), RAS (00-11-292) and INTAS-01-0245.

25.02

3-D MODELLING AND GENE EXPRESSION MAPPING OF THE DEVELOPING HUMAN BRAIN

JM Kerwin1, T Strachan1, MK Scott1, S Sarma1, J Sharpe2, , L Puelles3, D Davidson2, RA Baldock2 and S Lindsay1
1.Institute of Human Genetics, University of Newcastle upon Tyne NE1 3BZ2.MRC Human Genetics Unit, Crewe Road, Edinburgh EH4 2XU3.Dept.Human Anatomy and Psychobiology, Univ.Murcia, Spain.

During a period of approximately 4 weeks (from 26 to 56 days of development; Carnegie stage [CS] 12 to CS23) the major subregions of the human brain are established and development proceeds from a simple neural tube to a highly complex three-dimensional structure. We have used the new technique of optical projection tomography (OPT; Sharpe et al [2002]) to create 3-D reconstructions spanning this period of organogenesis. Gene expression patterns can be mapped and anatomical regions identified in these OPT reconstructions using MAPaint,a computer programme developed as part of the Edinburgh Mouse Atlas Project (http://genex.hgu.mrc.ac.uk/). MAPaint provides any number of simultaneous section views of planes that can be selected at any arbitrary viewing orientation and position angle through the reconstructions. The high resolution OPT reconstructions and sophisticated software provide much increased speed of analysis of gene expression and anatomical data and greatly facilitate comparisons between developmental stages and between the developing human brain and that of other species. In the long-term, our aim is to link the 3-D reconstructions to an anatomical database and embed both within a custom-designed gene expression database in order to create an Electronic Atlas of the Developing Human Brain (http://www.ncl.ac.uk/ihg/EADHB/).

25.03

THE ROLE OF SDF-1 IN THE DEVELOPING CEREBRAL CORTEX

A. Liapi1, J. Parnavelas1 and B.Nadarajah2
1Department of Anatomy, University College London and 2School of Biological Sciences, University of Manchester.

Recent studies have reported that SDF-1, a chemokine initially identified in the immune system, is expressed in many regions of the nervous system including the cerebral cortex. To investigate the role of SDF-1 in the developing cerebral cortex, we first examined its spatio-temporal expression pattern using in situ hybridisation and immunohistochemistry. Our analysis showed that SDF-1 is present in the developing cortex at a time when neuronal proliferation and migration are at their peak. To investigate whether it plays a role in neuronal proliferation and differentiation, dissociated cortical cells were cultured in the presence of exogenous SDF-1-containing medium. These experiments indicate that this chemokine affects the proliferation and differentiation of cortical neurons in a dose-dependent manner. Furthermore, in order to examine whether it plays a role in the radial and tangential migration of cortical neurons, embryonic brain slices were cultured in the presence of SDF-1-containing medium. Our results showed that the applied SDF-1 impedes both the tangential and radial movements of neurons in the developing cortex. Thus, our results demonstrate a multifunctional role for SDF-1 in the developing cerebral cortex that is likely to be mediated via its G-protein coupled receptor CXCR4.

25.04

PHOSPHORYLATION OF DELTA SUBUNIT OF THE ACETYLCHOLINE RECEPTOR BY THETA PROTEIN KINASE C ISOFORM DURING NEONATAL SYNAPSE ELIMINATION AT THE NMJ

María A. Lanuza, Carmen M. González, Neus Garcia, Manel M. Santafé, Phillip G. Nelson*, Josep Tomas.
*Unitat d'Histologia i Neurobiologia, Facultat Medicina i Ciencies de la Salut, Universitat Rovira i Virgili, C/Sant Llorenç, 21, 43201 Reus, Spain*Section on Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA.*

We have recently reported a PKC dependent period between P4 and P8 in the postnatal synapse elimination process including axonal loss and muscle AChR organization. We detected theta-PKC at the synaptic area of the muscle fibers by confocal immunofluorescence. Because theta-PKC has been shown to be postnatally regulated in skeletal muscle during the development, we analyzed endplate maturation and synapse elimination in theta-PKC knockout mice. Neonatal mouse Levator auris longus muscle was stained immunohistochemically to detect both AChRs and axons. Mice lacking theta-PKC showed a delay in the postsynaptic AChR clusters maturation (the appearance of AChR-free areas by destabilization of certain receptors was retarded) and an initial block of synapse elimination process. We propose that there is an initial theta-PKC dependent period in the synapse elimination process. If theta-PKC plays a role in this process affecting AChR stabilization, delta subunit AChR could be the target of their phosphorylation action. By using phosphospecific, subunit specific antibodies to react with the phosphorylated and nonphosphorylated forms of the delta subunit of the AChR, we observed a deficient phosphorilation of the delta-AChR subunit in the K.O. animals in vivo.

25.05

ACTIVITY-INDEPENDENT ASYNCHRONOUS SYNAPSE WITHDRAWAL INDUCED BY AXOTOMY IN SINGLE MOTOR UNITS OF WLD MUTANT MICE

T.H. Gillingwater, D. Thomson, M.P. Coleman* & R.R. Ribchester
*Division of Neuroscience, University of Edinburgh, Edinburgh EH8 9JZ and *Institute for Genetics, University of Cologne, Cologne, Germany.*

Competitive, activity-dependent mechanisms regulate asynchronous elimination of neuromuscular synapses during development. Axotomy in WldS mutant mice induces non-competitive, asynchronous withdrawal of nerve terminals from motor endplates (Gillingwater et al., 2002;J.Physiol. 543,739-55). To address whether synapse withdrawal occurs asynchronously within single Wld-expressing motor units, we examined the relative occupancy of endplates in lumbrical muscles supplied by collateral branches of the same motoneurone, 5 days after axotomy (ketamine/xylazine anaesthesia, i.p.). First we reconstructed the complete terminal arbor of single motoneurones that also expressed yellow fluorescent protein. Second, we measured fractional endplate occupancies in pairs of terminals supplied by the same motoneurone in immunostained preparations. Third, we tested the role of spontaneous activity, by combining axotomy at different points along the sciatic nerve, with local injections of botulinum toxin (Type A;0.1 ng/g body weight). All three groups showed random, asynchronous retraction of nerve terminals within single motor units. A long-nerve stump significantly retarded the overall rate of axotomy-induced synapse withdrawal ($P<0.01$;Kruskal-Wallis test). The data suggest that an 'intrinsic withdrawal' mechanism could contribute to the asynchronous remodelling of motoneurone terminal arbors.

25.06

A GAIN-OF-FUNCTION MUTATION IN FGFR3 CAUSES ABNORMAL NEOCORTEX DEVELOPMENT

Suzanne L Inglis and Tomoko Iwata
Department of Pathology, University of Glasgow, Beatson Research Laboratories, Garscube Estate.

FGF2 plays important roles in neocortex development. Neural density and cerebral cortex thickness is significantly reduced in Fgf2 null mice. Fgfr3 is one of the 4 members of high-affinity tyrosine kinase receptors for FGF. FGF2 has been indicated to be a potent mitogenic ligand for Fgfr3 in vitro. Despite its well-known function in bone growth, the function of Fgfr3 in the brain remains unknown. We have previously generated a mouse with a gain-of-function mutation (K644E) in the Fgfr3 gene (Iwata et al., 2000). The model mirrors the characteristics of Thanatophoric Dysplasia type II in humans, including skeletal abnormalities and macrocephaly. We observed a significant increase in the neocortex thickness in mice with the Fgfr3-K644E mutation starting at embryonic day 12, indicating that the macrocephaly is caused by abnormal brain development. In situ hybridisation and immunohistochemistry showed that Fgfr3 is expressed in developmentally specific regions of the neocortex. The increase in cortex size may be caused by abnormal proliferation, differentiation, and migration of neuroepithelial cells. This work is in accordance with the Animals (Scientific Procedures) Act 1986.

25.07

FACTORS AFFECTING THE GUIDANCE OF DEVELOPING CHICK TRIGEMINAL GANGLIA AXONS

S. Cattermole and L. Robson
Neuroscience Department, Medical Sciences Building, Barts and London, Queen Mary`s School of Medicine and Dentistry, Mile End Road, London, E1 4NS.

Developing axons are guided to their final destination by both attractive and repulsive cues. These cues can work over either a short or a long range. Long-range cues involve the development of a concentration gradient for a diffusible factor. Previous studies have identified NT3 and BDNF as factors involved in trigeminal ganglia axon guidance to the maxilla. However, the role of these two factors is more in neuron survival rather than in directing the axons initially. The factor or factors that control the initial trajectories of the trigeminal axons remain to be fully elucidated. The aim of this study is to investigate which factors are able to direct axon growth from the developing chick trigeminal ganglia. A collagen gel culture system was used to study the growth and direction of the trigeminal ganglia axons; beads soaked in the factor were placed at a known distance from the trigeminal ganglia. After 48 hours the axon growth was assessed and the direction of the axons either towards or away from the bead noted. A variety of growth factors that are present in the developing maxilla during trigeminal axon out growth and members of the neurotrophins were tested for their axon guidance properties.

25.08

NEONATAL INCREASE OF NEUROTENSIN IN RAT MESENCEPHALON: A POTENTIAL TROPHIC ACTION ON DEVELOPING DOPAMINERGIC NEURONES

N. Azmi, P. M. Wigmore & G. W. Bennett.
School of Biomedical Sciences, University of Nottingham Medical School, Nottingham, NG7 2UH, UK.

The ontogenic study of the neuropeptide, neurotensin (NT), in perinatal rat brain shows changes consistent with a trophic role(1). In the present study, neurotensin-like immunoreactivity (NT-LI) levels were measured within telencephalon, diencephalon and ventral mesencephalon of perinatal Lister-hooded rat using a highly sensitive radioimmunoassay (RIA) for NT and a C-terminally directed antibody(2). Consistent with previous findings(1), NT-LI levels were initially low at embryonic day 20 (E20) in the telencephalon and diencephalon of the prenatal rat but gradually increased to reach a maximum at postnatal day 0 (P0) and P2, respectively . In addition, a transient NT peak appeared at P3 in the ventral mesencephalon which was approximately four fold higher than the levels measured at E20, P0, P1 and P5. The developmental profile of NT-LI levels in the ventral mesencephalon may provide new insights on a trophic role of NT on the early development of dopaminergic neurones. To address this, ongoing studies are examining the effect of NT in primary mesencephalic cultures.

NA is sponsored by Universiti Kebangsaan Malaysia, Malaysia.
1. Bennett, G.W. et al. (1998). Dev. Brain Res. 111; 189-196.
2. Holtom, P.E. et al. (2000). J. Neurosci. Methods 100; 151-156.

25.09

THE ROLE OF SPHINGOSINE-1-PHOSPHATE IN SCHWANN CELL DEVELOPMENT

S.C. Blake (1), H. Mellor (2), S. Lupton (3), and N.J. Scolding (1)
1 Institute of Clinical Neurosciences, University of Bristol, Frenchay Hospital, Bristol, BS16 1LE; 2 Department of Biochemistry, University of Bristol; 3 Address on request.

The family of G protein-coupled sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) receptors, previously known as EDG receptors, have been implicated in Schwann cell development, although their precise roles are unclear. We have investigated possible functions of S1P and LPA in primary Schwann cells from humanely killed rats and in the rat Schwann cell line, SCL4.1/F7.
S1P2/EDG5, S1P3/EDG3, and LPA1/EDG2 were the most prominent EDG receptors expressing messenger RNA in both primary Schwann cells and SCL4.1/F7 cells, and were found along the myelin sheath, but not in the axon, in adult rat teased sciatic nerve fibres.
Treatment of SCL4.1/F7 cells with S1P or LPA caused (1) a transient increase in activation of Rho family GTPases, followed by (2) formation of geodesic actin wreathes and actin-rich lamellipodia, and (3) an increase in cell migration. These effects were mimicked by SCL4.1/F7 cell conditioned medium (F7CM). Analysis of F7CM lipid extracts by thin layer chromatography showed a band that co-migrated with S1P. No band co-migrated with oleoyl-LPA. The presence of S1P in F7CM lipid extracts was confirmed by fluorimetric high performance liquid chromatography.
The results suggest that S1P may form an autocrine signal involved in Schwann cell development.

25.10

EARLY EXPRESSION OF CALCIUM-BINDING PROTEINS IN HUMAN DIENCEPHALON

I Bystron 1,2, Z. Molnar 3, C Blakemore 1
1 University Laboratory of Physiology, Oxford, UK; 2 Department of Morphology IEM, St Petersburg, Russia and 3 Department of Human Anatomy and Genetics, Oxford, UK.

Analysis of expression of homeobox genes and segmental neuronal mapping have provided new insights into the organization of the embryonic vertebrate brain (Puelles and Rubinstein 1993, Puelles and Verney 1998). We have examined the distribution of microtubule-associated protein (MAP2), calretinin (CR) and calbindin (CB) expression across the several neuromeric domains of the diencephalon in the early human brain at Carnegie stage 14 (approximately 33 days post-fertilizational). Human embryos were obtained with consent after legal abortions, following national guidelines in Russia. Chemoarchitectural analysis of the wall of the neural tube allowed us to distinguish the earliest neuronal population of the human diencephalon. MAP-positive cells (immature neurons) are distributed along the marginal zone, decreasing in density along caudorostral and lateromedial axes. The network of their processes constitutes the first component of the neuropil along the entire axis of prosomers 1-3 (p1-p3). Zecevic et al., 1999 and Verney, 2001 did not detect CR- or CB-immunoreactivity in the diencephalon at this stage of development. However, in our material, CR- and CB-immunoreactivity was present in the basal plate of the caudal diencephalic segments. Post-mitotic CB-immunoreactive cells were seen in the marginal zone of the basal plate of p1-p2, decreasing in density from lateral to medial. In contrast, in the alar plate, CB-positive cells were also found in the ventricular zone, with a mediolateral gradient of density. CB-positive cells were more abundant than CR-positive at this stage in development of the diencephalon. Only a few CR-positive cells were found in the basal plate of p1 and p2.
The pattern of distribution of CB immunoreactivity in the ventricular zone of the alar plate is not correlated with the level of proliferation. The functional role of CB expression in the germinal epithelium of the alar plate is unclear.

Supported by the MRC and the Royal Society.

26.01

THE FUNCTION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE PHARYNX OF *CAENORHABDITIS ELEGANS*

Chris J. Franks, Ben A. Edwards and David B. Sattelle
MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

To date, only one of 29 putative nicotinic acetylcholine receptor (nAChR) subunits identified in C. elegans has been implicated in the functioning of the pharynx. Raizen et al. (1995), using an extracellular recording technique, the EPG, demonstrated that electrotonic potentials associated with activity of the pharyngeal motoneuron MC were absent in loss of function mutants of the non-a nAChR subunit, EAT-2. We have confirmed these observations in worms expressing an eat-2 null allele, ad465. In intracellular recordings from wild-type (Bristol N2) pharyngeal muscle, nicotine elicits a dose-dependent depolarisation, in addition to an increase in the rate of action potential firing. However, the eat-2 (ad465) pharynx, although lacking MC associated EPG potentials remains sensitive to nicotine. The depolarising responses elicited by 10 μM nicotine, in both ad465 and N2 worms are 39.0±2 and 38.0±1.6 mV respectively, indicating that either the postsynaptic nAChR that contains the null ad465 allele of eat-2 is not itself functionally null, or that more than one nicotinic receptor subserves a role in the function of the pharynx. We are currently investigating the identity and function of other nAChR subunits in the pharynx.

26.02

THE NICOTINIC ACETYLCHOLINE RECEPTOR FAMILY OF *CAENORHABDITIS ELEGANS*

Andrew K. Jones, Behrooz Esmaeili, Jose L. Garrido and David B. Sattelle
MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

Nicotinic acetylcholine receptors (nAChRs) are prototypical members of the ligand-gated ion channel super-family. They are pentameric membrane proteins, which mediate the fast actions of the neurotransmitter acetylcholine (ACh) at the neuro-muscular junction and in the nervous system. To date, 29 putative nAChR subunits (21 α and 8 non-α) have been identified in C. elegans, which is by far the largest nAChR family yet observed. Why nematodes have such a diverse nAChR family (mammals are known to possess 9 α and 7 non-α subunits) has yet to be fully resolved. Previously, the C. elegans nAChR subunits have been subdivided into five major groups (Mongan et al. 2002. Protein Science 11:1162). Recent analysis of the C. elegans genome has identified two more potential ACR-16-like non-α subunits and an additional DEG-3-like α subunit candidate. In addition, 20 "outlier" subunits have been identified including six clustered non-α subunits on the T01H10 cosmid. To gain further understanding of this diverse nAChR family, GFP constructs are used to determine expression patterns and gene silencing by RNA interference is being used as a first approach to determine subunit functional roles.

26.03

DYNAMIC REDISTRIBUTION OF SPECTRIN ALPHA II IS A DOWNSTREAM EVENT OF THE Gq ALPHA (Gqa)-PHOSPHOLIPASE C (PLC) PATHWAY

M. Street, S. J. Marsh*, D. A. Brown* and N. J. Buckley
*Sch. of Biochem. & Molec. Biol., Sch. of Biomed. Sci., Univ. of Leeds, Leeds LS2 9JT, UK and * Dept. of Pharmacology, University College London, London, WC1E 6BT, UK.*

Spectrins are important component of the membrane cytoskeleton linking organised membrane domain to the filamentous cytoskeleton, and are found in the multiprotein complexes including membrane proteins that control neuronal excitability. To investigate the Gqa mediated signalling mechanism involving spectrin alpha II, CHO cells expressing the M1 mAChRs (M1-CHO cells) were transfected with a YFP- tagged spectrin alpha II (YFP-spectrin a II), and changes in its distribution were monitored. Addition of an M1 mAChR agonist oxotremorine (Oxo-M) caused membrane blebbing, and dynamic cytoskeletal reorganisation was demonstrated by redistribution of YFP-spectrin a II. Agonist induced membrane blebbing was a delayed and lengthy response, starting at 71s after the Oxo-M application and lasting for 369s. Both membrane blebbing and spectrin redistribution were dependent on the activation of PLCb. Although membrane blebbing is often associated with apoptosis, agonist induced membrane blebbing is not apoptotic. Neither phosphatidylserine externalization of plasma membranes nor nuclear condensation was observed in the Oxo-M stimulated M1-CHO cells. Our results suggest the involvement of membrane cytoskeleton reorganisation in the signal transduction pathway elicited by neurotransmitter.

This work is supported by the Wellcome Trust

26.04

MODULATION OF NICOTINIC RECEPTORS BY THE ACETYLCHOLINESTERASE INHIBITOR GALANTAMINE: POTENTIATION OF CALCIUM SIGNALS AND NEUROTRANSMITTER RELEASE

F. Dajas-Bailador and S. Wonnacott
Dept. of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, UK.

Neuronal nicotinic ACh receptors (nAChR) play an important role in learning and memory. The degeneration of basal forebrain cholinergic projections and the decrease of nAChR transmission could contribute to the imbalance of synaptic excitability and the cognitive impairments observed in Alzheimer's disease. Of the acetylcholinesterase (AChE) inhibitors clinically used, galantamine can allosterically modulate nAChR-mediated currents. This dual action (AChE inhibition-nAChR potentiation) might offer significant therapeutic advantages. Given the crucial role of calcium signals in neurones, we examined the role of galantamine in the modulation of intracellular calcium and a downstream cellular event: neurotransmitter release, in SH-SY5Y cells and hippocampal slices. Galantamine increased nicotine-evoked modulation of intracellular calcium and [3H]noradrenaline release. The potentiation of nAChR-mediated calcium signals and [3H]noradrenaline release showed a bell-shaped dose response; no potentiation was observed for KCl-evoked responses or when galantamine was added alone. These results support an allosteric interaction with nAChR and provide the first direct evidence for galantamine as a potentiator of nAChR-dependent calcium signals and transmitter release, processes that mediate many of the physiological consequences of nAChR activation.

Supported by Sanochemia Pharmazeutika.

26.05

α7 NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) SUBUNIT DISTRIBUTION IN HUMAN THALAMUSM

A. Ray, A. J. Graham, R. H. Perry, E. Jaros, E. K. Perry, J. A. Court
Joint MRC-University of Newcastle upon Tyne Centre Development in Clinical Brain Ageing, MRC Building, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, UK.

The homomeric α7 nicotinic acetylcholine receptor (nAChR) is an ionotropic receptor with reported roles in sensory and cognitive pathways. Previous ^{125}Iα-bungarotoxin (α-BTX) binding (1) and mRNA studies have identified the lateral geniculate nucleus (LGN) and reticular nucleus (Rt) as thalamic nuclei of high α7 nAChR expression. The present study investigates the distribution of the α7 nAChR subunit in post mortem human thalamus (n=6) using immunohistochemistry. Highest numbers of α7-immunoreactive (IR) neurons occurred in the LGN. In the Rt, α7 immunoreactivity was present on soma and neuropil but was not as marked as α-BTX autoradiography would suggest. Moderate numbers of α7-IR neurons occurred in the mediodorsal (MD), paraventricular (PV), ventral anterior (VA), lateral dorsal (LD) and ventral lateral (VL) nuclei with neuropil immunoreactivity ranging from absent (MD) to intense at the pial surface of PV. α7-IR astrocytes were observed in PV and MD. Immunofluorescent confocal microscopy suggested α7 co-localizes with GFAP but not synaptophysin in the Rt. These results may help to elucidate the role of the α7 nAChR in thalamic function.

1. Spurden et al, J. Chem. Neuroanat. 13:105-113 (1997).

26.06

A HUMAN α6α4 SUBUNIT CHIMERA FORMS FUNCTIONAL nAChRs WHEN COEXPRESSED WITH THE β4 SUBUNIT

Ruud Zwart, Giovanni Benedetti, Non M. Evans, Peter J. Craig, Suchira Bose, Jason P. Benton, Kathy P. Pearson, Gordon I. McPhie, Emma Bell, Steve G. Volsen, Lisa M. Broad and Emanuele Sher
Eli Lilly and Company Ltd., Sunninghill Road, Windlesham, Surrey, GU20 6PH, UK.

The nAChR α6 subunit distribution in the brain suggests a role for α6 in the nicotinic modulation of dopaminergic transmission, making them possible targets for anti-Parkinson's drugs. Functional expression of α6 subunit-containing nAChRs has been proven difficult, and therefore very little is known about the functional and pharmacological properties of α6 subunit-containing nAChRs.
We have tried to express the human α6 subunit together with a range of other human nAChR subunits in Xenopus oocytes and in HEK-293 cells, but no functional nAChRs were formed. In order to obtain nAChRs with α6 subunit-specific pharmacological properties, a chimeric subunit was constructed consisting of the extracellular domain of the α6 subunit, linked to the remainder of the human α4 subunit. In both Xenopus oocytes and in HEK-293 cells functional nAChRs were formed when the α6α4 chimera was coexpressed with the β4 subunit, but not with the β2 subunit. 100 nM α-conotoxin MII blocked the ACh-induced responses mediated by α6α4/β4 nAChRs, but not α4/β4 nAChR-mediated responses, demonstrating that both subtypes of nAChR have different pharmacological properties.The human α6α4/β4 chimeric nAChR stably transfected in HEK-293 cells may provide a valuable tool for the identification of potential therapeutic agents acting at α6 subunit-containing nAChRs.

26.07

INFLUENCE OF ALPHA1 AMINO ACIDS 185-196 ON THE PROPERTIES OF MOUSE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS

Purohit, P.G.[1] , Hartley, M.[2], Heinemann, S.F.[2], and Connolly, J.G.[1]
[1]Dept. of Physiology and Pharmacology, SIBS, University of Strathclyde, Glasgow, G4 0NR, U.K. [2]Molecular Neurobiology Laboratory, Salk Institute, La Jolla, U.S.A.

The mouse muscle nicotinic acetylcholine receptor (AChR, α1β1γδ) expressed in Xenopus oocytes is readily blocked by α-bungarotoxin, whereas the neuronal α3β2 AChR is not. Also, the relative potency of acetylcholine (ACh): nicotine(Nic) is 100:1 for muscle AChR but only 2:1 for α3β2.
To investigate these distinctions, a mutant a1 subunit (SM1) was constructed in which amino acids 185-196 of α1 were mutated to the corresponding α3 sequence. Following 11 minutes incubation with 10-100 nM toxin, the % inhibitions of control ACh responses of the mutant (SM1β1γδ) receptor were broadly similar to those of wild type muscle AChR, although recovery from blockade appeared to be more rapid for the mutant receptor. Also, the ACh:Nic potency ratio of the mutant receptor remained 100:1. Thus the 185-196 region of α1 does not completely determine toxin or nicotine sensitivity. However individually estimated Hill coefficients for the SM1 receptor were 0.83 ± 0.02 (± S.E.M) and 0.84 ± 0.05 for acetylcholine and nicotine respectively, (n=4). These slopes were significantly lower ($p<0.01$, Student's-t) than corresponding values for wild type muscle (1.53 ± 0.05, 1.73 ± 0.04) or neuronal (1.46 ± 0.11, 1.37 ± 0.01) receptors.

We acknowledge the support of the MRC and Royal Society.

26.08

MOLECULAR CLONING OF NOVEL DROSOPHILA NICOTINIC RECEPTOR SUBUNITS AND EXPRESSION OF SUBUNIT CHIMERAS IDENTIFIES NOVEL BUNGAROTOXIN-BINDING SUBUNITS

Stuart J. Lansdell and Neil S. Millar
Department of Pharmacology, University College London, London, UK.

Nicotinic acetylcholine receptors (nAChRs) play an important role in mediating fast synaptic transmission in the insect brain and are also target sites for neonicotinoid insecticides such as imidacloprid. To date, ten putative nAChR subunits have been identified from Drosophila melanogaster, but there have been no reports of the successful heterologous expression of recombinant nAChRs from this or any other insect. However, the ability of some cloned insect nAChR subunits to form functional recombinant receptors when co-expressed with vertebrate nAChR subunits, raises the possibility that these difficulties may be attributable to a requirement for additional, but as yet unidentified, insect subunits. We report here the molecular cloning and characterisation of two Drosophila subunits, Dalpha6 and Dalpha7 (the latter reported previously only as a partial cDNA clone). Heterologous expression of subunit chimeras containing the extracellular domains of Dalpha6 and Dalpha7 (as homomeric and heteromeric complexes) has revealed their ability to form a high affinity binding site for nicotinic ligands such as alpha-bungarotoxin. Construction of a series of additional subunit chimeras (between insect and mammalian nAChR subunits) is underway in an attempt to identify in more detail the influence of domains (present within these and other subunits) upon receptor folding and assembly.

26.09

PHARMACOLOGICAL CHARACTERISATION OF ALPHA9/ALPHA10 NICOTINIC RECEPTORS: EVIDENCE FOR HETEROMERIC CO-ASSEMBLY REVEALED BY EXPRESSION OF SUBUNIT CHIMERAS

Elizabeth R. Baker and Neil S. Millar
Department of Pharmacology, University College London, London, UK.

The nicotinic acetylcholine receptor (nAChR) alpha9 and alpha10 subunits are expressed primarily within hair cells of the inner ear and have been implicated as playing a role in auditory processing. Whilst functional recombinant alpha9 /alpha10 nAChRs have been described previously by expression in oocytes, there have been no reports of their successful heterologous expression in cultured cell lines. As with other nAChR subunuts (such as alpha7) this has been attributed to inefficient subunit folding/assembly and has hindered a detailed pharmacological characterisation. Chimeras, containing the extracellular domain of the alpha9 or alpha10 subunits fused to the C-terminal domain of the 5-hydroxytryptamine 3A subunit have been constructed and expressed. Specific binding of the nicotinic radioligand [3H]-methyllycaconitine was detected in cells cotransfected with both chimeras. In contrast little or no specific binding was detected with either subunit chimera alone, providing evidence of a requirement for heteromeric subunit co-assembly. Equilibrium radioligand binding studies provides evidence for an atypical pharmacological profile, in agreement with previous electrophysiological studies. Specific binding of several non-nicotinic ligands has been identified, including strychnine (a GlyR antagonist), bicuculline (a GABAAR antagonist) and atropine (a muscarinic AChR antagonist).

26.10

THE NICOTINIC ACETYLCHOLINE RECEPTOR FAMILY OF THE PUFFERFISH, *FUGU RUBRIPES*

Andrew K. Jones*, Greg Elgar** and David B. Sattelle*
MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK. Fugu Genomics, United Kingdom Human Genome Mapping Project Resource Centre, Wellcome Genome Campus, Hinxton Hall, Hinxton, Cambridgeshire, CB10 1SB, UK.*

Nicotinic acetylcholine receptors (nAChRs) mediate fast cholinergic synaptic transmission at nerve muscle junctions and in the brain. However, the complete gene family of nAChRs has not so far been reported for any vertebrate organism. We have identified the complete nAChR gene family from the reference genome of the pufferfish, Fugu rubripes. It consists of 16 α and 11 non-α candidate subunits, making it the largest vertebrate nAChR gene family known to date. The gene family includes an unusual set of muscle-like nAChR subunits comprising of two α1s, two β1s, one ε, one γ but no functional δ. However, one of the β1 subunits possesses an aspartate residue and N-glycosylation sites hitherto shown to be necessary for δ subunit function. Potential Fugu orthologs of neuronal nAChR subunits α2-4, α6 and β2-4 have been identified. Interestingly, the Fugu α5 counterpart appears to be a non-α subunit. Fugu possesses an expanded set of α7-9-like subunits and no α10 ortholog has been found. Two new candidate β subtypes, designated β5 and β6, are subunit types not so far found in the human genome. Comparison of the Fugu nAChR gene structures show they are considerably more diverse than those of higher vertebrates.

26.11

FUNCTIONAL GENOMICS OF THE DROSOPHILA MELANOGASTER NICOTINIC ACETYLCHOLINE RECEPTOR GENE FAMILY

1Jepson J, 1Brown L, 1Grauso M, 2Salvaterra PM, 1Sattelle DB.
1 = MRC Functional Genetics Unit, Dept. of Human Anatomy and Genetics, Oxford2 = Beckman Research Instituite, City of Hope, Duarte, USA.

There are ten genes encoding nicotinic acetylcholine receptor (nAChR) subunits (7α and 3β) in Drosophila. Molecular and functional diversity is also greatly increased by the presence of different alleles, alternative splicing and RNA editing.
We are investigating the functional roles of nAChRs within the Drosophila nervous system using optical, electrophysiological and molecular approaches. We have examined the prevalence and characteristics of nicotinic responses in a cha-1 GAL4: UAS-GFP Drosophila line, in which cholinergic neurones in the fly brain are tagged with GFP. Using fura-2 based CCD imaging on GFP cells from 3rd instar larvae, we observed an elevation of [Ca2+ii in response to 0.5uM nicotine in approximately 30% of GFP-tagged cells. These responses to nicotine were reversibly inhibited by 1uM mecamylamine. To study the nAChR subunits responsible for these responses we are combining microarray analysis, dsRNA-mediated gene silencing and physiology. Initial microarray data indicates that three alpha subunits are significantly up-regulated in cholinergic cells. We have therefore incubated Drosophila embryos in dsRNA corresponding to these and other nAChR-encoding genes, in order to examine how the silencing of particular subunits effects nicotinic responses.

26.12

CAN THE YEAST TWO-HYBRID SYSTEM BE USED TO STUDY ASSEMBLY DOMAINS OF GABA-A RECEPTOR SUBUNITS?

M.J. Smith M. Beck and F.A. Stephenson
School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK.

Assembly domains in multi-subunit membrane proteins such as K+ channels and Na+/K+ ATPase have been studied using the yeast two-hybrid system. Assembly motifs for GABA-A receptors are contained within subunit extracellular N-termini. The yeast two-hybrid system was thus investigated for use in delineation of these domains. The most abundant GABA-A receptor in adult brain contains 2α1, 2β2, 1γ2 subunit. The GABA-A receptor sequences, α1(1-224) and β2(1-220), were cloned in-frame into fish and bait plasmids of both the GAL4 and modified (m) LexA two-hybrid systems. Various combinations of GAL4 and mLexA fish and bait vectors were co-transformed into yeast strains AH109 (GAL4) and L40 (mLexA). Protein-protein interactions were assayed by colony growth on selective media and β-galactosidase reporter gene activity. Appropriate empty vector controls were carried out in parallel. Although reporter gene activity was produced following co-transformation of α1(1-224) and β2(1-220) both activities were evident when the bait vector contained no insert implying that both N-terminal sequences yielded auto-activation of reporter genes. It is concluded that unfortunately neither the GAL4 nor the mLexA yeast two-hybrid system are suitable for the study of assembly domains of GABA-A receptor α1 and β2 subunits.

26.13

THE PUTATIVE GABA-A RECEPTOR TRAFFICKING FACTOR, GRIF-1, IS A HOMO-OLIGOMER

G.S. Ojla, M. Beck, K. Brickley and F.A. Stephenson.
School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK.

GABA-A receptor interacting factor (GRIF-1) is a novel 913 aa protein that has been proposed to function as a trafficking molecule for [beta]2 subunit-containing GABA-A receptors (1). To gain insight into the function of GRIF-1, GRIF-1(218-913) was used as a bait in a rat brain yeast two-hybrid screen to identify GRIF-1 interacting proteins. Among others, a 1.8 kb interacting clone was isolated. Nucleotide sequencing revealed that the insert encoded GRIF-1 (8-633) implying that GRIF-1 is able to dimerize. This was investigated further by an immunoprecipitation strategy. GRIF-1 was epitope-tagged at the N- and C-termini with the c-Myc and FLAG epitopes respectively. Tagged constructs were co-expressed in human embryonic kidney (HEK) 293 cells. Anti-FLAG and non-immune Ig antibody immunoprecipitations were carried out from soluble fractions of transfected cell homogenates and immune pellets analysed by immunoblotting. It was found that anti-FLAG antibodies immunoprecipitated both GRIF-1-FLAG and GRIF-1-c-Myc. Control experiments in which GRIF-1-FLAG and GRIF-1-c-Myc were expressed independently in HEK 293 cells, the respective soluble fractions mixed and anti-FLAG immunopreciptations carried out, showed no co-association of GRIF-1 FLAG and GRIF-1-c-Myc.
These two approaches suggest that recombinant GRIF-1 is at least a homo-dimer.

1. Beck, M et al., (2002) J. Biol. Chem. 277.

26.14

GABA(A) RECEPTOR ASSEMBLY SIGNALS EXPRESS SUBUNIT SELECTIVITY

K.Bollan; H.Tang; C.N.Connolly
Departement of Pharmacology & Neuroscience, Dundee University,Dundee,UK.

GABA(A) receptors are the major inhibitory ionotropic receptors in the CNS. These receptors are constructed from a range of available subunits(α1-6, β1-3, γ1-3, δ, ε and θ.) It is clear that GABA(A) receptor assembly is normally a prerequisite for cell surface expression. Unassembled subunits are retained in the endoplasmic reticulum (ER) by an association with the ER chaperone proteins, BiP and calnexin. Despite the enormous potential for receptor diversity, relatively few functionally distinct receptors are thought to exist. How could receptor diversity be limited? One possible mechanism would be that preferential interactions between certain subunit combinations occur at the expense of other combinations.
Assembly signals in GABA(A) receptors have been identified in the α1, β2/3 and γ2. Multiple signals appear to exists, with distinct domains being responsible for interaction with specific receptor subunit classes. We have determined that differences between assembly signals of α 1 and γ 2 determine their differential ability to assemble with β subunits.

26.15

CEREBELLAR GRANULE CELL-SPECIFIC GABA-A RECEPTOR ABNORMALITIES IN STARGAZER MICE

J.H. Ives1; P. Tiwari1; W. Sieghart2; J.M. Lucocq3, C.E. Young1, H. Payne1, C.L. Thompson1
(1) School of Biological and Biomedical Sciences, University of Durham, Durham, UK.(2) Division of Biochemistry and Molecular Biology, Brain Research Institute, Vienna, Austria.(3) School of Life Sciences, University of Dundee, Dundee, UK.

Stargazin expression is completely ablated in the cerebellum of the spontaneously ataxic mouse, stargazer (stg). We provide compelling evidence that stargazin plays an intimate role in the assembly and/or trafficking of GABA-A receptor (GABAR) subtypes in cerebellar granule cells (CGCs). The BMAX of high-affinity [3H] muscimol binding sites in stg was only 39 ± 7% of control (+/+) level. In situ autoradiography revealed that these binding sites were specifically down-regulated in the CGC layer of stg. Total specific Ro15-4513 binding sites (g2-containing GABARs) was not significantly affected. Thus, expression of a6d-containing GABARs appeared to be specifically impaired by the stargazer mutation. Steady-state mRNA levels for GABAR α1, α6, β2, β3, γ2 and δ in stg was not different to +/+. However, cell surface expression of α6 and δ was down-regulated in cultured CGCs from stg. By EM immunocytochemistry we have shown that cell surface expression of extrasynaptic and, to a lesser extent, synaptic GABAR alpha6, in situ, is severely impaired. A proteomics approach has been activated to prove our concept that stargazin plays a direct role in the assembly and trafficking of the exclusively extrasynaptic α6/δ-subtype of GABARs, *in vivo*.

Funded by The Wellcome Trust and AstraZeneca UK Ltd.

26.16

MODULATION OF [35S]TBPS BINDING IN MOUSE BRAIN

L.R.Fish; E.Philipps; A.D.Smith; S.M.Cook; E.Egan, T.A.Brown; K.Wafford; J.R.Atack
Merck Sharp & Dohme, Terlings Park, Essex, CM20 2QR.

GABAA receptors are pentameric GABA-gated ion channels that have a TBPS binding site located in the channel pore. [35S]TBPS binding can be modulated in vitro by compounds acting at the GABAA receptor. The aim of this study was to determine if [35S]TBPS binding could be modulated in vivo by compounds acting at allosteric sites on the GABAA receptor using mouse brain and an in vivo receptor occupancy assay (Atack et al., 1999). Maximal [35S]TBPS binding was observed 1 min after i.v. administration in a time course experiment. Dose dependent inhibition of [35S]TBPS binding by etomidate was demonstrated with significant decrease in [35S]TBPS binding between vehicle and 3 and 30 mg/kg (48 ± 6% and 82 ± 9% respectively, $P<0.01$; n=5). Other compounds acting on the GABAA receptor were investigated such as an agonist (diazepam), partial agonist (bretazenil) and antagonist (Ro15-1788) of the benzodiazepine site, loreclezole and the convulsant pentylenetetrazole. The results demonstrate that [35S]TBPS binding at the GABAA receptor can be modulated in vivo.

Atack JR et al., (1999) Neuropsychopharm. 20: 255-262.

26.17

PROBING THE ZN^{2+} POTENTIATING SITE IN GLYCINE RECEPTORS

P. Miller & T. Smart
Pharmacology Department, Medical Sciences Building, UCL, Gower Street, London, WC1 6BT.

The divalent cation Zn2+, at low concentrations, has previously been shown to potentiate submaximal glycine evoked whole cell responses on ionotropic glycine receptors (GlyR). Here, low potentiating concentrations of Zn2+ in the presence of the chelator tricine were co-applied with glycine to cells expressing either human GlyR alpha1 or alpha2 subtypes. This revealed a 10-fold shift in sensitivity to Zn2+ potentiation between the two receptors. A site-directed mutagenesis strategy was implemented to replace alpha2 residues with alpha1 equivalents at points of variation within the N-terminus. Using electrophysiological whole cell recording, a critical aspartate residue was found to be responsible for this differential sensitivity. The alpha1 N-terminus was modelled upon the crystal structure of the AChBP to identify other potential Zn2+ binding residues positioned spatially within the region of the critical aspartate. This approach identified two more potential binding residues, a histidine and a glutamate. Additionally, an essential threonine was also discovered, which reversed the phenotype of the potentiating Zn2+ site to that of an inhibitory site. These data identify residues that are involved in Zn2+ binding to the potentiation site and the subsequent signal transduction in GlyRs.

26.18

THE *C. ELEGANS LEV-8* GENE ENCODES A MULTIFUNCTIONAL NICOTINIC ACETYLCHOLINE RECEPTOR A SUBUNIT, ACR-13, EXPRESSED IN NEURONS, MUSCLE AND EPITHELIAL CELLS

Paula R. Towers and David B. Sattelle*.
MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

We have cloned and sequenced ACR-13, a nicotinic acetylcholine receptor (nAChR) a subunit from the ACR-8 like group of nAChR subunits in the nematode Caenorhabditis elegans. ACR-13 shares with other members of this subunit group an unusual YxxCC motif in loop C of the acetylcholine binding site and the presence of a histidine instead of a glutamic acid residue at the –1' position in the second transmembrane (M2) channel lining region. The acr-13 gene is located on Chromosome X and its position on the physical map is close to that of lev-8 on the corresponding genetic map. A single base pair change in the first intron of ACR-13 in lev-8 mutant worms leads to alternative splicing which introduces a premature stop codon. The lev-8 worms are partially resistant to levamisole-induced egg laying and paralysis. This phenotype is rescued by injection of the wild-type gene and its promoter region into the gonad of the worm. The lev-8 worms also show reduced rates of pharyngeal pumping and defecation. An ACR-13::GFP reporter construct shows ACR-13 expression in motor neurons, body wall muscle and the muscles of the vulva. There is extensive neuronal expression in the head of the worm as well as in the epithelial socket cells involved in sensory perception. Thus, ACR-13 is the first C. elegans nAChR subunit shown to be expressed in neurons, muscle cells and epithelial-derived cells.

27.01

CEREBELLAR PURKINJE CELL VOLTAGE-GATED POTASSIUM CURRENTS ARE SIGNIFICANTLY REDUCED IN Kv3.3 KNOCKOUT MICE

N.P. Morris1, E. Veale2, N. Heintz3, B. Rudy3 & B. Robertson4.
1 School of Biomedical Sciences, University of Leeds, Leeds. 2 Department of Biological Sciences, Imperial College London. 3 Department of Physiology & Neuroscience, New York University School of Medicine, New York. 4 Department of Physiology & Pharmacology, University of Strathclyde, Glasgow.

We investigated the effect of Kv3.3 subunit knockout (KO) on the electrophysiological properties of Purkinje cells. Experiments were performed using outside-out patch recordings in cerebellar slices obtained from Kv3.3 KO mice, and age-matched wildtype (WT) littermates, using procedures in accordance with the Animals (Scientific Procedures) Act 1986.
Peak voltage-gated K+ conductance in WT animals was 9.1 ± 1.7 nS (mean ± S.E.M; n = 6 cells) on voltage steps to +60 mV, but only 2.5 ± 0.4 nS ($P < 0.01$, Student`s T test, n = 13) in Kv3.3 KO. Potassium conductance - voltage curves from WT mice were well-fitted with a single, first order Boltzmann function yielding a mean V1/2 of -13.5 ± 3.2 mV (n = 5) and slope factor (k) of 13.5 ± 1.4 mV (n = 5). In Kv3.3 KO animals, similar fits yielded a more negative V1/2 of -29.1 ± 2.5 mV (n = 9) with k = 11.9 ± 1.9 mV (n = 9).
The results suggest that Kv3.3 channel subunits constitute a major component of the outward potassium current in cerebellar Purkinje cells, which may have an important functional role in contributing to their complex firing patterns.

Our work is supported by The Wellcome Trust.

27.02

ANANDAMIDE (AEA) PRODUCES DIVERSE ACTIONS INVOLVING CANNABINOID AND VANILLOID RECEPTORS ON RAT CULTURED DRG NEURONES

Rhian Evans, Ruth Ross & Roderick Scott
Dept. of Biomedical Sciences, IMS, The University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD.

There is evidence that the endogenous cannabinoid, AEA activates cannabinoid CB1, vanilloid TRPV1 and novel CB-like receptors. We investigated the receptors mediating effects of AEA in neonatal rat cultured dorsal root ganglion (DRG) neurones using whole-cell patch clamp recording. Extracellular application of 100nM AEA inhibited high voltage-activated Ca2+ currents (VACCs) by 22 ± 7% (n=8). The CB1 receptor inverse agonist, SR141716A (100nM) did not produce an enhancement of the VACCs, and failed to attenuate the inhibition of VACCs by AEA (100nM; n=4). Intracellular application of 100nM AEA elicited TRPV1 receptor-mediated inward currents, the mean population response was –0.85 ± 0.21pA (n=21; 13 responding neurones). Under control conditions and when AEA was applied with capsazepine (1μM) to the intracellular environment the mean population inward currents were -0.09 ± 0.05pA (n=13; 3 spontaneously active neurones) –0.01 ± 0.01pA (n=14; 1 responding neurone) respectively. In contrast, extracellular application of AEA evoked currents, only at higher concentrations of 10 and 100μM, suggesting that TRPV1-mediated responses are most effectively activated by intracellular AEA compared with extracellular application. In conclusion, we have evidence that CB-like (resistant to SR141716A) and TRPV1 receptors are involved in AEA-evoked responses in DRG neurones.

28.01

MLCK INHIBITORS BLOCK STYRYL DYE LOSS BUT NOT TRANSMITTER RELEASE AT RAT NEUROMUSCULAR JUNCTIONS

G. S. Bewick, J. E. Rudling, A. Cuthbert, R. McWilliam, B. Reid.
Department of Biomedical Sciences, Institute of Medical Sciences, University of Aberdeen, AB25 2ZD, UK.

MLCK inhibition blocks synaptic vesicle (SV) mobilisation from the reserve to the readily releasable pool (RRP) (Ryan, 1999, J.Neurosci., 19: 1317-1323). Effects of specific MLCK inhibitors on neurotransmitter release have not been demonstrated, although staurosporine (broad spectrum kinase inhibitor) increases end-plate potential (EPP) rundown during, and slows amplitude recovery after, tonic stimulation at frog neuromuscular junctions (Becherer et al, 2001, J.Neurosci., 21: 782-787). We evaluated the effects of several kinase inhibitors on SV mobilisation (by FM1-43 and FM2-10 loss) and transmitter release (quantal content (QC) rundown) in rat extensor digitorum longus (EDL) motor terminals during 20Hz stimulation in vitro. EDL preparations excised from adult (>350g) male Sprague-Dawley rats killed by Schedule 1 (Animals (Scientific Procedures) Act 1986) were paralysed with u-conotoxin GIIIB (2uM) to permit recording. MLCK-active inhibitors (staurosporine, 2uM - broad spectrum; wortmannin, 1uM - MLCK & PI3-kinase; ML-9, 30uM – MLCK specific) all abolished dye loss from labelled terminals, but PI3-kinase inhibition (LY294002, 1uM - PI3-kinase specific) had no effect. None of these inhibitors affected initial QC, QC rundown or miniature EPP amplitude. The data indicate reserve pool mobilisation is not required to maintain transmitter release during tonic activity and suggest rapid RRP recycling can suffice under these conditions.

28.02

MODULATION OF ADENOSINE RESPONSES BY NITRIC OXIDE IN HIPPOCAMPAL SLICES

Ali Shahraki, Trevor W. Stone
Room 330, West Medical Building, Glasgow University, Glasgow G12 8QQ.

Activation of NMDA receptors has been shown to suppress neuronal responses to adenosine, so we have examined whether NO is able to modify neuronal responses to adenosine and mediate the actions of NMDA. Experiments were performed on hippocampal slices in which evoked field excitatory postsynaptic potentials (fEPSPs) were recorded extracellularly from the CA1 region. Adenosine 10μM depressed single fEPSPs by 54.46% ±5.6 (n = 4) of the control size. The superfusion of S-nitroso-N-acetylpenicillamine (SNAP) at 100μM induced a long-lasting potentiation (LTP) of fEPSP slope (141% * 10.98, n = 4) compared with baseline. During applications of SNAP two adenosine responses 10 mins apart were significantly smaller than before SNAP, (30.5% ±3.29 and 20.6% ±2.02, n = 4, respectively). Superfusion of xanthine (100μM) / xanthine oxidase (0.02U/ml) (X/XO) induced LTP (126.2% ±6.17, n=5) and significantly suppressed responses to adenosine (32.6% ±3.02, $P<0.05$; 28.5% ±4.15, n=5, $P<0.001$), compared with controls (48.7% ±4.12, n=5). The guanylate cyclase inhibitor ODQ (10μM) and superoxide dismutase (120U/ml) prevented the inhibitory effects of SNAP and X/XO on adenosine responses and their induction of LTP, suggesting that the action of NO is mediated through cyclic GMP.

28.03

NICOTINIC RECEPTOR SUBTYPES INVOLVED IN THE MODULATION OF GLUTAMATE AND GABA RELEASE IN RAT NATIVE PREPARATIONS

Tristan Baldwinson, Giovanna De Filippi, Emanuele Sher.
Eli Lilly and Company Ltd, Lilly Research Centre, Windlesham , Surrey, GU20 6PH, UK.

The contribution of nAChR subtypes activation to the modulation of Glu and GABA release in cerebellar slices and hippocampal neurones has been investigated with the patch-clamp technique by using recently developed selective ligands.
Cerebellar slices from P10-P15 Wistar rats and primary cultures of hippocampal neurones from P1-P2 Wistar rats were prepared following procedures in compliance with the UK Animal (Scientific Procedures) Act 1986.In cerebellar granule cells, ACh application induces Glu release by activation of presynaptic α7 receptors (De Filippi et al., 2001). Recently developed selective α7 agonists were not able to induce Glu release when focally applied but caused sustained desensitisation of further ACh applications.
In cultured hippocampal neurones, the application of the same selective α7 agonists, mimicked ACh in inducing both a fast somatic current and GABA release. Prolonged application of low agonist concentrations caused sustained α7 receptor desensitisation. In addition, by using selective agonists for β2 and β4 –containing nAChRs, we found that activation of these receptor subtypes also contribute to modulation of GABA release.
These findings confirm that nAChR subtypes play an important modulatory role in the CNS and could therefore represent therapeutic targets for several neuro-psychiatric indications.

28.04

REGULATION OF CALCIUM-DEPENDENT EXOCYTOSIS VIA A CALMODULIN AND LIPID-BINDING DOMAIN OF SYNAPTOBREVIN (VAMP 2)

S. Quetglas1, L. De Haro1, C. Iborra1, G.Ferraci2, R. Miquelis2, S. Opi3, N. Sasakawa4, K. Kumakura4, C. Leveque1,M. Seagar1
1INSERM Unité 464 & 2UMIM, Faculté de Médecine Secteur Nord, 13916 Marseille, 3CNRS Unité 9027, 13402 Marseille, France; 4Laboratory of Neurochemistry & Neuropharmacology, Sophia University, Tokyo 102-8554, Japan.

Neurotransmitter release requires the assembly of SNARE complexes formed by the vesicle protein synaptobrevin (VAMP 2) and two plasma membrane proteins, syntaxin 1 and SNAP-25. Ca++-dependency is probably conferred by the association of Ca++-binding proteins with SNAREs. Ca++/calmodulin or acidic phospholipids bind in a mutually exclusive fashion to VAMP77-90, a domain close to the membrane anchor (1). The residues involved were identified by introducing mutations into synthetic VAMP peptides and plasmon resonance spectroscopy (Biacore). Micro-injection of bovine chromaffin cells with wild type but not mutant VAMP77-90 peptides inhibited evoked catecholamine release detected by amperometry. Tetanus toxin (TeNT) suppressed Ca++-dependent human growth hormone release from permeabilized PC12 cells. Transfection of TeNT-resistant (Q76V, F77W) VAMP restored secretion in the presence of TeNT. However additional mutations in VAMP77-90 that reduce calmodulin and lipid binding abolished it's ability to rescue release (2). Thus calmodulin and phospholipid-binding to a membrane-proximal domain of VAMP 2 is required for Ca++-dependent exocytosis, possibly to regulate trans SNARE complex assembly.
(1) Quetglas et al. (2000) Proc. Natl. Acad. Sci USA 97, 9695-9700
(2) Quetglas et al. (2002) EMBO J. 21, 3970-3979

28.05

NEUROMUSCULAR TRANSMISSION IS ALTERED IN A NOVEL TRANSGENIC MOUSE MODEL WITH THE FAMILIAL HEMIPLEGIC MIGRAINE TYPE-1 R192Q MUTATION

Simon Kaja (1,3), Arn M.J.M. van den Maagdenberg (2), Ludo Broos (2), Rob C.G. van de Ven (2), Michel D. Ferrari (3), Rune R. Frants (2), and Jaap J. Plomp (1,3).
Departments of Neurophysiology (1), Human Genetics (2), and Neurology (3), Leiden University Medical Centre, Wassenaarseweg 62, 2333 AL Leiden, The Netherlands.

Mutations of CACNA1A, encoding the α1A (Cav2.1) subunit of P/Q-type calcium channels, cause a spectrum of diseases, including familial hemiplegic migraine type-1 (FHM1). We generated a transgenic FHM1 mouse model carrying the clinical R192Q mutation. Heterozygous and homozygous mice are viable and do not show an overt clinical phenotype.
P/Q-type calcium channels are expressed presynaptically at central synapses and the peripheral neuromuscular junction (NMJ), and mediate neurotransmitter release. Thus, Cav2.1 mutations are likely to induce synaptic malfunction, and our previous studies indeed revealed synaptic defects at NMJs of the Cav2.1 mutants tottering, Rolling Nagoya and leaner.Ex vivo electrophysiological analysis of NMJs of R192Q mice showed ~100% increase in spontaneous quantal acetylcholine (ACh) release, compared with wild-type controls. Slight depolarization by 10 mM K+ increased release in wild-type NMJs about 6-fold. However, the increase at R192Q NMJs was more pronounced, about 12-fold. Increased ACh release at R192Q NMJs was sensitive to Ω-Agatoxin-IVA, confirming P-type calcium channel involvement.Our results strengthen the hypothesis that synaptic dysfunction contributes to FHM1, and show that the R192Q mutant mouse is valuable for FHM1-related pathophysiological studies. A more detailed electrophysiological NMJ analysis is underway.

28.06

CO-OCCURRENCE OF CA^{2+} CHANNEL AND METABOTROPIC RECEPTOR SUBTYPE GIVES RISE TO SYNAPSE-SPECIFIC MODULATION OF GABA RELEASE FROM INTERNEURONS

D. A. Rusakov, A. Wuerz, D. M. Kullmann
Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK.

GABA release from interneurons shows a marked heterogeneity in its dependence on Ca2+ channel types and in its sensitivity to diffuse messengers including glutamate. How this heterogeneity provides for precise modulation of transmission at subsets of synapses is not known. Here we show that blocking N-type Ca2+ channels occludes the effect of group III metabotropic glutamate receptor (mGluR) activation on IPSCs recorded in CA1 interneurons. Activation of group III mGluRs inhibits action potential-driven Ca2+ transients in individual axonal varicosities of interneurons when these are mediated by N-type, rather than P-type, channels. Among varicosities supplied by the same interneuron the contributions of N-type channels to these presynaptic Ca2+ transients tend to be similar, whereas the effects of group III mGluR activation diverge. Specific glutamatergic modulation of GABA release from individual synapses thus depends on co-occurrence of presynaptic Ca2+ channel subtype and target cell-dependent expression of mGluRs.

28.07

ONE FOURTH OF NMDA RECEPTORS ON CA1 PYRAMIDAL CELLS COULD DETECT GLUTAMATE RELEASED AT MORE THAN ONE SYNAPSE

A. Scimemi1, A. Fine2, D. M. Kullmann1, D. A. Rusakov1,2
1Institute of Neurology, University College London, Queen Square, London WC1N 3BG; and 2National Institute for Medical Research, Mill Hill, London NW1 6AA.

Evidence for glutamate spillover in the hippocampus at physiological temperature remains controversial. We recorded from CA1 pyramidal cells (whole-cell mode) in acute rat hippocampal slices at 35°C and examined EPSCs mediated by NMDA receptors (NMDARs). We divided stratum radiatum into two separate pathways that converged on the same cell but could be stimulated independently. After obtaining stable responses to single stimuli at each pathway, we applied the use-dependent NMDAR blocker MK801 and continued stimulation of only one (test) pathway until the EPSCs decreased to ~15% of baseline. When we resumed stimulation of the other (silent) pathway, its responses were reduced to 48+/-7% of baseline. This reduction could not be explained by the spontaneous activation of NMDARs in the presence of MK801, or by the activity-dependent changes in the occupancy of the NMDAR glycine-binding sites. The MK801 dependent block of NMDARs at the silent pathway was exacerbated ~4-fold when glutamate uptake was blocked, and ~2-fold when five stimuli at 20 Hz were applied to the test pathway. Our data lead to an estimate that, on average, ~25% of NMDARs at CA1 pyramidal cells can be activated by glutamate released at more than one synapse supplied by stratum radiatum fibres.

28.08

DOES THE MOLECULAR MAKEUP OF THE OXIDE RECEPTOR INFLUENCE ITS KINETIC CHARACTERISTICS?

Victoria Wykes, Barry Gibb and John Garthwaite
The Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT.

Physiologically, nitric oxide (NO) signal transduction occurs through activation of guanylyl cyclase (GC)-coupled receptors, which catalyse cGMP formation. Knowledge of the molecular dynamics of the GC-coupled receptors is currently limited and incoherent; for example EC50 values for NO have ranged from 2 nM to 300 nM. The proteins exist mainly as heterodimers and two isoforms ($\alpha1\beta1$ and $\alpha2\beta1$) have been shown to exist in the brain and elsewhere. This study examined the activation profile and NO sensitivity of these two isoforms expressed in COS-7 cells, using clamped NO concentrations.
The GC activation profile was rapid, with a peak activity occurring within 2 s, followed by desensitisation to 50% of maximum by 12 s. In concert with phosphodiesterases this profile of activity may generate diverse cGMP signals, enabling the second messenger to engage different downstream receptors in different cells. Half-maximal activity was observed at 1 nM for the $\alpha1\beta1$ GC isoform and 2 nM for $\alpha2\beta1$ isoform. This suggests that NO is more potent for GC than reported in several previous studies using varying NO concentrations. The results further suggest that the GC isoforms are functionally similar and so may have evolved to target different sub-cellular locations.

28.09

DOES NITRIC OXIDE REGULATE THE NMDA RECEPTOR?

Rachel Hopper and John Garthwaite
Wolfson Institute for Biomedical Research, University College London, Gower Street, London, WC1E 6BT, UK.

Nitric oxide (NO) functions widely as a neural messenger and exerts many effects through guanylyl cyclase activation, resulting in cGMP formation. An alternative proposed NO transduction pathway is the modification of protein function by nitrosation of thiol groups. The N-methyl-D-aspartate receptor (NMDAR) is a classical example of a protein supposedly regulated by S-nitrosation, the result being an inhibition of receptor function that reverses only slowly, if at all. The present study aimed to determine if this mechanism has physiological relevance to synaptic function in the brain.
Electrophysiological recordings were made from the CA1 region of rat hippocampal slices. Perfusion of either the NO synthase substrate, L-arginine, or the inhibitor, L-nitroarginine, did not modify NMDAR function. Exogenous NO in concentrations well in excess of those needed to activate guanylyl cyclase, also had no effect. However, in accordance with published evidence, release of NO from a caged compound by UV light inhibited NMDAR function. Tests on HEK-293 cells transfected with NMDAR subunits confirmed these findings with the caged derivative. However, the effect could not be replicated using NO, except in the presence of UV light. The results cast doubt on the physiological relevance of S-nitrosation.

29.01

IS PHOSPHOLIPASE-C BETA-1 NECESSARY FOR 5-HT- AND NORADRENALINE-MEDIATED MODULATION OF gK^+ LEAK IN MOUSE FACIAL MOTONEURONES?

*P.M.Larkman, *E.M.Perkins, ^P.Kind.
*Divisions of *Neuroscience and ^Biomedical and Clinical Laboratory Sciences, University of Edinburgh. UK.*

5-HT2 or alpha-1 receptor activation inhibits a resting potassium conductance (gK+Leak), in rat facial motoneurones (FM`s). The mechanism coupling receptor to channel is unknown. Both receptors stimulate PLC-beta-mediated hydrolysis of PIP2 to diacylglycerol and IP3. The role of PLC-beta 1 in receptor-mediated modulation of gK+Leak was investigated in brain slices prepared from PLC-beta 1-/- mice and their littermate controls (7-14 days old). Whole-cell patch-clamp recordings indicated that inhibition of gK+Leak by 5-HT or NA was reduced in FM`s of PLC-beta 1-/- animals, though modulation of the hyperpolarisation-activated current, Ih, was retained (n = 3). Responses to 5-HT and NA in wildtype or heterozygous animals were normal (n = 7). Immunohistochemistry, employing a PLC-beta 1-specific antibody, confirmed the presence of PLC-beta 1 in wildtype FM`s. Recent reports suggest that PIP2 depletion, rather than production of IP3 or diacylglycerol, promotes KCNQ-type K+ channel closure (Neuron, (2002), 35, 507-520). Recordings from rat FM`s in vitro (6-14 days old) showed that buffering intracellular Ca2+ (BAPTA, 20 mM or EGTA, 10 mM) or inhibiting PKC (H-7, staurosporine or chelerythrine) did not prevent receptor-mediated inhibition of gK+Leak. These results suggest a role for PLC-beta 1 and PIP2 in the regulation of gK+Leak.

Supported by the MRC and EU.

29.02

GLUTAMATE IMMUNOREACTIVITY IS SEVERELY REDUCED IN THE CEREBELLAR CELLS OF STARGAZER MUTANT MICE WHICH SHOW A SPECIFIC DEFICIT IN BDNF MRNA EXPRESSION

Christine, A. Richardson and Beulah Leitch
Department of Biological & Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, UK.

The brain derived neurotrophic factor (BDNF) is known to influence neuronal survival, differentiation and maturation. Recently, its role in synapse formation and plasticity has also emerged. In the cerebellum of the spontaneous recessive mutant mouse stargazer there is a specific and pronounced deficit in BDNF mRNA expression. BDNF protein levels in the cerebellum as a whole are reduced by 70%; in the granule cell layer there is a selective and near total reduction in BDNF mRNA expression.
Recently, we published data demonstrating that inhibitory neurons in the cerebella of stargazer mutants have significantly reduced levels of GABA (50%) and fewer, smaller inhibitory synapses compared to wild-type controls. Our current investigations indicate that the stargazer mutation has an even more pronounced effect on the phenotype of glutamatergic neurons in the cerebellum. We have found that there is a profound decrease in the levels of glutamate-immunoreactivity (approximately 70%)in mossy fibres terminals and granule cell dendrites in stargazers compared to wild-type controls. The number and length of excitatory synapses, however, appear to be unchanged. Whether the reduction in glutamate content is directly attributable to the lack of BDNF in the cerebellum of the stargazer mutant is yet to be proven.
Acknowledgements:

Supported by The Wellcome Trust and University of Dur

29.03

SELECTIVE BLOCK OF AF-DX BINDING TO HUMAN MUSCARINIC RECEPTORS DIFFERENTIATING M2 AND M4 BY DICYCLOMINE, PIRENZEPINE, TROPICAMIDE, PG1000 AND PD102807

Margaret Piggott, George Wake, Elaine Perry
MRC/University of Newcastle Centre Development for Clinical Brain Ageing, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne, NE4 6BE.

AF-DX384 has high affinity for muscarinic receptors M2 and M4. To measure these receptors separately in human brain tissue autoradiographically would help determine the influence of these receptors in dementia symptoms.
In cloned human M2 and M4 mAChR subtypes expressed in CHO cells, 100nM Pirenzepine blocked 70% of [3H]AF-DX 384 binding to M4, 120nM fully blocking M4. 100nM Pirenzepine blocked 20% of M2 binding.
100nM Tropicamide blocked M4 binding by 50%. 1microM Tropicamide blocked M2 binding completely. PG1000 blocked 80% AF-DX M2 binding at 50pM, but 500pM fully blocked M4. PD102807 blocked M4 receptor binding at 100nM, and 80% at 50nM. PD102807 also had high affinity to M2 receptors, blocking 50-60% of M2 binding at 100nM.
Dicyclomine at 10nM completely blocked AF-DX binding to M4, while 1nM blocked 60% of M4 binding. A higher concentration of dicyclomine (1microM) was needed to block M2 receptor binding by 90%, while 100nM dicyclomine blocked M2 binding by only 25%. This differential affinity of dicyclomine has been exploited to discriminate [3H]AF-DX384 M2 and M4 binding autoradiographically in cingulate cortex in a series of prospectively clinically assessed dementia with Lewy body cases.
Acknowledgement - Thanks to Sebastian Lazareno for helpful advice, and sample of PG1000. Thanks to Pfizer for PD102807 sample.

29.04

D3 RECEPTOR MODULATION OF DOPAMINE EFFLUX IN THE RAT NUCLEUS ACCUMBENS

Claire Roberts; Robert Cummins; Zaira Gnoffo; James N.C. Kew
GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW.

The nucleus accumbens (NAc) can be anatomically divided into shell and core regions which receive projections from the ventral tegmental area and sunstantia nigra, respectively. In this study we investigated the modulation of in vitro dopamine release in the NAc shell and core. Dopamine efflux was electrically evoked from rat NAc shell or core and measured using fast cyclic voltammetry. In the NAc shell, clozapine (300nM), haloperidol (30nM) and sulpiride (300nM) increased dopamine efflux. In contrast, the D3 selective compound, SB-277011 (30nM-300nM), inhibited efflux. The D3/D2 receptor agonist, 7-OH-DPAT (10nM–100nM), inhibited dopamine efflux in a concentration-dependent manner. All 4 antagonists attenuated the 7-OH-DPAT-induced inhibition with pA2`s of 8.6, 7.6, 7.3 and 8.2 for haloperidol, sulpiride, clozapine and SB-277011, resectively. In the NAc core, 7-OH-DPAT also inhibited dopamine efflux in a concentration-dependent manner. However, SB-277011 (30nM) did not attenuate the 7-OH-DPAT-induced inhibition. These data indicate that the 7-OH-DPAT-induced inhibition of dopamine efflux in the NAc shell is a D3 receptor-mediated response.

29.05

EXAMINATION OF (S)-(-)-COTININE, THE MAJOR METABOLITE OF NICOTINE, ON DOPAMINE RELEASE IN THE RAT STRIATUM, USING A 96-WELL PLATE ASSAY

G. Pashmi, S. Parks, S. Wonnacott
Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY.

Smoking-related diseases kill one in two life-long tobacco smokers. In rats, nicotine injection results in the presence of its metabolite cotinine in the brain, therefore cotinine may play a role in nicotine addiction. As dopamine mediates many rewarding effects of nicotine, the ability of cotinine to affect dopamine release was examined in rat striatal minces using a 96-well plate assay.
Briefly, rat striatal minces were incubated for 30min with 50nM tritiated dopamine. The tissue was dispensed into a 96-well plate. After pre-incubation for 5min +/- antagonist, cotinine or nicotine was applied for 5min, outflow collected and released tritium measured. Concentration-response relationships for nicotine- and cotinine-evoked dopamine release gave EC50 values of 0.1uM and 100uM respectively, suggesting cotinine is a weak agonist. Pre-incubation with cotinine or nicotine reduced nicotine-stimulated dopamine release dose-dependently. Increase of pre-incubation times were investigated, to examine the impact of receptor desensitization on evoked release. Cotinine responses were mediated by nicotinic receptors, as they were blocked by mecamylamine. Subtype-selective antagonists were used to study receptors involved in dopamine release. These results contribute to the elucidation of the role of cotinine in the reward pathway.

This study was supported by BBSRC and GlaxoSmithkline.

30.01

EFFECTS OF INTERLEUKIN-1 ON EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL SLICES

H.C. Patel, S. Lucas, F.M. Ross and S.M. Allan
School of Biological Sciences, 1.124 Stopford Building, University of Manchester, Manchester, UK.

Polymorphisms of the pro-inflammatory cytokine interleukin-1ß (IL-1ß) gene are associated with temporal lobe epilepsy and hippocampal sclerosis. In addition an increased production of IL-1 has been shown in human temporal lobe epilepsy. This is reflected in rat models of epileptic activity using kainic acid or bicuculline methiodide to induce seizures. The aim of this study was to investigate the effect of IL-1ß and the endogenous IL-1 receptor antagonist (IL-1ra) on epileptiform activity generated in vitro in rat hippocampal slices.
Population spikes were recorded from the CA1 pyramidal cell layer of hippocampal slices (450µm) prepared from male Sprague-Dawley rats (100-150g). Slices were stimulated to check slice viability and electrode positioning. The aCSF was changed to one containing no added magnesium or 4-aminopyridine (100µM). Spontaneous inter-ictal like activity was monitored continuously. Control burst rate was calculated as the mean rate during the last 3 minutes before drug addition.
The addition of IL-1ß (10-50 ng/ml) to the perfusate had no consistent effect on the rate of spontaneous inter-ictal activity generated by either model. This suggests that IL-1ß does not affect the number of inter-ictal episodes per se however this does rule out a potential effect on ictal activity.

Work supported by MRC

30.02

KINETICS OF NITRIC OXIDE INACTIVATION IN THE BRAIN

C. Hall, C. Griffiths and J. Garthwaite.
Wolfson Institute for Biomedical Research, University College London, London WC1E 6BT, UK.

At low nanomolar concentrations nitric oxide (NO) activates guanylyl cyclase, producing cGMP. At high (micromolar) levels, however, NO can be toxic, making control of concentration critical. NO formation through NO synthases is well-characterised, but only recently has a specialised inactivation mechanism for NO been found (Griffiths and Garthwaite, J.Physiol. 563.3: 855-862, 2001). The experiments aimed to determine the kinetic properties of this mechanism in brain tissue, using either dispersed cells or intact slices from rat cerebellum. In dispersed cells, inactivation of NO followed Michaelis-Menten kinetics, yielding a Km of 62 nM and a Vmax of 0.43 nmol (mg protein.s)-1. In the slices, cGMP levels provided an index of the penetration of NO into the tissue. Half-maximal cGMP accumulation required 1.0 uM external NO, 500-fold greater in dispersed cells. Correspondingly, immunocytochemistry for cGMP revealed marked gradients of NO across the slice thickness at steady-state, following application of lower external NO concentrations. Diffusional modelling suggested that the Vmax value for intact cerebellar tissue is at least 1 µM s-1. This is more than 2-fold higher than the value predicted from isolated cells after correcting for the different protein concentrations, and would impose a half-life of under 40ms on physiological NO signals (0.5-20nM).

30.03

PI 3-KINASE DOES NOT MEDIATE ACTIVATION OF LARGE CONDUCTANCE CA^{2+} -ACTIVATED K^{+} (BK) CHANNELS BY INSULIN

Dervla O'Malley, Michael L.J. Ashford and Jenni Harvey
Department of Pharmacology & Neuroscience, University of Dundee, Dundee, DD1 9SY, UK.

Insulin is largely associated with energy homeostasis but growing evidence suggests it may also have a role as a modulator of neuronal function. In this study, we have examined the actions of insulin, using HEK293 cells stably transfected with the [alpha] (hSlo) subunit of the BK channel. Inside-out recordings in symmetrical 140mM K+, displayed a channel with a linear I-V relationship, a slope conductance of around 210pS and reversal potential close to 0mV. Channel open probability increased with membrane potential depolarisation and increasing [Ca2+]i. During cell attached recordings, insulin caused a rapid increase in channel activity, as did the BK channel opener NS-1619. As insulin and insulin like growth factor–1 (IGF-1) receptors have a high sequence homology, a comparison of potencies of insulin and IGF-1 was used to determine the receptor identity. Both insulin (10nM) and IGF-1 (10nM) enhanced BK channel activity, suggesting that IGF-1 receptors are activated by insulin. Strong evidence links PI 3-kinase to the insulin-signalling cascade, so the cells were incubated with LY294002 and wortmannin. The PI 3-kinase inhibitors failed to block insulin activation of BK channels indicating that insulin, through IGF-1 receptors stimulates BK channels in a PI3K – independent manner. This process may be a novel mechanism for regulating excitability.

31.01

POST-PULSE INHIBITION OF HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY IN THE RAT

Atkinson HC, Wood SA, Bate E & Lightman SL
URC Neuroendocrinology, University of Bristol, Bristol, UK.

Ultradian rhythmicity of glucocorticoids is a characteristic feature of the hypothalamic-pituitary-adrenal (HPA) axis in rodents and man. We have previously shown that in rats, the response to a mild white noise stress (100 dB, 10 min) is greater during the rising phase of an endogenous corticosterone pulse when compared to the falling phase. We propose that the HPA axis displays alternate periods of activation and inhibition with respect to basal adrenocortical activity. In our current studies we have developed a model to examine this post-pulse inhibition of HPA activity. Male rats were cannulated and five days later connected to our automated blood-sampling system. With this system blood levels of corticosterone can be determined in conscious freely-behaving animals. Animals were exposed to an initial noise stress (100 dB, 10 min) to synchronise pulsatile release of corticosterone, thereafter a second similar noise stress was applied 20, 40, 60 or 80 minutes later. When the second noise stress is applied 60 or 80 minutes after the initial stress a distinct pulse of corticosterone is detectable. When the second noise stress is applied 20 or 40 minutes after the first noise, however, corticosterone levels are variable and no obvious pulse was detected. These preliminary results provide evidence of post-pulse inhibition in HPA activity in the rat.

31.02

CO-EXPRESSION OF PROGESTERONE RECEPTOR AND LH IN THE PITUITARY GLAND OF DOMESTIC FOWL

B Das*, R W Lea* & P J Sharp**
University of Central Lancashire, Preston Roslin Institute, Roslin, Edinburgh.*

Progesterone is vital to the induction of the preovulatory luteinizing hormone (LH) surge in the domestic hen and progesterone receptors (PR) expression in the pituitary gland has been demonstrated.
The aim of this study was to determine whether progesterone acts directly upon pituitary gonadotrophs in the fowl.Laying and broody hens were terminally anaesthetized and perfused with Zamboni`s fixative. Anterior pituitary gland sections (16mm) were treated with mouse monoclonal antibody (raised against chicken PR, dilution 1:1000, H-928, Calbiochem) and anti-mouse IgG (dilution 1:1000, Alexa Fluor 488) to localize PR expression. The same sections were subsequently treated with rabbit anti turkey LH (dilution 1:10,000; 783, Roslin Institute) and anti rabbit IgG (dilution 1:2500; Alex Fluor 568) to identify LH immunofluorescence (IF).
PR-IF and LH-IF were both widespread in the anterior pituitary gland of the laying hen but significantly depressed ($p<0.01$, t-test) in pituitary glands of brooding birds (n=4).
Furthermore, LH-IF and PR-IF was co-expressed in gonadotrophs throughout the caudal and cephalic lobes. This indicates that progesterone may be acting directly on LH cells in the domestic fowl anterior pituitary gland to affect sensitivity of the cell to GnRH.0

31.03

DECREASED ANXIETY-RELATED BEHAVIOUR AND INCREASED SPATIAL MEMORY RETENTION IN 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 KNOCKOUT MICE

Joyce L.W. Yau1, Carina Hibberd1, Janice Paterson2, John J. Mullins2 and Jonathan R. Seckl1
1. Molecular Endocrinology laboratory, Molecular Medicine Centre, Western General Hospital, Edinburgh, UK ; 2. Molecular Physiology Laboratory, Wilkie Building, Teviot Place, University of Edinburgh, Edinburgh, UK.

11ß-Hydroxysteroid dehydrogenase type 1 (11ß-HSD-1) is a key enzyme which amplifies intracellular levels of active glucocorticoids within specific tissues. The hippocampus highly expresses both corticosteroid receptors and 11ß-HSD-1, and plays an important role in fear/anxiety behaviours and memory. We examined the anxiety-related behaviours (elevated plus maze and open field) and spatial memory (Y-maze) in young (3 mths) and middle-aged (12 mths) male mice homozygous for targeted disruption of the 11ß-HSD-1 gene on the C57BL/6J background and wild type controls. Young 11ß-HSD-1 knockout mice displayed more open-protected stretches (43%, $P < 0.05$) in the elevated plus maze and showed greater locomotion (38%, $p<0.01$) in the open field test than young wild type controls, suggesting subtly reduced anxiety related behaviour. Spatial memory retention [percentage time spent in the novel arm of the Y-maze after a 2 hour inter-trial interval (ITI)] was impaired ($P<0.01$) in middle-aged wildtype mice (31 ± 3%) but maintained in middle-aged 11ß-HSD-1 knockout mice (46 ± 4%). Basal plasma corticosterone levels were elevated in the 11ß-HSD-1 knockout mice compared to wildtype controls ($P<0.01$). These data suggest that circulating glucocorticoid levels are not the sole signal for glucocorticoid actions on brain function and that intracellular regeneration plays an important role.

32.01

ABERRANT GATING OF PHOTIC INPUT TO THE SCN CLOCK IN Vipr 2-/- MICE

Hughes AT, Fahey B, Cutler DJ, Coogan AN, Harmar AJ, & Piggins HD
School of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, UK;Department of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ, UK.

Vasoactive intestinal polypeptide and its receptor, VPAC2, play important roles in the functioning of the circadian clock located in the hypothalamic suprachiasmatic nuclei (SCN). Mice lacking VPAC2 receptors (Vipr2-/-) fail to generate circadian rhythms in clock gene expression, neuronal firing rate and locomotor behaviour. It is unclear from these studies whether all clock processes are disrupted by this mutation. The SCN clock gates its sensitivity to photic stimuli such that light pulses delivered during the subjective night engage the molecular machinery of the clock, while similar pulses given during the subjective day do not. A key intracellular event in photic resetting is upregulation of phosphorylated extracellular signal-regulated kinase (P-ERK). We have investigated gating of clock sensitivity to photic stimuli in Vipr2-/- and WT mice through analysis of photic induction of P-ERK in the SCN. In WT mice, light induced P-ERK in the SCN during the subjective night and not the subjective day. By contrast, light pulses to Vipr2-/- mice elicited significant increases in P-ERK during both night and day phases. These results demonstrate that gating of the sensitivity of the circadian clock to photic stimuli is lost in Vipr2-/- mice, providing further evidence for the absence of circadian clock function in these animals.

32.02

CIRCADIAN AND PHOTIC REGULATION OF ERK1/2 AND Elk-1 IN THE SYRIAN HAMSTER SUPRACHIASMATIC NUCLEI

Andrew N. Coogan, Hugh D. Piggins
3.614 School of Biological Sciences, University of Manchester, Oxford Road, M13 9PT.

Daily rhythms in physiology and behaviour in mammals are temporally ordered by the master circadian clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus. The intracellular systems that determine the phase of this clock remain obscure. To this end we examined circadian and photic regulation of extracellular protein kinase 1/2 (ERK) and the transcription factor Elk-1 in the Syrian hamster SCN.
Under a light/dark cycle or in constant dark (DD), levels of phosphorylated ERK (P-ERK) in the SCN were high during the subjective day and low during the subjective night. No such variation was noted for phosphorylated Elk-1 (P-Elk-1). Following a light pulse during the subjective night, expression of P-ERK and P-Elk-1 were rapidly upregulated. Light pulses during the subjective day failed to elicit any change in P-ERK or P-Elk-1. Total levels of ERK1/2 or Elk-1 did not vary under any condition examined. Treatement with the MEK inhibitor U0126 or the NMDA-receptor channel blocker MK-801 attenuated the photic induction of P-ERK and P-Elk-1. U0126 also significantly attenuated phase-advances in running wheel behaviour following a light pulse during the subjective night. Thus, Elk-1 is a putative effector of the ERK pathway in the SCN, and activation of this pathway appears to be required for phase-resetting of the circadian clock.

33.01

SUBSTANCE P INHIBITS GABA-A CURRENTS OF PARAVENTRICULAR SPINALLY PROJECTING NEURONS

Richard Barrett-Jolley
Dept. Physiology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT.

The neurotransmitter substance P (SP) has been proposed as a hypothalamic mediator of the cardiovascular response to stress (Culman & Unger, Can. J.Physiol.Pharmacol 73:885-891,1995)
In this work we have begun to investigate whether spinally projecting "pre-sympathetic" neurons of the rat paraventricular nucleus (SPNs) express SP receptors (NK1).
Methods were similar to those used previously (Barrett-Jolley, et al. J.Neurosci.Met 102:25-33,2000), but briefly: PVN SPNs were labelled by injection of retrograde tracer (DiI) into the spinal-cord IML (T2) of rats under general anaesthesia (metatomidine/ketamine 0.3%). One week later, rats were humanely killed by overdose of anaesthesia (sagittal) and hypothalamic slices prepared. Results are given as mean+/-SE (p-values from ANOVA).
Using standard immunohistochemical methods in combination with retrograde labelling we found 19/22 SPNs to express NK1-like immunoreactivity. In addition, we found that 1μM SP inhibited whole-cell GABAA currents to 46+/-13% (n=3 $p<0.05$). This effect was mimicked by addition of 100nM PMA and okadaic acid (60+/-8% n=3 $p<0.05$), a combination known to increase PKC dependent membrane receptor phosphorylation.
Further experiments with selective NK1 antagonists and inhibitors of PKC will be required to confirm the mechanism by which SP modulates the activity of SPNs.

Funded by BHF Fellowship FS2000019.

33.02

P2X4 RECEPTOR SUBUNIT IMMUNOREACTIVITY IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) OF THE RAT

Fathia Ashur and Jim Deuchars
School of Biomedical Sciences, University of Leeds, LS2 9NQ.

ATP as a fast synaptic transmitter acts via ionotropic (P2X1-7) receptors. Of these, the P2X4 receptor subunit (P2X4 RS) is widely expressed throughout the CNS. Here we have studied the immunohistochemical distribution of the P2X4RS in the NTS of humanely killed rats. Using 2 separate antisera against different epitopes (Alomone Labs, 1:500; Santa Cruz, 1:500) we observed a similar distribution. Using fluorescence microscopy neuronal somata in the dorsal vagal neucleus (DVN) contained moderate levels of P2X4 receptor subunit immunoreactivity (P2X4RS-ir) while somata in the NTS were very lightly labeled. The subpostremal NTS showed an intense immunoreactivity. In the NTS conventional light microscopy revealed fine punctuate staining of the neuropil, of which some elements appeared to be glial processes, although glial somata were not evident. Electron microscopy revealed immunoreactivity in neurones postsynaptically at synaptic junctions while presynaptically the P2X4RS-ir was distal to the release site. P2X4RS-ir was also detected in endosomes. In addition, glial cell processes surrounding presynaptic terminals were also labeled. We are currently attempting to determine the origin and chemical nature of the presynaptic terminals bearing the P2X4RS, as well as those are adjacent to P2X4RS-ir glial processes.

33.03

NEUROCHEMISTRY OF NEURONES IN THE INTERMEDIUS NUCLEUS (InM) OF THE ADULT RAT MEDULLA OBLONGATA.

Carol J. Milligan1, Trevor F.C. Batten2 and Jim Deuchars1
School of Biomedical Sciences1and School of Medicine2, University of Leeds, Leeds, LS2 9NQ, UK.

The nucleus tractus solitarius (NTS) in the dorsomedial medulla oblongata is critically involved in autonomic regulation. In preliminary studies injections of retrograde tracers into the NTS revealed retrogradely labelled neurones in the intermedius nucleus of the medulla (InM). In a parallel study we provide electrophysiological evidence for a novel pathway from the InM to the NTS (this meeting). In light of these connections we are investigating the phenotype of neurones within this poorly characterized nucleus. Using in situ hybridisation with digoxigenin-UTP-labelled sense and antisense cRNA probes for GAD65, GAD67 and VGluT2 we have demonstrated the presence of mRNA for both inhibitory (GAD) and excitatory (VGluT2) neurones within the InM. Using immunohistochemistry we have observed immunoreactivity for the glutamate receptor subunit (GluR1, an AMPA preferring subunit), neuronal nitric oxide synthase (nNOS), the calcium-binding protein calretinin and the angiotensin II type 1 receptor subunit (AT1) in neurones of the InM. Our current investigations are determining the co-localisation of transmitters in, and the projections of, these neurones to gain evidence in support of the hypothesis that the InM plays a role in central autonomic regulation.

33.04

ELECTROPHYSIOLOGICAL EVIDENCE FOR A NOVEL PATHWAY BETWEEN THE NUCLEUS INTERMEDIUS AND THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT

Mark L.Dallas, Susan A.Deuchars, David I.Lewis and Jim Deuchars.
School of Biomedical Sciences, University of Leeds, Leeds LS2 9NQ, UK.

The nucleus of the solitary tract (NTS) plays a pivotal role in the regulation of autonomic function through widespread central connections. Here we provide evidence for a novel pathway to the NTS from the nucleus intermedius (InM). Male Wistar rats (15-21 days) were terminally anaesthetized, humanely killed and 300um coronal slices of medulla oblongata prepared. Whole cell patch clamp recordings were made from dorsal and dorsomedial NTS neurones. Electrical stimulation of both the solitary tract and the InM elicited responses in 10/12 neurones. The solitary tract evoked glutamatergic EPSPs were 6.4±1.4mV in amplitude (AMP) with a half amplitude width (HW) of 53.3±10.2ms and a 10-90% rise time (RT) of 0.79±0.3mV/ms (n=12). In 12 neurones, a GABAergic IPSP (AMP=6.4±1.2mV, HW=44.2±15.2ms, RT=0.55±0.2mV/ms) from the solitary tract was observed. Glutamatergic EPSPs (AMP=6.5±1.6mV, HW=51.3±16.4ms, RT=0.75±0.3mV/ms(n=10)) and GABAergic IPSPs (AMP=6.3±1.3mV, HW=53.3±4.9ms, RT=0.5±0.1mV/ms(n=10)) could be evoked from the InM in NTS neurones. The function and the pharmacology of these novel excitatory and inhibitory synaptic pathways are currently being investigated.

The University of Leeds and The Wellcome Trust supported this work

34.01

NOVEL STRATEGIES FOR MODIFYING TGFß-INDUCED SCARRING IN THE INJURED SPINAL CORD

C. Lagord, P. Ellahee, S. Coutts, M. Berry and A. Logan
Molecular neurosciences, Department of Medicine, University of Birmingham, Birmingham, B15 2TT. UK.

A role for TGFß has been demonstrated in various fibrotic pathologies, including lung fibrosis and glomerulonephropathy. We have previously shown that after traumatic brain or SCI there is an increased expression, both at mRNA and protein level, of TGFß1 and TGFß2 at the injury site. We have also shown that inhibiting TGFß activity using specific blocking antibodies suppresses ECM protein deposition after brain injury. In the spinal cord, TGFß2 isoform expression is upregulated mainly by reactive astrocytes around the lesion site in a time frame corresponding to the deposition of scar material. Here, we present the strategies we are now using to inhibit scar formation after SCI: ● - Investigating the expression after SCI of CTGF (connective tissue growth factor) which is thought to act down stream of TGFß and to mediate TGFß effects in fibrosis. This would allow more specific targeting of the pro-fibrotic effects of TGFß, as TGFß possesses multiple other physiological roles. ● - Evaluating the possible involvement of the angiotensin axis in the modulation of the TGFß fibrogenic pathway. ● - Developing a method to deliver DNA encoding anti-fibrogenic agents (including the pan-TGFß antagonist decorin or the recombinant TGFß2-specific antibody) to the reactive astrocytes around the lesion site.

34.02

IMMUNOHISTOCHEMICAL LOCALISATION OF Kv3 POTASSIUM CHANNEL SUBUNITS IN THE RAT THORACIC SPINAL CORD

Ruth Brooke, Sue Deuchars and Jim Deuchars
School of Biomedical Sciences, University of Leeds, Leeds, LS2 9NQ.

The Kv3 subfamily (Kv3.1-Kv3.4) of voltage gated potassium channels activate rapidly at depolarised potentials (approx. -10mV), generally slowly inactivate and mediate action potential repolarisation. Here we used immunohistochemistry to determine their distribution in the thoracic spinal cord of humanely killed rats. Kv3.1b immunoreactivity (Kv3.1b-IR) was observed within the somatic membrane of neurones throughout the grey matter. Labelled neurones were observed around and occasionally within the intermediolateral cell column (IML) however, this region was predominantly unlabelled. Kv3.2-IR could only be detected in putative fibres in the superficial dorsal horn. Kv3.3-IR and Kv3.4-IR was present throughout the grey matter with the majority of staining punctate, suggestive of labelled terminals. Co-localisation of Kv3.3 and Kv3.4 with the synaptic vesicle protein, SV2 and the glutamate vesicle markers, vGlut1(with Kv3.4) and vGlut2 (with Kv3.3 and Kv3.4), confirmed this. In addition, Kv3.3 and Kv3.4 were co-localised with the glycine transporter, GlyT2. Kv3.3 was also observed in the somatic membrane of neurones in the dorsal horn and around the IML region. These results suggest that Kv3.1b and Kv3.3 may regulate the firing properties of neurones at their somata whereas Kv3.3 and Kv3.4 influence release of neurotransmitters from presynaptic terminals.

34.03

PRELIMINARY EVALUATION OF METHODS FOR THE STUDY OF IDENTIFIED EXCITATORY AND INHIBITORY NEURONES IN LI-LIII OF THE RAT SPINAL DORSAL HORN

A. L. Stewart, R. Morris, B.A. Bannatyne, S.L.G. Gordon, A. G. Singleton and D. J. Maxwell
Department of Veterinary Preclinical Sciences, University of Liverpool, Liverpool, L69 7ZJ, and Spinal Cord Group, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ.

The circuitry of the outer dorsal horn of the spinal cord remains elusive. We have recently developed methods, which may help in unravelling aspects of this complex region of the nervous system.
Juvenile rats (15-25 days old) were anaesthetised deeply with ether and decapitated. Parasaggital or transverse slices (400-600 [micron]m thick) of the lumbar spinal cord with attached dorsal roots, DRG and peripheral nerves, were prepared and used for sharp microelectrode recording of membrane potential and conductance, peripheral nerve stimulation evoked synaptic responses and changes in these to drug application. Neurobiotin was injected into the neurones on completion of characterisation. Slices were fixed with 4% paraformaldehyde overnight and then stained en-block with streptavidin conjugated to Cy3. The neurones were reconstructed using Laser confocal microscopy and Neurolucida software. The slices were then resectioned on a vibrating microtome into 50[micron]m thick sections and restained immunocytochemically. Anti-VGLUT2 was used to identify axon terminals of putative excitatory neurones and anti-GAD and anti-GLYT2 to identify GABAergic or glycinergic neurones respectively. To date 5 neurones have been imaged with the confocal microscope and reconstructed. Preliminary evidence suggests that at least a proportion of these cells are GABAergic.

35.01

FM1-43 LABELLING OF MECHANOSENSORY ENDINGS IN MOUSE SKIN

N. Kain, C.R. Slater
School of Neurology, Neurobiology & Psychiatry, University of Newcastle upon Tyne.

Nociceptor endings release peptides during an inflammatory response, presumably by calcium-mediated exocytosis. Here we ask whether membrane cycling (coordinated exocytosis and endocytosis) also occurs in cutaneous mechanosensory endings. We used the fluorescent dye FM1-43, used extensively to study membrane cycling during chemical transmitter release (Cochilla et al., 1999, Ann. Rev. Neurosci. 22:1-10), together with quantitative fluorescent imaging analysis, to study the lanceolate mechanosensory endings associated with the hairs of mouse skin.
A preparation made from the inner surface of the pinna was labelled in vitro at 32degC. The sensory endings took up FM1-43 in a time- and concentration-dependent manner (near saturation at 10microM, 30 min labelling period) in a standard mammalian bathing solution. FM1-43 uptake was 55% of control values when calcium was omitted from the bathing medium, 20% of control when the calcium channel blocker cobalt (5mM) was added and less than 10% of control in a medium with 3mM cobalt and no calcium or magnesium. The conclusion that FM1-43 uptake was occurring by calcium-dependent membrane cycling was supported by the finding that the mechanosensory nerve terminals contain synaptophysin, a component of synaptic vesicles at chemical synapses. These observations support the view that membrane cycling occurs continuously in cutaneous mechanosensory endings.

35.02

ACCUMULATION OF SODIUM CHANNELS AT POSTSYNAPTIC SITES IN RAT MUSCLES REGENERATING IN THE ABSENCE OF THE NERVE IS ASSOCIATED WITH A LOCAL INCREASE IN ABUNDANCE OF THEIR mRNAs

S.S. Awad, A. Buckel, C. Young, H.R. Brenner*, C.R. Slater
*School of Neurology, Neurobiology & Psychiatry, University of Newcastle upon Tyne; *Department of Physiology, University of Basel, Switzerland.*

Voltage-gated sodium channels (NaV1) are concentrated in the postsynaptic membrane at mammalian neuromuscular junctions (NMJs) where they facilitate action potential generation. When rat muscles are damaged by injection of notexin, and allowed to regenerate in the original basal lamina (BL) sheath without the nerve, NaV1s accumulate in the regenerating muscle fibres at the original postsynaptic sites (Lupa & Caldwell, 1994, J. Cell Biol. 124: 139-147). We have used this situation to study the events underlying NaV1 accumulation at the NMJ.
We found that RNA species encoding NaV1 are already concentrated in the postsynaptic region 3 days after NTX treatment, (1 day after the first new muscle fibres form), and remain there for up to 4 weeks. Ectopically expressed neural agrin, resulting from local transfection with cDNA, also causes accumulation of NaV1 mRNA. NaV1 itself is first detectable by imunolabelling 5 days after NTX and is immediately concentrated at the NMJ and in the surrounding perijunctional region. AnkyrinG, whose distribution closely parallels that of NaV1 during NMJ development, has a similar distribution. These results suggest that neurally-derived agrin, which persists in the BL after NTX, induces expression of NaV1 mRNA and triggers accumulation of NaV1 in association with ankyrinG, much as during normal development.

35.03

THE OLFACTORY RECEPTION OF THIOL COMPOUNDS: THE BIOCHEMISTRY OF BAD SMELLS

Rachel H. Spice, Robert J. Beynon, Krishna C. Persaud*
*Faculty of Veterinary Sciences, University of Liverpool, Liverpool L69 7ZJ*Department of Instrumentation and Analytical Science, UMIST, Manchester M60 1QD.*

The mammalian olfactory system exhibits acute sensitivity to thiol odorants. This sensitivity is often associated with a conserved aversive response to specific thiol compounds, exemplified by the constituents of the skunk defensive spray. The work described is a proteomics-based investigation into the molecular mechanisms that result in high levels of sensitivity to thiol odorants and the aversive response they induce at high odour intensities. Proteomics tools were used to isolate and identify candidate proteins with a possible role in reception of thiols in the mammalian olfactory epithelium. The targeting of exposed cysteine residues highlighted proteins in the olfactory cilia theoretically capable of strong interactions with free thiol groups of odorants by disulphide linkages. Differential protein labelling experiments were also performed to enable observation of interactions between thiol odorants and proteins in the cilia membrane of olfactory receptor neurons. Identification of candidate proteins was achieved by MALDI-ToF and tandem mass spectrometry.

This work is funded by RAM Research Ltd.

35.04

PERSISTENT GAMMA-FREQUENCY OSCILLATIONS IN RAT SUPERFICIAL PRIMARY AUDITORY *IN VIVO* INDUCED BY KAINATE

A. Roopun, R.D. Traub, M. O. Cunningham, F.E.N. LeBeau, E.H. Buhl & M.A. Whittington
School of Biomedical Sciences, The Worsley Building, University of Leeds, LS2 9JT; Dept. Physiology & PharmacologySUNY Health Sciences Center, Brooklyn, NY 11203 USA.

Gamma frequency oscillations (30 - 60 hz)occur continuously in EEG recordings from human primary sensory cortices during the awake, alert state. Here we present an in vitro model of these oscillations induced in rat primary auditory cortex by kainate.
Horizontal, 450u thick slices were prepared from male rat (150 – 200g) and maintained at the interface between warm, wet 95%O2/5%CO2 and aCSF at 32 – 35 oC. Following bath application of kainate, 400 nM, field oscillations were seen with peak power in LIII (9.2 ± 3.3 uV2.Hz-1, 31 ± 2 Hz). Frequency was significantly reduced by 20 uM pentobarbital to 20 ± 2 Hz ($P < 0.02$) and power in the gamma band was abolished by blockade of AMPA receptors (20 uM SYM2206), GABA(A) receptors (bicuculline, 20 uM) and reduction in gap junction conductance (carbenoxolone 0.2 mM). Intracellular recordings from pyramidal neurons revealed gamma frequency trains of IPSPs phase-locked to the local field. EPSPs were almost absent and pyramidal neuronal spiking was sparse. Fast-spiking interneurons generated single spikes on nearly all periods of the on-going gamma oscillation and received large-amplitude trains of EPSPs at gamma frequency, again phase-locked to the local field.
This preliminary analysis of primary sensory cortical gamma oscillations demonstrated many similarities to gamma oscillations characterised in more detail in the hippocampus.

35.05

THE RAT'S TACTILE DISCRIMINATIVE ABILITIES

Filip Aggestam and Peter Cahusac
Department of Psychology, Stirling University, Stirling FK9 4LA, Scotland.

The rat, as a nocturnal animal, relies heavily on its whiskers for near-object identification and navigation. This study investigated the rat's ability to discriminate between different materials. Ten rats were trained over 5 weeks in daily sessions (5/7 per week) to a criterion performance (80% success) on a simple two-choice roughness discrimination task using a Lashley Jumping stand. Subsequently, the rats were introduced to four different materials: wood, aluminium, plastic and stone. Within 2 weeks exposure to the new materials it was found that the rats were reliably able to discriminate between them. This occurred when olfactory, auditory, visual, surface roughness, size and shape cues were controlled for. Finally, 4 different metals were used: brass, iron, copper and aluminium. Here the rats were unable to discriminate between them. The results are consistent with what might be expected from the rat's behaviour in its natural environment where, for example, discriminating between wood and stone would be important. However, there appear to be limits to their abilities. Materials with similar hardness and surface properties (excluding roughness), such as the 4 different metals, could not be discriminated from each other.

Supported by a Nuffield Undergraduate Research Bursary.

36.01

CHANGES IN STRIATAL DOPAMINE RELEASE AND NICOTINIC ACETYCHOLINE RECEPTOR SUBUNIT MRNA EXPRESSION IN THE SUBSTANTIA NIGRA PARS COMPACTA FOLLOWING CHRONIC NICOTINE TREATMENT

1N. P. Visanji, 2S.N. Mitchell, 2M.J. O'Neill and 1S. Duty.
1Neurodegenerative Disease Research Group, GKT School of Biomedical Sciences, King's College London, U.K.2Eli Lilly & Co. Ltd., Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey, U.K.

Whilst dopaminergic neurones in the striatum are sensitive to local application of nicotine, a systemic administration often fails to produce a consistent increase in dopamine release. Since chronic administration of nicotine is known to upregulate the expression of nicotinic acetylcholine receptors (nAChRs), a series of experiments were conducted to assess whether the response to systemic nicotine was enhanced by chronic pre-treatment and, if so, what molecular mechanisms might underlie this enhancement.
Initial microdialysis studies indicated that an acute systemic nicotine (0.8 mg/kg s.c.) challenge in animals pre-treated with nicotine (0.8 mg/kg s.c.) for 8 days produced a significantly higher dopamine efflux compared to 8 day vehicle pre-treated controls. Complementary in situ hybridisation studies revealed that 8 days treatment with nicotine also produced significant increases in $\alpha 6$ and $\beta 3$ nAChR mRNA in the substantia nigra pars compacta when compared to vehicle.
These data demonstrate that nicotine-evoked dopamine release in the striatum is significantly enhanced following chronic nicotine pre-treatment, in parallel with an upregulation of certain nAChR subunits in the nigrostriatal pathway. Further investigation of these molecular mechanisms and the availability of more selective nAChR agonists may aid in the development of new therapies for the treatment of Parkinson's disease.

Supported by an MRC CASE Award (Eli Lilly & Co. Ltd.)

36.02

CHARACTERISATION OF THE MORPHOLOGY, CONNECTIVITY AND NEUROCHEMISTRY OF SINGLE CELLS IN THE GLOBUS PALLIDUS

Sadek, A.R., Magill, P.J., Bolam, J.P.
MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, University of Oxford, Oxford, UK.

The globus pallidus (GP) occupies a pivotal location within the basal ganglia. It has the potential to relay topographically and functionally diverse information to all other nuclei of the basal ganglia. In order to characterise single neurons of the GP, we used the juxtacellular method to label cells with Neurobiotin in different regions of the GP. Neurobiotin was revealed using nickel-diaminobenzidine. Some sections were immunostained for parvalbumin (PV), whilst others were subjected to immunofluorescence to reveal PV and choline acetyltransferase. The full dendritic and local axonal arborisations of each GP cell were reconstructed in three dimensions using Neurolucida. Local axon collaterals of GP neurons were observed to appose PV-positive and unstained perikarya in basket-like arrangements of 6-10 boutons. In addition, axons formed multiple appositions with single PV-positive dendrites. Laterally located cells were found to have discoidal dendritic arborisations. Their local axon collaterals had 350-450 boutons. Medial cells exhibited a radial dendritic pattern with 550-650 axonal boutons. These findings imply that there are at least 2 groups of GP neurons, which via their local axon collaterals have the potential to affect the behaviour of other GP neurons. Electron microscopic analysis of axonal boutons and dendritic terminations is in progress.

36.03

DOPAMINERGIC AND NORADRENERGIC INNERVATION OF GLOBUS PALLIDUS IN THE RAT

P.D. Prime, J. Boyes, P.M. Magill, J.P. Bolam
MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford, OX1 3TH.

Anatomical and physiological data suggest that dopaminergic neurons of the ventral midbrain innervate, in addition to the striatum, other regions of the basal ganglia. The aim of this study was to further characterise the dopaminergic innervation of the globus pallidus (GP) at light and electron microscopic levels. Sections of perfuse-fixed rat brain were immunostained to reveal tyrosine hydroxylase (TH), dopamine transporter (DAT) or dopamine-β-hydroxylase (DβH) and processed for light and electron microscopic analysis. In confirmation of previous studies, the GP was found to contain TH-positive fine axonal arbours and courser axons of passage, and a smaller number of DβH-positive fibres (Parent et al 1987 Brain Res., 426 397-400; Rodrigo et al 1998 J. Chem. Neuroanat., 15 1-20). The sections immunostained to reveal DAT were also found to contain axonal arborisations with prominent varicosities and larger axons of passage. Electron microscopic analysis revealed TH-immunoreactive boutons forming both symmetrical and asymmetrical synapses onto dendrites in the GP. Preliminary electron microscopic analysis of the DAT-immunostained sections has revealed the presence of vesicle-filled immunoreactive synaptic boutons. These data suggest that the GP receives a dopaminergic innervation as well as a less abundant input from noradrenergic fibres.

36.04

THE SUBCELLULAR DISTRIBUTION OF IONOTROPIC GLUTAMATE RECEPTORS IN NEURONS OF THE BASAL GANGLIA IN THE 6 HYDROXYDOPAMINE MODEL OF PARKINSON`S DISEASE

Claude Blondeau and J.Paul Bolam
MRC Anatomical Neuropharmacology Unit, Mansfield road, Oxford OX1 3TH.

In Parkinson`s disease (PD), overactivity of the glutamatergic pathways from the cortex to the striatum and the subthalamic nucleus, and from the subthalamic nucleus to the basal ganglia output nuclei may, in part, underlie the motor symptoms of the disease. The objective of this study was to determine whether there are alterations in the subcellular distribution of NMDA (NR1 and NR2B subunits) and AMPA (GluR2/3 subunits) receptors in neurons of the striatum, subthalamic nucleus and substantia nigra in the 6-hydroxydopamine model of PD. This was addressed using the quantitative immunogold technique on freeze-substituted, lowicryl-embedded tissue obtained from rats two weeks after the lesion. The proportion of immunogold particles for NR1 and NR2B subunits (not GluR2/3) in the membrane of dendrites and spines (excluding the postsynaptic membrane) was lower in the striatum on the lesioned side. However, there was no change of labelling in the cytoplasm and in the postsynaptic membrane between the two sides. These results suggest that alterations in glutamate transmission in the striatum is not reflected in changes of the abundance of ionotropic glutamate receptors at synaptic specializations.

36.05

ACTIVATION OF CANNABINOID CB1 RECEPTORS REDUCES THE RELEASE AND REUPTAKE OF GLUTAMATE IN THE RAT STRIATUM

Brown, T., Brotchie, J., Fitzjohn, S.
1.124 Stopford Building, Oxford Rd, Manchester, M13 9PT.

Cannabinoids produce profound effects on locomotor activity in rodents, in part by an action of striatal cannabinoid CB1 receptors. CB1 receptors are present on the terminals of glutamatergic cortical neurons that innervate the striatum. Electrophysiological studies suggest that activation of CB1 receptors reduces the release of glutamate from corticostriatal neurons, although such an action has not been shown directly. Furthermore, the effect of cannabinoids on glutamate reuptake has not been investigated. We have examined the effects of the plant derived cannabinoid, δ9-tetrahydrocannabinol (THC) on the uptake and K+-stimulated release of [3H] glutamate in rat striatal slices. THC dose-dependently reduced the K+-stimulated release of [3H] glutamate from rat striatal slices with an EC50 of 2.0μM (maximal inhibition: 54±8%, $P<0.05$). Incubation with THC also dose-dependently reduced the uptake of [3H] glutamate by rat striatal slices with an EC50 of 3.7μM (maximal inhibition: 51±8%, $P<0.05$). The effects of 10mM THC on both the uptake and release of [3H] glutamate were fully prevented by 100nM SR141716A, a CB1 receptor antagonist. These findings indicate that CB1 receptors modulate both the uptake and release of glutamate in the rat striatum. Further work will be required to establish the physiological significance of these apparently opposing effects.

37.01

SUB-CHRONIC PCP ADMINISTRATION SIGNIFICANTLY ALTERS PARVALBUMIN-IMMUNOREACTIVE INTERNEURONES IN THE PREFRONTAL CORTEX (PFC) AND HIPPOCAMPUS OF THE RAT

Z. Abdul-Monim, J.C. Neill , G.P. Reynolds1
Bradford School of Pharmacy, University of Bradford, BD7 1DP and 1Department of Biomedical Science, The University of Sheffield, S10 2TN, UK.

Sub-chronic treatment with NMDA antagonists has been shown to profoundly decrease GABA synthesis (Qin et al, , Mol. Brain Res., 1994, 21: 293-302)1994), an effect that may equate to the loss of markers for subtypes of GABAergic interneurones observed in post-mortem schizophrenic patients in cortex (Reynolds & Beasley, J. Neurochem. Anat., 2001, 22: 95-100) and, more profoundly, in hippocampus (Zhang & Reynolds, Schiz. Res., 2002, 55: 1-10). The present study investigated the effects of sub-chronic PCP on parvalbumin (PV)-immunoreactive GABAergic interneurones in rat PFC and hippocampus.Rats (n=12) were sub-chronically treated with PCP (2mg/kg, ip) or vehicle (ip) twice daily for 7 days and brains removed 6 weeks later. Following fixation and immunochemical staining for PV, two-dimensional image analysis was used to determine the size and density of PV-immunoreactive cell bodies. PV interneurone density was significantly reduced ($P<0.01$) in M1 region of the PFC, with significant increases ($P<0.05$) in both the M2 region and the cingulate cortex. Additionally, a significant decrease in both cell size and density in both the dentate gyrus ($P<0.01$) and the CA2/3 region of the hippocampus ($P<0.001$) were observed. These preliminary evidence suggest that sub-chronic PCP can induce a deficit in PV-containing GABAergic neurones in the PFC and, particularly, the hippocampus, equivalent to the neuronal pathology of schizophrenia.

We thank Pfizer Inc for financial support.

37.02

α-CαMKII TRANSLOCATION MECHANISMS IN HIPPOCAMPAL PYRAMIDAL NEURONES

Paul A.A. Grant, Sabine L, Best, Richard Thorogate, and Katalin Torok
Dept. Basic Medical Sciences, St. George's Hospital Medical School, London, SW17 0RE, U.K.

The mechanisms of Ca2+-dependent accumulation of αCaMKII in post-synaptic densities (PSD) is investigated to understand the role of αCaMKII in memory. The time course of intracellular Ca2+ and αCaMKII translocation and the role of Thr286-autophosphorylation were investigated in hippocampal pyramidal neurones. The fluorescences of fluo-4 and EGFP/αCaMKII were monitored in parallel cultures. Intracellular Ca2+ transients were triggered by bath application of CaCl2 (2mM). Fluo-4 fluorescence rose 2.8 ±0.3 (S.E.M.)-fold in the dendritic shafts (n=5) at 27±5s and decayed with t1/2 101±24 s. αCaMKII translocation in dendritic shafts and cell body began at 170±43s (n=9) and increased 3.2±0.6-fold (n=6) at 540±128s (n=5) persisting for 40 min. The non-phosphorylatable T286A-αCaMKII mutant showed early translocation in the processes at 27±5s persisting for 35±6s (n=5). This was followed by delayed translocation (n=2) in which spots appeared in processes and the cell body at 93±19s. The delayed accumulations of wild type αCaMKII were irreversible whereas those of the T286A mutant were reversed by Ca2+ sequestration. These data suggest that initiation of αCaMKII translocation is Ca2+-dependent, however Ca2+-independent permanent localisation of αCaMKII requires Thr286-autophosphorylation.

This work is funded by the MRC.

37.03

NETWORKS OF MITOCHONDRIA AND SMOOTH ER IN HIPPOCAMPAL DENDRITES OF RAT AND GROUND SQUIRREL: A 3D-ELECTRON MICROSCOPE RECONSTRUCTION STUDY

V.I. Popov1,2 and M.G. Stewart11
The Open University, Milton Keynes MK7 6AA, UK; 2Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino 142290, Russia.

Three dimensional (3D) reconstructions of mitochondria and cisterns of smooth endoplasmic reticulum (sER) in dendrites and axons from hippocampus of both rats and ground squirrels were performed via serial ultrathin sections. One giant branched mitochondrion was observed in each dendritic segment examined whereas axonal boutons contained mitochondria only as discrete entities. The maximal length of mitochondria in dendritic segments examined was ~20 um; axonal mitochondria were short, <3 um. The participation of sER cisterns in the structure of giant dendritic mitochondria was demonstrated. Mitochondria were not observed to penetrate into dendritic spines in either dentate gyrus or CA1, whereas CA3 thorns (which represent short dendritic branches) contained mitochondrial segments that did not penetrate into spines. Cisterns of sER originating from the outer membrane of the mitochondrial envelope penetrated into every spine examined. 2h of long-term potentiation (LTP) in rat dentate gyrus in vivo stimulated the fusion of the outer membrane of the mitochondrial envelope with the cell membrane through sER cisterns. Sites of membrane fusion were located directly in apposition to similar fusion sites in: adjacent dendrites; dendrites and presynaptic boutons; and dendrites and glial cell processes.

V.I.P. was supported by The Leverhulme Trust (grantF00269G) and RFBR02-04-48890.

37.04

ISOLATION OF A SLOW CHOLINERGIC SYNAPTIC RESPONSE IN CA1 HIPPOCAMPAL INTERNEURONS

L. Ferrigan, M.J. Khoshnood and S.R. Cobb
Division of Neuroscience and Biomedical Systems, IBLS, University of Glasgow, G12 8QQ.

The dense cholinergic innervation from the medial septal nucleus is believed to be an important regulator of hippocampal function. The purpose of the present study was to investigate the effect of cholinergic septo-hippocampal fibre stimulation upon the diverse population of interneurons. Hippocampal slices (250um) were prepared from Wistar rats (P16-28) and whole-cell current clamp recordings made from visually identified CA1 interneurons. In the presence of 2uM NBQX, 50uM CGP40116 and 10uM bicuculline, to block fast amino acid transmission, electrical stimulation of cholinergic fibres elicited a slow, often suprathreshold, depolarising response (mean amplitude 7.805 +/- 0.6512mV, latency 13.91s) in 20 of 32 cells tested. Application of the muscarinic antagonist, atropine (5uM), produced a complete inhibition of the response (n=8 of 9). 7 cells unresponsive to afferent stimulation were further tested with bath application of carbachol (10uM), 4 responded with a depolarisation of 11.69 +/- 3.09 mV whilst 3 showed no change in membrane potential. Post-hoc anatomical analysis revealed cholinergic responses in several distinct subtypes of interneuron with respect to soma location and dendritic/axonal arborisation. This data demonstrates sufficient synaptic release of acetylcholine to produce slow depolarising responses in interneurons, similar to that seen in pyramidal cells.

37.05

POSSIBLE COMPENSATORY EFFECTS OF OXYTOCIN AND ARGININE-VASOPRESSIN ON HIPPOCAMPAL PLASTICITY

B. Dubrovsky1, A. Tatarinov 1, A. Tsiodras1, J. Harris1 and K. Gijsbers2.
1 McGill University Health Centre, Montreal, Canada; 2 Department of Psychology, Stirling University, Stirling, Scotland.

There is strong evidence that the classical steroids corticosterone, which is released during stress, affects both Long Term Potentiation (LTP) in the hippocampus of rats as well as tasks involving spatial memory. However there is also evidence that other corticosteroids as well as neurosteroids have similar effects on the hippocampus. Apparently LTP can be enhanced as well as diminished by the action of different steroids. In addition, as is shown here, simultaneous injection of both corticosterone and the neurosteroid dehydroepiandrosterone results in counter-balancing effects. Evidence is also presented which indicates that the neurosteroids oxytocin and arginine-vasopressin could be involved in a similar compensatory relationship in modulating neuronal plasticity in the hippocampus.
It is suggested that it is the overall steroid profile release that is significant for understanding any memory effects of stress.

38.01

HALOPERIDOL, BUT NOT CLOZAPINE OR LAMOTRIGINE, IMPROVES THE IMPAIRMENT IN REVERSAL LEARNING INDUCED BY D-AMPHETAMINE IN THE RAT

N.F. Idris1, C.H. Large2, G.P. Reynolds3, J.C. Neill1
1Bradford School of Pharmacy, University of Bradford, West Yorkshire, BD7 1DP, 2Dept. of Biology, Psychiatry CEDD, GSK S.p.A., Verona, Italy 3Department of Biomedical Science, The University of Sheffield, S10 2TN, UK.

Recently we have shown that the psychotomimetics PCP and d-amphetamine induce a selective reversal learning impairment in the rat. The effect of PCP is improved by clozapine, and the anticonvulsant, lamotrigine (Abdul-Monim et al. 2003 J. Psychopharmacology 17(1): 57-66; Idris et al. 2003 Br J Pharmacol. In press) which has some efficacy in bipolar disorder (Calabrese et al 1999 J. Clin. Psychiat. 60: 79-88) but not by haloperidol. The aim of this study was to explore the mechanism of d-amphetamine's effect by comparing the efficacy of haloperidol, clozapine and lamotrigine against d-amphetamine in this paradigm.
Female hooded-Lister rats (Harlan, UK) were trained to perform an operant reversal learning task to 90% criterion. D-amphetamine at 0.5mg/kg produced a significant reduction in performance of the reversal task ($p<0.01$). Lamotrigine (25mg/kg), clozapine (5mg/kg), and haloperidol (0.025mg-0.05mg/kg) had no effect on cognitive performance alone, however, haloperidol (0.075mg/kg) disrupted overall task performance. The combination of lamotrigine or clozapine with d-amphetamine did not prevent the impairment in reversal task performance. In contrast, haloperidol at 0.05mg/kg significantly improved the impairment in reversal task performance induced by d-amphetamine ($p<0.01$). The results suggest differing mechanisms for the cognitive impairment induced by PCP and d-amphetamine and support a role for involvement of the dopaminergic system in the effect of d-amphetamine, but not PCP.

38.02

APP-RELATED PEPTIDES AND MEMORY RESCUE

Lancashire CL, Mileusnic R and SPR Rose
Department of Biological Sciences, Open University, Milton Keynes, MK7 6AA, UK.

Chick APP resembles very closely its human counterpart (95% homology), with the Amyloid beta sequences being identical. Using a one-trial learning task in day old chicks we found that blocking either the synthesis of APP with antisense, or its function with antibodies to its external domains, results in the rapid (<1hr) onset of amnesia. This early onset is compatible with a signalling rather than an adhesion role for APP. Analysis of the manifold activities of APP led to the identification of the small stretch of amino acids (APP319-335) containing the RERMS sequence C-terminal to the KPI insertion site of sAPP-695 as the active domain responsible for neurotrophic activity, growth promotion and neurite extension. Synthetic APP328-332 in both orientations (NH2-RERMS and NH2-SMRER), injected 30 min before training, prevents the memory deficit induced by APP antisense or APP antibody administration. Both RERMS and SMRER contain the palindromic sequence RER, and this tripeptide will itself prevent amnesia. Injected RER rapidly binds to neurons, and is partially displaced by a 17mer APP peptide fragment but not by beta Amyloid, suggesting that RER and Amyloid beta have different binding sites.

38.03

RESTORATIVE PROPERTIES OF AN APP-DERIVED PEPTIDE ON THE INDUCTION OF HIPPOCAMPAL LTP IN BETA-AMYLOID TREATED BRAIN SLICES

A. J. Milner, G. M. Dallerac, D. M. Cummings, S. P. R. Rose, R. Mileusnic & K. P. S. J. Murphy
Department of Biological Sciences, Open University, Walton Hall, Milton Keynes, MK7 6AA, UK.

The amyloid precursor protein (APP) is implicated in learning and Alzheimer's disease (AD). In the latter, the APP-derived peptide, beta-amyloid (Ab), is believed to be pathogenic. Whilst the role of APP in learning is not fully known, there is evidence to suggest that APP may serve in a variety of ways; as a signalling and/or adhesion molecule.
Perturbation of APP, using either antisense or antibodies to APP, induces amnesia in chicks trained in a one-trial learning task. Remarkably, injection of another APP-derived peptide (RERMS) prior to training prevents amnesia, suggesting that the RERMS moiety is capable of mimicking the signalling function of APP in learning. Further experiments have revealed that this property resides in the palindromic sequence RER (Mileusnic, Lancashire & Rose, this meeting).
Injection of Ab has been shown to impair learning in several animal models. in vitro, hippocampal long-term potentiation (LTP) is widely held to be a model of learning. We report here that exposure to the Ab fragment (Ab25-35) blocks the induction of LTP at CA3-CA1 synapses in mouse hippocampal slices. However, if the slices were also exposed to RER, then LTP could be successfully induced. The ability of RER to rescue LTP in a mammalian system suggests that this novel peptide may have efficacy as a cognitive enhancer in humans, particularly with reference to AD.

38.04

REDUCTION OF CELL PROLIFERATION AFTER PASSIVE AVOIDANCE LEARNING IN CHICK HIPPOCAMPUS

Ageliki M. Nikolakopoulou1, Catherine R.Dermon2 and Michael G. Stewart1
1Dept. of Biological Sciences, The Open University, Milton Keynes, MK7 6AA, UK2.Dept. of Biology, University of Crete, Heraklion 714 09, Crete, Greece.

Neurogenesis persists in adult life in the avian brain (Alvarez-Buylla, 1990a Experientia 46: 948-55). Newborn cells can be identified by the administration of 5-bromo-2-deoxyuridine (BrdU), which is an analog of thymidine and incorporates into the DNA during the S-phase of the cell cycle.
In the present study, the objective was to examine cell proliferation in the hippocampus (HP) after passive avoidance training. The domestic chick hippocampus can be divided into dorsal and ventral regions, based on the response to ischemia (Horner et al., 1998 Eur. J. Neurosci. 10: 3913-3917). Cell proliferation was measured from the same chick groups as in the previous proliferation study (Dermon et al., 2002, Europ. J. Neurosci., 16, 1-10) at 24h and 9 days post BrdU injection.
In contrast to data from the IMHV and LPO where neurogenesis increases after passive avoidance training, in the hippocampus there is a reduction in cell proliferation. The explanation for this finding is unclear, but one possibility is that avoidance training experience may be causing stress which is expressed in chick hippocampus by a reduction in cell proliferation which occurs in the rat hippocampus as a result of stressful experiences (Gould and Tanapat, 1999 Biol Psychiatry. 46(11): 1472-9).

38.05

EFFECTS OF INTRAPERITONEAL DEHYDROEPIANDROSTERONE SULPHATE ON LEARNING AND MEMORY IN CHICK

Sujkovic E, Fry* JP, Mileusnic R, Rose SPR
*The Open University, Department of Biological Sciences, Brain and Behaviour Research Group, Walton Hall, Milton Keynes, MK7 6AA, UK; *Department of Physiology, UCL, London WC1 6BT, UK.*

Previous evidence has suggested a role for the steroid dehydroepiandrosterone sulphate (DHEA-S) in the regulation of learning and memory in rodents. Subsequent work from our group has demonstrated that DHEA-S is present in the brain of day old chicks and has memory enhancing effects following intracerebral administration (Migues et al., Neurosci., 109(2): 243-251, 2002; Johnston and Migues, Neural Plasticity, 8(4): 255-270, 2001).
In the present study, the effects of peripherally administered DHEAS on the acquisition and consolidation/retrieval stages of memory formation were studied in day old chicks, using a one-trial passive avoidance learning task. This task employs taste aversion and has been used extensively in studying the mechanisms of memory formation, as it is rapid, precisely timed and reproducible. Intraperitoneally administered DHEAS (20 mg/kg) before or after training enhanced recall 24 hours following training in a weak version (usually recalled for less than 9 hrs) of the passive avoidance task. The memory enhancing effects of DHEAS were observed at 30 min before and 30 min or 4.5 hrs after the training session, but not at 30 min prior to the 24 hrs retention test.
These findings provide further evidence that DHEAS enhances learning and memory and may be involved in the temporal biochemical cascade of long-term memory formation.

38.06

OESTRUS-CYCLE DEPENDENT EFFECTS ON MOUSE OLFACTORY MEMORY AND NMDA-EVOKED PLASTICITY

Gabriela Sánchez-Andrade and Keith Kendrick
Laboratory of Cognitive and Developmental Neuroscience, The Babraham Institute, Babraham, Cambridge CB2 4AT.

Ovarian hormones influence adult cognitive functions. This study tests whether there are oestrus cycle-dependent effects on olfactory memory tasks in mice and if they are associated with altered NMDA-evoked plasticity changes within the olfactory bulb (OB) and piriform cortex (PC).
Two NMDA-dependent behavioural tests were used: social transmission of food preference and a habituation-dishabituation task with a long-term retention test. In both, proestrous mice (C57Bl6/129SV) formed a robust long-term olfactory memory (24h) while oestrous and dioestrous females didn't. Short-term habituation-dishabituation responses weren't influenced by cycle stage.
In vivo microdialysis in anaesthetised mice showed a potentiation of neurotransmitter release as result of repeated NMDA receptor activation in the OB. Significant enhancement of evoked glutamate, serotonin and noradrenaline release was detected in the OB of proestrous mice in response to a third NMDA (250mM) challenge 4h after initial challenge. This was absent in dioestrous and oestrous mice.
Similar enhancement in levels of glutamate, GABA and dopamine were seen downstream in the ipsilateral PC of proestrous mice and of GABA in oestrous animals. No effects were found in dioestrous mice.
Results suggest a role for sex hormones in facilitating NMDA-dependent plasticity changes associated with olfactory memory in female mice.

38.07

DIFFERENTIAL REGIONAL AND GLUTAMATE RECEPTOR-DEPENDENT ENCODING, CONSOLIDATION AND RETRIEVAL OF PAIRED-ASSOCIATE LEARNING

R.G.M. Morris, M. Day and R. Langston
Division and Centre for Neuroscience, The University of Edinburgh,1 George Square,Edinburgh EH8 9JZ.

Paired-associate learning is often used to examine episodic or event memory in humans. The best animal model to date is the recall of 'what, where and when' by scrubjays after food-caching (Clayton and Dickinson, Nature, 1998), but this species unsuited to neurobiological analysis. We report a new paradigm in which rats are trained to encode paired-associates during single sample trials on each successive training day. The effectiveness of this encoding is displayed daily as above chance recall of one item of either pair (a spatial location) when cued by the other (a specific flavour of food). In Exp 1, using unique 'what-where' pairings, blocking NMDA receptors in the hippocampal formation impaired memory encoding but had no effect on recall. Inactivating hippocampal neural activity by blocking AMPA receptors impaired both encoding and recall of one-trial pairs. The latter deficit may be secondary to a strictly spatial impairment. However, in Exp 2, paired-associates that had been trained repeatedly were recalled successfully during hippocampal AMPA receptor blockade. Thus, representation of a familiar space can be stored outside the hippocampus and then retrieved to enable unique 'what-where' associations to be automatically encoded within a hippocampal memory space.

38.08

SPATIAL LEARNING AND MEMORY IN RCS RATS USING THE WATER MAZE

Lianne Robinson1, Tom E. Salt2 and Gernot Riedel1
1 Dept. Biomed. Sci., Univ. Aberdeen, Aberdeen; 2 Inst. Ophthalmology, UCL, London.

Royal College of Surgeons (RCS) rats have a progressive loss of rods during the first few weeks/months of life. Here we assessed their spatial learning and memory in a reference memory task using the water maze. Experiments were in accordance with Animals (Scientific Procedures) Act 1986 used four groups of rats (control-pre-trained, control-naïve, RCS-pre-trained, RCS-naïve: N=8 each). Pre-trained rats were tested at 3 weeks of age and again at 3 months, naïve groups were tested at 3 months only. Paradigm: 4 trials per day on 4 days (max. 90s, 30s on platform in a fixed location); probe trial on day 5 (platform removed, max. 60s).
Results showed no difference between groups at 3 weeks of age. Three months later, the RCS pre-trained subjects were profoundly impaired in reversal learning compared with pre-trained controls. At 3 months of age naïve RCS and control rats also performed worse than pre-trained controls. Learning was evident in all 4 groups with final performance levels not different on day 4 and no difference in the probe trial. Results suggest that the RCS rats can learn and remember a spatial paradigm. Subtle deficits in dystrophic RCS rats may be due to a lack of visual input.

38.09

CANNABINOID-INDUCED LEARNING DEFICIT: IS IT SPATIAL?

L. Robinson, R. G. Pertwee and G. Riedel
Department of Biomedical Sciences, Institute of Medical Science, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD.

The synthetic cannabinoid HU210 is a potent CB1 receptor agonist with long lasting pharmacological effects in vivo. HU210 induced a spatial learning deficit in a reference memory task using the water maze (Ferrari et al, 1999). This experiment tested if the deficit could be reversed by CB1 antagonists SR141716A (3mg/kg)and AM281 (0.5mg/kg, 1.5mg/kg). In Exp.1, rats were trained 4 days, 4 trials a day (90s max, 30s on platform), all inter trial intervals (ITI) were 30s. Probe trials were performed 1, 4 and 7 days following acquisition. In Exp. 2, rats were pre-trained on a delayed-match-to-position task (DMTP) with ITI (30s or 1hr) between trial 1 and 2, all others were 30s. HU210 (100ug/kg) injected on alternating days (Exp. 2A) or with a 2 day interval (Exp. 2B). All experiments were performed in accordance with Animals (Scientific Procedures) Act 1986. HU210 induced a learning and memory deficit in acquisition and probe trials (Exp. 1) that was not reversed by the antagonists. With deficits in spatial and procedural elements of the task. Pre-training in the DMTP task removed procedural deficits and there were no spatial learning deficits (Exp. 2A and B). These results question whether the action of HU210 on learning is mediated by CB1 receptors. With the lack of effect in Exp. 2 suggesting that learning deficits are non-spatial and may not be mediated by the hippocampus.

38.10

THE DENTATE GYRUS IS ESSENTIAL FOR SPATIAL WORKING MEMORY IN MICE

Dominic V. Harbaran & Gernot Riedel
Dept. of Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland AB25 2ZD.

We have recently shown that the dentate gyrus (DG) is essential in the acquisition phase of spatial reference memory in mice (Harbaran & Riedel, FENS Abstr. vol1, A107.15, 2002), and consequently investigated its role in spatial working memory. Naïve C57Bl/6 mice were trained in a delayed-match-to-position (DMTP) paradigm using an open-field water maze with four trials a day and a 90 s intertrial interval (ITI). The location of the submerged platform was altered daily. Once the task was acquired, the interval between trials one and two was increased to 20 min and the mice were tested for three days. After training and testing, ten mice received bilateral intra-dentate colchicine infusions (2.5mg/ml; 3 sites per hemisphere) in order to ablate the DG; 10 mice received sham surgery. Adequate measures were taken to minimise pain and discomfort in accordance with the Animals (Scientific Procedures) Act 1986. After 10 days recovery, the mice were retested in the DMTP task with ITIs of 0 s, 90 s and 20 min for three days in each ITI condition. Lesioning of the DG impaired mice on the task independent of interval between the first and second trial. There was neither a trial one deficit nor alteration in swim speed indicating a purely spatial impairment and not a procedural deficit. Results suggest that the DG in mice supports spatial working memory in a delay-independent manner.

38.11

AMYLOID PRECURSOR PROTEIN AND A PROTEIN KINASE C SUBSTRATE (MARCKS) IN LONG-TERM MEMORY: A STUDY OF DIFFERENTIAL GENE EXPRESSION

R. O. Solomonia (1), K. Morgan (2), A. Kotorashvili (1), B. J. McCabe (3), A. P. Jackson (2) and G. Horn (3)
(1) Inst. Physiology, Georgian Academy of Sciences, 14 L. Gotua Str., Tbilisi 38600, Georgia(2) Dept. Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK(3)Sub-Dept. Animal Behaviour, University of Cambridge, Dept. Zoology, Madingley, Cambridge CB3 8AA, UK.

The IMHV is a region of the chick brain storing information acquired through the learning process of imprinting. We have examined in this region changes in expression of candidate genes involved in memory. Chicks were exposed to a rotating red box and the strength of their preference for it, a measure of learning, determined. Brain samples were removed ~24 h after training. Candidate genes whose expressions were different in IMHV samples derived from strongly imprinted chicks relative to those from chicks showing little or no learning were identified using subtractive hybridization. The translation products of two candidate genes [amyloid precursor protein (APP) and myristoylated alanine rich C kinase substrate (MARCKS)] were investigated further in the IMHV and in a region not previously implicated in imprinting, the posterior neostriatum. In left IMHV, levels of the two proteins increased with strength of learning. The effects in right IMHV were not significantly different from those in the left. There were no effects of learning in posterior neostriatum. This is the first study to relate changes in amounts of MARCKS and APP proteins to strength of learning in a region known to be a memory store and indicates that systematic identification of proteins involved in memory is possible.

38.12

EXPRESSION OF THE GABA(A) RECEPTOR GAMMA4-SUBUNIT GENE IN THE ZEBRA FINCH BRAIN DURING AND AFTER SONG LEARNING

Christian Thode, Hans-Rudolf Guettinger and Mark G. Darlison
Neuroscience and Signal Transduction Laboratory, School of Science, The Nottingham Trent University, Nottingham NG11 8NS, England (CT and MGD), and Abteilung fuer Allgemeine Zoologie, Fachbereich Biologie, Universitaet Kaiserslautern, 67663 Kaiserslautern, Germany.

GABA(A) receptors are the major inhibitory neurotransmitter receptors in brain. In mammals, they exist as pentameric assemblies of different combinations of 16 subunits (alpha1–alpha6, beta1–beta3, gamma1–gamma3, delta, epsilon, pi and theta); two additional polypeptides, beta4 and gamma4, are present in certain vertebrates. In the chick, the gamma4-subunit transcript, which is found in brain regions that process sensory information, is down-regulated after imprinting training on a visual stimulus, a form of recognition memory. These data are consistent with previous observations that GABA(A) receptor ligands modulate learning and memory in a variety of species. The song system of the zebra finch (Taeniopygia guttata) comprises an anterior forebrain pathway (AFP) that is required mainly for song acquisition, and a set of nuclei that form the vocal motor pathway. Both circuits undergo a variety of biochemical, electrophysiological and morphological changes as the juvenile bird learns to sing. We have shown that the gamma4-subunit mRNA is localised in several parts of the AFP, and that the level of this transcript differs in juveniles vs. adults. These data suggest that receptors containing the gamma4 subunit play a role in the establishment of the neuronal circuits that control the stereotypic process of song production.

39.01

GENE THERAPY FOR CONGENITAL MYASTHENIC SYNDROMES

Amr Abdelgany, Angela Vincent, Matthew J. A. Wood1 and David Beeson.
Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DS, UK; 1 Department of Human Anatomy, South Parks Road, Oxford.

The slow channel congenital myasthenic syndrome (SCCMS) is a dominantly inherited disorder of the neuromuscular transmission. It is caused by excitotoxic mutations the genes encoding the subunits of the muscle acetylcholine receptor (AChR).
Selective suppression of the mutant alleles may restore normal receptor kinetics. Here, we study the potential of allele-specific posttranscription gene suppression using hammerhead ribozymes and their DNA analogue, DNAzymes. These catalytic nucleic acids can be directed to cleave RNA transcripts at sites created by a mutation.
We demonstrate mutation-specific cleavage of full-length cRNA transcripts harbouring the slow channel syndrome mutation αS226F by hammerhead ribozymes, and of a second slow channel syndrome mutation, αS269I, by DNAzymes. For a third mutation, αV156M, which does not create an easily targeted cleavage sites, we identified putative cleavage sites near the mutation and designed the DNAzyme binding arms to specifically target the mutant sequence by creating a mismatch with the wild-type sequence. A significant discrimination in cleaving the mutant cRNA was observed both in vitro and in HEK293 cells transfected with mutant and wild type AChR. Our results suggest that in vitro catalytic nucleic acids can be designed to give allele-specific down-regulation of most AChR missense mutations.

39.02

ENHANCED TRANSFECTION OF REACTIVE GLIA IN THE INJURED CNS: NOVEL METHODS FOR OPTIMISING DELIVERY OF THERAPEUTIC GENES

Lee Barrett, Ajaz Ahmed, Mike Allan, Martin Berry and Ann Logan
Molecular Neuroscience, Division of Medical Sciences, University of Birmingham, Birmingham, B15 2TT, UK.

Traumatic CNS injury leads to a cascade of events that inhibit axonal regeneration and ultimately lead to neuron death. One crucial aspect of the injury response determining axonal regeneration, is glial scarring at the site of injury. Transfecting activated glial cells at the injury site with cDNA encoding anti-fibrotic agents, such as the TGF-ß antagonist decorin should block scar tissue formation thereby facilitating axonal regeneration. Transfection of mixed primary adult dorsal root ganglion (DRG) cultures with PEI22, at an N:P ratio of 1.0, revealed a 14.1±1.01% level of GFP expression. Double staining revealed reporter gene expression in GFAP-positive glia and also in occasional neurons. Injection of the optimised PEI22/DNAGFP polyplexes directly into the rat spinal cord at a concentration of 0.5mg/ml DNA led to significant levels of GFP expression in glia bordering the injection site at 5 days. In relation to other polycationic polymers and lipids, which have limited success only in the CNS, these results suggest that efficient transfection of post-mitotic reactive glia in the injured CNS is possible with therapeutic nucleic acids using linear PEI22.

39.03

"STEALTH" ADENOVIRUS ENTRY TO CNS NEURONS AND GLIA

Karen Doyle, Mark Stevenson, Kerry Fischer, Len Seymour, Martin Berry, Vivian Mautner, Ann Logan
Molecular Neuroscience Group, Division of Medical Sciences, University of Birmingham; The Wolfson Research Laboratories, Queen Elizabeth Medical Centre, Edgebaston, Birmingham B15 2TH, UK.

Adenoviruses used in gene therapy are flawed by two major problems: recognition by the immune system and loss of cell specificity due to CAR and av integrin promiscuity. Fisher et al. (2001) coated adenovirus using pHPMA (poly-[N-(2-hydroxypropyl)metacrylamide]) producing stealth adenoviruses that are taken up only by pinocytotic cells. Results using Ad5-CMV-GFP (green fluorescence protein) coated with Texas Red labelled pHPMA (TRDpHPMA) (surface charge -30mV) at 20mg/ml show that, stealth adenovirus is preferentially taken into highly pinocytotic DRGN (dorsal root ganglion neurons) and RGC (retinal ganglion cells), but not glia or C6 cells. DRGN, RGC and C6 cells were tested with the polycation polylysine-FITC to test cell surface charge to see if stealth adenovirus pinocytotic uptake is charge dependent. 50% of DRGN, 100% of surrounding glia and C6 cells were negatively charged. RGC were 80% positively charged. Microscopy after 5 days showed 50% of DRGN and >90% RGC showed adenovirus-TRDpHPMA uptake, suggesting that stealth adenovirus enters positively charged neurons by pinocytosis. Lysotracker showed that DRGN and RGC pinocytotic vesicles have the acidic environment required for adenovirus capsid dismantling, but the coated virus does not express GFP.

Ref: Fisher et al. Gene Therapy (2001) 8, 341-348

39.04

VIRAL MEDIATED DELIVERY OF CO-CHAPERONE MOLECULES REDUCES POLYGLUTAMINE AGGREGATION IN A MODEL OF SBMA

Howarth J.L, Glover C.P.J, Cheetham M.E, Bienemann A.S, Hobson R.J, Cosgrave A.S, Gallo J-M and Uney J.B.
Bristol University Research Centre for Neuroendocrinology, Marlborough Street, Bristol, BS2 8HW, UK; Department of Neurology, Institute of Psychiatry, King`s College London, De Crespigny Park, London SE5 8AF, UK; Division of Molecular Genetics, Institute of Ophthalmology, UCL, London, UK.

Spinal and Bulbar Muscular Atrophy (SBMA) is a degenerative disease of motor neurones linked to an expansion within the polyglutamine tract of the androgen receptor (AR) gene. The expansion ultimately results in the formation of protein inclusions (containing AR aggregates, chaperone molecules and components of the ubiquitin/proteasome degradation pathway) that cause toxicity and/or sequester cell survival proteins. Recent studies have demonstrated that some chaperone molecules, including hsp70 and hsp40, can inhibit the formation of these protein aggregates and may therefore prevent SBMA.
In this study we have used highly efficient adenoviral mediated transfection to study chaperone function in N2a Neuroblastoma cells transfected with wild-type AR (hAR) or expanded AR (hARk). The presence of hsp70 alone had no significant effect on the level of aggregation observed by immunofluorescence microscopy, but cotransfection with various co-chaperones, including neuronal hsp40s, caused a decrease in cytosolic aggregates. Similar results were observed after transfection of co-chaperones alone. In summary, we have shown that a number of previously uncharacterised co-chaperones can reduce the level of aggregation of expanded AR, supporting the hypothesis that such molecules may represent therapeutic targets in the treatment of SBMA and other triplet repeat diseases.

39.05

ENHANCING EXPRESSION FROM A NEURON-SPECIFIC DUAL VECTOR TET-OFF SYSTEM USING THE WOODCHUCK ENHANCER WITH NO LOSS OF TRANSGENE REGULATION

Youn-Bok Lee, Colin P. J. Glover, Alison S. Bienemann, James B. Uney
Bristol University Research Centre for Neuroendocrinology (URCN), Marlborough Street, Bristol BS2 8HW, UK.

Most viral vectors used to study gene function in the brain cannot target expression specifically to neurons or mediate regulatable transgene expression. In this study we have used the Woodchuck Hepatitis virus posttranscriptional enhancer (WPRE) to augment the expression mediated by the dual-adenoviral neuron-specific tetracycline regulatable system. This involved the 3` addition of the WPRE to the Ad vectors encoding the CMV-rtA (Tet-off) transactivator, the synapsin-rtA transactivator and the tetracycline regulatable element (TRE)-EGFP cassette. The results demonstrated that the inclusion of the WPRE did not alter the regulatability of the Tet-off system when used to transfect hippocampal neurons. Furthermore, EGFP-expression mediated by the neurone-specific (synapsin promoter driven) and pleitropic (CMV-promoter driven) Tet-off Ad system containing the WPRE could mediate strong neuronal transgene expression at all MOI`s(100, 50, 10)) used. However, without the addition of the WPRE no expression at the lower MOIs could be seen with either system. This WPRE enhanced- tet-regulatable Ad system can therefore be used at MOIs that cause little cytotoxic effects in vitro and reduced immune response in vivo. The optimization of this vector system should facilitate its use in studies of neuronal gene function and as a gene therapy agent.

40.01

GROWTH AND DIFFERENTIATION CHARACTERISTICS OF MYC IMMORTALISED HUMAN NEURAL STEM CELL LINES

Miljan E.A., Pollock, K., Rodrigues, T ,Patel S, and Sinden J.D
ReNeuron, 10 Nugent Road, Surrey Research Park, Guildford, Surrey, GU2 7AF.

Immortalised neural stem cell lines that can be expanded in culture and are capable of differentiation to a more mature phenotype are useful for a variety of neuroscience research and development applications. Primary neural stem cells isolated from early (first trimester) fetal brain obtained under national ethically approved protocols, were plated on laminin and expanded using the growth factors EGF and bFGF. Cells were immortalised using a retrovirus carrying a myc immortalising gene. Colonies were selected and expanded into cell lines which have been maintained over 30 passages. Cells were characterised with respect to phenotype using immunoctytochemistry and confocal microscopy. In standard growth conditions the cell line 197VM (ventral mesencephalon) and the cell line CTX027 (cortex) have a flattened polygon morphology, are nestin positive; beta-III tubulin and GalC negative. They retain stable diploid karyotype and consistent phenotype over multiple passages and grow readily as adherent monolayers in all common culture formats. Within 4-14 days after removal of growth factors both lines undergo growth arrest and acquire a more neuronal morphology with extensive networks of beta-III tubulin-positive cells. In addition GFAP-positive astrocytes are also present but in lower abundance. The 197VM line also generates low levels (<1%) of TH-positive neurons. We are currently investigating the utility of these cells in a range of neuropharmacology and toxicology applications.

40.02

TRANSCRIPTIONAL REGULATION OF PAX GENES DURING THE DIFFERENTIATION OF NEURONS BY HUMAN PLURIPOTENT STEM CELLS

S.A. Przyborski
School of Biological and Biomedical Sciences, University of Durham, Science Laboratories, South Road, Durham. DH1 3LE.

Pax genes encode proteins that have been implicated as regulators of cell fate specification and development. To identify whether particular members of the Pax gene family are differentially expressed during the development of the nervous tissue in man, we examined the transcriptional profile of several Pax family members during the differentiation of human neurons using oligonucleotide micro-arrays. Human pluripotent embryonal carcinoma (EC) stem cells were grown as adherent monolayers and induced to differentiate using retinoic acid. Total RNA was collected from differentiating cells over 21 days and from purified populations of EC-derived neurons. Labelled RNA was hybridised against a custom oligonucleotide micro-array representing several hundred human target sequences including those specific for seven members of the Pax gene family. Only one member of this family of transcripts showed differential regulation. The transcriptional profile and upregulation of Pax6 expression correlated with the appearance of neurons and expression of other neural transcripts. Induction of Pax6 expression during retinoic acid-induced differentiation of human EC cells was also confirmed by RT-PCR. The transcription of Pax6 and absence of the dorsal marker Pax7 suggest that neurons generated from human pluripotent stem cells are ventral in character.

40.03

ENHANCING THE PRODUCTION OF NEURONS FROM HUMAN STEM CELLS

S.A. Przyborski
School of Biological and Biomedical Sciences, University of Durham, Science Laboratories, South Road, Durham. DH1 3LE and ReInnervate Limited, OldShire Hall, Old Elvet, Durham, DH1 3HP.

Human embryonal carcinoma (EC) stem cells derived from germ cell tumours are valuable tools for the study of embryogenesis and closely resemble embryonic stem cells. We have previously described neural differentiation by the EC lineage, TERA2.cl.SP12, in response to retinoic acid (Przyborski 2001, Stem Cells, v19, p500). When TERA2.cl.SP12 cells are exposed to retinoic acid and grown as adherent monolayers, only 10-15% of cells commit toward the neuronal lineage whilst the remainder of cells produce non-neuronal cell types including epidermal tissues. Using established protocols it is possible to isolate and purify neurons from such cultures. Such a process takes several weeks and the numbers of neurons produced is relatively low. In this study, we describe the development of novel procedures to enhance neuronal productivity with increased efficiency using a combination of selective growth conditions and cell sorting. This approach will result in the production of large numbers of neurons from human EC cells at high levels of purity, which will be useful for research purposes and drug discovery programmes.0

40.04

PROTEIN EXPRESSION PROFILING AND THE IDENTIFICATION OF NOVEL CELL MARKERS ASSOCIATED WITH NEURAL DIFFERENTIATION BY HUMAN PLURIPOTENT STEM CELLS

M.W. Hayman and Stefan A Przyborski
School of Biological and Biomedical Sciences, University of Durham, Science Laboratories, South Road, Durham. DH1 3LE.

The characterisation of protein markers that distinguish stem cells and differentiated neural cell types would be useful in any study which attempts to identify the biochemical changes associated with human stem cell differentiation and the formation of neurons. Moreover, this information may permit precise localisation of a variety of cell types and could ultimately lead to early in vivo identification of anomalies in stem cell populations such as carcinomas and tumours of the central nervous system. Here we report the analysis of soluble protein preparations from TERA2.cl.SP12 human embryonal carcinoma stem cells (Przyborski, 2001, Stem Cells, v19, p500) and their differentiated derivatives, including mature neural cells. Analysis via SELDI TOF mass spectrometry and 1D PAGE has yielded several target cell specific markers that show almost exclusive expression in specific populations of cultured cells. Work is currently being performed to isolate and characterise these target proteins using a combination of chromatography and mass spectrometry. Identification of proteins expressed in specific cell populations will provide valuable tools for monitoring cell differentiation and molecules involved in particular pathways of development.

40.05

NEURAL DIFFERENTIATION BY HUMAN PLURIPOTENT STEM CELLS EXPRESSING GREEN FLUORESCENT PROTEIN

R. Stewart, M. Lako and S.A. Przyborski.
School of Biological and Biomedical Sciences, University of Durham, Science laboratories, South Road, Durham, DH1 3LE.

Neural differentiation by pluripotent human embryonal carcinoma (EC) stem cells in vitro closely resembles the process of neurogenesis in the embryo (Przyborski et al, 2000, EJN, 12, p3521). In this study, we have derived a novel human EC cell line, TERA2.cl.SP12-GFP, which stably expresses green fluorescent protein (GFP). To test whether GFP-positive stem cells were able to form neural derivatives, we exposed cultures to retinoic acid and grew cells either as adherent monolayers or as suspended aggregates. Cell differentiation was monitored by phase microscopy and by the expression of stem cell and neural markers using cytometry, western analysis and immunocytochemistry. TERA2.cl.SP12-GFP cells expressed pluripotent stem cell markers (eg. SSEA-4) that were rapidly down-regulated in response to retinoic acid but were replaced by the expression of neural markers (eg. N-CAM). Cells grown as aggregates formed neurospheres whilst those cultures grown as monolayers formed morphologically identifiable neurons after 2-3 weeks of differentiation. In addition, TERA2.cl.SP12-GFP cells formed well differentiated xenograft tumours when transplanted into nude mice. In all cases, stem cells and their differentiated derivatives remained GFP-positive. This cell system provides a value resource for cell tracking in vivo, co-culture experiments and the study of human neural development.

40.06

NEURAL DIFFERENTIATION BY MAMMALIAN MESENCHYMAL STEM CELLS *IN VITRO*

A.P. Croft and S.A. Przyborski
School of Biological and Biomedical Sciences, University of Durham, Science Laboratories, South Road, Durham DH1 3LE.

.Recent research has demonstrated that mesenchymal stem cells (MSCs) isolated from mammalian bone marrow have the capacity to differentiate into neural cell lineages in vitro. In our laboratory we are investigating the induction of neural differentiation by mammalian MSCs under various defined culture conditions previously shown to a induce neural phenotype (e.g. exposure to ß-mercaptoethanol (BME), dimethylsulphoxide) and conditions consistent with those that induce neural differentiation of embryonic stem cells in vitro. Cells were exposed to induction agents for various periods ranging from 5hrs to 7 days. Treated cultures were harvested in the appropriate manor for immunocytochemistry and western blot analysis to determine the expression of neural cell-type markers (e.g. NF-68, GFAP, NSE, nestin, ß-tubulin III). A variable number of cells were responsive to each of the induction agents and a range of neural-like morphologies resulted in response to either BME, retinoic acid, or brain derived neurotophic factor. This data suggests that there may be differences in the mechanisms of neural differentiation by MSCs under various culture conditions. To address this issue, we are currently examining the expression of various proneural transcription factors, such as neuroD1, during the differentiation of neural derivatives from mammalian MSCs in vitro.

40.07

CELL FATE DETERMINATION IN THE HUMAN EMBRYONIC ECTODERM AND THE INDUCTION OF THE NEURAL LINEAGE

G.M. Horrocks, V.B. Christie, L. Lauder, M.W. Hayman and S.A. Przyborski
School of Biological and Biomedical Science, University of Durham, Science Laboratories, South Road, Durham, DH1 3LE.

Cell signaling is critical during embryonic development. Previous experiments indicate that neural induction is the default pathway of differentiation and ectodermal cells will form neural tissues unless instructed otherwise. The formation of epidermal tissues is partly dependent on the activity of bone morphogenetic proteins (BMPs) that signal to inhibit neural induction and induce the formation of epidermal progenitors. Antagonism of BMP activity results in the formation of neural progenitors. Similarly, dissociation of cells disrupts BMP signaling giving rise to neural differentiation. Whilst much is known about these signaling events in lower vertebrates, there are few data concerning their role in humans. Using a pluripotent embryonal carcinoma (EC) stem cell model, we describe how TERA2.cl.SP12 human EC cells differentiate in response to retinoic acid using two alternative approaches that dramatically change cell fates decisions. Culturing differentiating EC cells as confluent adherent monolayers results in the formation of few neurons (<15% total cell population) and the expression of epidermal markers. However, growing dissociated EC cells in suspension results in tissues that are almost entirely neural in nature. We are currently using this model to investigate the role of BMP signaling during the differentiation of human pluripotent stem cells.

41.01

IS THE NEURONAL APOPTOSIS INHIBITOR PROTEIN (NAIP) REALLY A NEURONAL PROTEIN?

Tracy A Lynch*, Nguyen thi Man*, Thanh Le, Aurthur H.M. Burghes, Carolina Soler-Botija, Eduardo Tizzano and Glenn E. Morris*.
**Biochemistry Group,North East Wales Institute, Wrexham LL11 2AW, UK.Dept of Medical Biochemistry, Ohio State University, COLUMBUS, OH43210, USA.Genetics and Research Institute, Hospital Sant Pau, Barcelona, Spain.*

The gene for human neuronal apoptosis inhibitory protein (NAIP) was isolated from a human fetal brain cDNA library during a search for the genes responsible for spinal muscular atrophy (SMA). Subsequent antibody studies suggested that NAIP is a 150kD cytoplasmic protein in neuronal cells, especially cerebellar Purkinje cells, but some inconsistencies remained. NAIP mRNA levels were found to be very low in brain and much higher in liver and placenta. Several mouse NAIP genes are also expressed mainly in non-CNS tissues.
Using a panel of monoclonal antibodies raised against a recombinant NAIP fragment, we have identified a 150kD protein expressed exclusively in rabbit and rat CNS cells. This protein is expressed at very high levels in Purkinje neurons but it is a cross-reacting protein and not NAIP itself. SMA fetal spinal cord with a genetic deletion of exons 5 and 7 in both NAIP alleles did not show any decrease in levels of 150kD protein or in neuronal staining by antibody. We suggest that NAIP shares a dominant epitope with a more abundant protein, and that this cross-reacting protein is responsible for the apparent abundance of NAIP in rabbit neurons. Attempts to characterize and identify this Purkinje cell protein will be described.

41.02

MICROGLIAL ACTIVATION IS ASSOCIATED WITH INCLUSIONS IN DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA OF A CHRONIC MOUSE MODEL OF PARKINSON`S DISEASE

G. E. Meredith, H. Milligan, A.G. Dervan, P. Hariharan, S. Totterdell
Department of Cellular and Molecular Pharmacology, Chicago Medical School, North Chicago, IL USAUniversity Department of Pharmacology, Oxford OX1 3QT, UK.

Parkinson's disease (PD) is characterised, pathologically, by a persistent inflammatory response, mediated by microglia. Inflammation has been found in acute animal models, but because these models do not emulate the progressive nature of the disease, experimental results are difficult to compare to PD. We have developed a chronic model in C57/bl mice using MPTP (25 mg/kg) with probenecid (250 mg/kg) (MPTP/P). This model exhibits a progressive loss of nigral dopaminergic (DA) neurons, persistent motor impairment and inclusion formation. The animals were deeply anaesthetised and perfused transcardially at three time points post-treatment. Fifty micron thick sections were immunoreacted with Mac-1 (microglia), tyrosine hydroxylase and alpha-synuclein antibodies. The dual-labeled tissue was processed using the ABC system with DAB and nickel-intensified DAB. The triple-labeled tissue was processed for confocal microscopy using flurophore-labeled secondary antibodies (Cy2, Cy3, Cy5); nigral microglia were counted using unbiased stereology. There was no difference in the number of microglia between MPTP/P and control mice. However, activated microglia were located surrounding DA neurons with alpha-synuclein-immunopositive inclusions following MPTP/P treatment only. Therefore, inflammation may play an important role in inclusion formation.

41.03

CHARACTERIZATION OF MOLECULAR MECHANISM OF NEURONAL CELL DEATH INDUCED BY THE EXTRACELLULAR ALPHA-SYNUCLEIN

J.Y. Sung1,4*; J.W. JAHNG2; Y.S. AHN2; J. KIM3; J.E. LEE1; K.C. CHUNG4
1. Medical Science and Brain Korea 21 Project for Medical Sciences, Yonsei Univ. Coll. of Med., Seoul, South Korea2. Pharmacology, Yonsei Univ Coll Med, Seoul, South Korea3. Microbiolgy, Yonsei university coll. of Med., Seoul, South Korea4. Biology, Yonsei University, Seoul, South Korea.

Parkinson`s disease (PD) is caused by doparminergic neuronal cell loss in CNS substantia nigra area and characterized by the intracytoplasmic formation of protein inclusions known as Lewy bodies (LBs) and Lewy neurites. Although alpha-synuclein, major protein component of LBs, has been implicated in the pathogenesis of familial PD forms, its functional role is not clearly characterized yet. Previously we have shown that the extracellular addition of alpha-synuclein causes cell death in hippocampal progenitor cell line, and it was rapidly translocated via the binding of small GTPase, Rab5A. In the present study, we examined whether alpha -synuclein has the same capability to penetrate into neighboring neuronal cells in vivo. When alpha-synuclein was injected into rat brain, alpha-synuclein positive neurons were observed in the neighboring neuronal cells of brain slices. Furthermore, the N-terminal regions of alpha-synuclein were mapped to be critical for the endocytosis of alpha-synuclein. We will present data to provide detailed mechanism of intracellular transport of alpha-synuclein.

Supported by: Basic Research Program grant from KOSEF

41.04

PRIMARY MOTONEURONS FROM mSOD1 TRANSGENIC MICE DO NOT SHOW AN INCREASED VULNERABILITY TO APOPTOTIC AND EXCITOTOXIC STIMULI *IN VITRO*

Kieran D, Kalmar B, Dekkers J, Burnstock G, Greensmith L.
The Graham Watts Lab, Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology,University College London, Queen Sq, London WC1N 3BG.

Mutations in Cu/Zn superoxide dismutase (mSOD1) account for 10-20% of the familial form of Motor Neurone Disease. Here, using primary motoneuron cultures from transgenic mSOD1 mice, we examined the relative vulnerability of transgenic and wild type motoneurons to excitotoxic and apoptotic stimuli.
At 7 DIV, motoneuron cultures were exposed to either AMPA (100uM), staurosporine (200nM) or hydrogen peroxide (100uM). Twenty four hours later, in some cultures cell viability was assessed using an MTT assay. Surprisingly, there was no difference in cell viability between transgenic and wild type cultures following exposure to either AMPA, staurosporine or hydrogen peroxide.
In other cultures, following immunostaining for MAP2 and trypan blue, neuronal death was determined by counting the number of trypan blue and MAP2 stained neurons. The results showed that the extent of neuronal death was the same in both transgenic and wild-type cultures. For example, following exposure to staurosporine, 24.2% (+/- 2.0 S.E.M., n=24) of neurons had died in wild-type cultures and 25.8% (+/- 2.3S.E.M., n=24) had died in transgenic cultures. These results show that primary motoneurons from mSOD1 transgenic mice are no more vulnerable to excitotoxic or apoptotic stimuli than wild-type motoneurons.

41.05

DJ-1 AND SYNPHILIN EXPRESSION IN HUMAN BRAIN & NEUROBLASTOMA CELLS

Rina Bandopadhyay*, Andrew Reid*, Ann Kingsbury*, Mark Cookson^, Andrew Hope*, Rohan de Silva* John Hardy^ and Andrew Lees*
**Reta Lila Weston Institute of Neurological Studies, Royal free and University College Medical School, Windeyer Building, 46, Cleveland Street, London W1T 4JF. ^Laboratory of Neurogenetics, National institute of Aging, NIH, Bethesda MD 20892, USA.*

DJ-1 (PARK7) is the most recent Parkinson's disease (PD) gene identified to have mutations in isolated families with autosomal recessive inheritance. Synphilin-1 (SYN) is an alpha-synuclein interacting protein and is present in Lewy bodies (LBs), the pathological hallmark of sporadic PD. Here we present evidence of the expression of these two PD-related genes in human brain and in neuroblastoma cells. Our results are: 1) DJ-1 is an abundant protein in frontal cortex of PD, progressive supranuclear palsy and control cases shown by Western blotting whilst SYN also is expressed in significant amounts in this region 2) DJ-1 is endogenously expressed by neuroblastoma cells; preliminary data suggest that its expression is influenced by factors involved in neuronal differentiation 3) SYN immunoreactivity is localised to LBs in the substantia nigra and frontal cortex in PD cases shown by light and electron microscopy 4) Light immunolabelling by SYN is seen in cytoplasm of some frontal cortex pyramidal neurones and in midbrain dopaminergic nerve fibres and the nigral neuropil. Expression of DJ-1 and SYN in regions vulnerable to formation of LBs suggest they might be involved in common pathogenic pathways leading to PD.

41.06

MUTATION LENGTH AND NEURONAL FUNCTION IN A MOUSE MODEL OF HUNTINGTON`S DISEASE

Edward J. Coote1, Selma Dadak2, Peggy F. Shelbourne1 and Stuart Cobb2
Division of Molecular Genetics1, Division of Neuroscience and Biomedical Systems2 , Faculty of Biomedical and Life Sciences, University of Glasgow, G11 6NU.

Huntington`s disease (HD) is an inherited neurological disease characterised by movement, psychiatric and cognitive disturbances. It is caused by the unstable expansion of a glutamine-encoding CAG trinucleotide repeat in a gene encoding a ubiquitously expressed protein called huntingtin. The size of the CAG repeat tract on HD chromosomes can change in both germline and somatic tissue. The inverse correlation between mutation length and age at onset of symptoms means the size of the CAG repeat tract has a major influence on the clinical picture.
Given the faulty protein is present in all tissues of the body, the selective striatal pathology in HD remains a puzzle. We have used an accurate genetic mouse model of the HD to demonstrate that mutation length variability occurs in somatic tissues and the largest repeat stretches reside in the striatum. To further test the hypothesis that longer mutation lengths may contribute to the regional selectivity of HD pathology, we are developing approaches to directly relate neuronal function and mutation length at the single cell level. Current progress on combining whole cell patch clamp techniques that facilitate electrophysiological recording and single nucleus retrieval with PCR-based methodology to determine mutation length will be reported and discussed.

This work is funded by a grant from the Huntington`s Disease Society of Ameri.

41.07

EVIDENCE FOR DIFFERENT MOLECULAR ROUTES TO ABNORMAL TAU PHOSPHORYLATION ARISING FROM FTDP-17 AND FAD MUTATIONS

Sarah L. Lambourne*, Lynda A. Sellers*, Piers C. Emson*, Michel Goedert†, Maria Grazia Spillantini‡, Toby G. Bush§ and Lawrence S. Wilkinson*
**Babraham Institute, Cambridge CB2 4AT; †MRC Laboratory of Molecular Biology, Cambridge CB2 2QH; ‡Brain Repair Centre and Department of Neurology, University of Cambridge, Cambridge CB2 2PY;§University of Nevada, School of Medicine, Anderson Medical Building, MS 352, Reno NV 89557-0046*

It is now well established that there is a strong genetic basis for small proportion (1-2%) of Alzheimer`s disease (AD) cases (familial AD), with fully penetrant autosomal dominant mutations in three genes, APP, PS1 and PS2. In addition, pathogenic mutations in the tau gene are implicated in frontotemporal dementia with Parkinsonism linked to chromosome 17(FTDP-17), a disorder with a high degree of clinical and pathological overlap with AD.
We have produced combined transgenic mice, expressing mutated human tau (V337M FTDP-17 mutation) and human CT-100 APP (FAD V717I mutation), together with hemizygous transgenics expressing the single transgenes. We have shown that whilst abnormal tau phosphorylation occurs in the tau V337M mouse, tau phosphorylation in the combined transgenic mouse is significantly augmented, indicating a role for CT-100 or some other carboxy-terminal APP fragment in this process. In addition, a study of the activity of the major kinases known to phosphorylate tau has revealed similar kinetic profiles in the combined transgenic and CT-100 V717I mouse, which are distinct from those obtained for the tau V337M and littermate wildtype mice.
We hypothesise that both FAD APP and FTDP-17 mutations can lead to abnormal tau phosphorylation, but via different qualitatively distinct mechanisms.

41.08

CALCIUM-BINDING PROTEIN PARVALBUMIN PROTECTS MOTONEURONES FROM DAMAGE BY IgG PURIFIED FROM SERA OF PATIENTS WITH MOTOR NEURONE DISEASE

*Pullen A.H, *Demestre, M., *Greensmith, L., #Schwaller, B.
** Institute of Neurology, University College London, Queen Square, WC1N 3BG, #Division of Histology, Dept. Medicine, University of Fribourg, Switzerland.*

Passive transfer of serum-IgG from patients with Motor Neurone Disease (MND)to mice, but not from healthy controls, evokes lesions in spinal motoneurones (MN) and enhances intracellularCa2+. To test whether ectopic expression of the calcium binding protein parvalbumin (PV) in MN prevents these lesions, IgGs from 10 MND patients and 10 healthy controls were injected i/pinto (a) Balb/C mice, (b)wild-type (Wt) C57BL/6J and (c) Transgenic (Tg) Thy-PV mice on a C57BL/6J background ectopically expressing PV in MN. Glutaraldehyde-90mM oxalate fixative localised Ca2+ in MN. In Balb/C mice, compared to MN of control-IgG injected mice, MND-IgG treated MN showed distended Golgi ER, disrupted Nissl bodies and mitochondria. Numbers of Ca2+ containing Golgi complexes increased by 30% ($p=0.001$) and presynaptic terminals by 20% ($p=0.01$). MN of MND-IgG treated Wt mice also developed lesions and increased numbers of Ca2+containing Golgi ER ($p<0.001$) and presynaptic terminals ($p<0.002$). Relative to these Wt MN, Golgi distension in MN of MND-IgG injected Thy-PV Tg mice was reduced by 24% ($p<0.002$), abnormal Nissl bodies declined by 90%($p=0.001$, and MN had 41% fewer Ca2+ containing Golgi complexes($p<0.01$) and 17% less presynaptic terminals($p<0.01$). Thus, ectopic expression of PV in MN significantly attenuates the morphological lesion and Ca2+ enhancement associated with passive transfer of MND-IgG.

41.09

INVOLVEMENT OF sHSPs IN MUTANT SOD INCLUSION FORMATION IN FALS

Victoria Licence, Ory Quinlan
Department of Biological and Biomedical Sciences, University of Durham, South Rd, Durham, DH1 3LE.UK.

Mutations in SOD account for 15-20% of FALS cases. Such mutations result in protein misfolding and inclusion formation and it is thought that these inclusions are toxic to motor neurons. Chaperone proteins function within the cell to support the correct folding of nascent proteins. A number of chaperones have been identified as associated with mutant SOD1 (HSP72/70, HSP27, aB-crystallin) and several hypotheses have emerged to suggest this association is important to disease development. To test this, we have investigated SOD inclusion formation in the presence and absence of mutant sHSPs – specifically HSP27 and aB-crystallin. A cell culture system was established using proteosome inhibitors to drive inclusion formation of ectopically expressed SOD. The inhibitory effect of sHSPs upon SOD inclusion formation was monitored by immunofluorescence microscopy. Inclusion containing cells were counted and expressed as a percentage of transfected cells. Using this system we have shown that SOD inclusion formation occurs more readily in cells expressing mutant aB-crystallins and that the co-expression of mutant aB-crystallin and mutant SOD causes an increased occurrence of inclusions. We conclude that sHSPs do inhibit SOD inclusion formation and our data suggest that any additional load upon the protein degradation machinery could exacerbate SOD inclusion formation.

41.10

THE DEVELOPMENT OF ORGANOTYPIC SLICE CULTURES FOR THE STUDY OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPY ASSOCIATED NEURODEGENERATION

1 G. J. Clarke,2 J. W. Ironside, and 1 J. R. Fraser
1 Institute for Animal Health, Ogston Building, West Mains Rd, Edinburgh, EH9 3JF; 2 National CJD Surveillance Unit, University of Edinburgh, Edinburgh, EH4 2XU.

The transmissible spongiform encephalopathies (TSEs) are fatal infectious neurodegenerative diseases characterized by the accumulation of PrPSc, an abnormal isoform of the host prion protein (PrP). A synthetic peptide corresponding to residues 106-126 of PrP (PrP106-126) has in vitro, many of the physiochemical properties of PrPSc including proteinase resistance, fibril formation, a predominantly beta-sheet structure and the ability to induce apoptosis in dissociated neuronal cultures. Organotypic hippocampal cultures maintain the cytoarchitecture and synaptic connections of the brain, permitting the differentiation of neurons and preserving the physiological and morphological characteristics of the tissue in vitro. This study was designed to investigate the cytotoxic properties of PrP106-126 and the associated pathways of neurodegeneration in this model system. Since the toxicity of PrP106-126 is dependent upon the environment in which it is dissolved, the beta-sheet content and fibril formation of peptide solutions have been examined by circular dichroism and electron microscopy, and MTT assays are being performed to determine their cytotoxicity. The optimally cytotoxic peptide will then be added to organotypic cultures and the effects analysed using TUNEL, immunocytochemical and Western blot techniques to study neuronal death and associated glial reactions. The identification of neurodegenerative pathways may identify intervention strategies to repair or block the brain damage caused by these fatal diseases.

41.11

ANALYSIS OF PROAPOPTOTIC MARKERS AND CYTOSKELETAL CHANGES IN THE HIPPOCAMPUS IN MURINE SCRAPIE

Debbie A. Brown1, James W. Ironside2, and Jan R. Fraser1
1 Institute for Animal Health, West Mains Road, Edinburgh EH9 3JF2 National CJD Surveillance Unit, University of Edinburgh EH4 2XU.

Scrapie is a member of a group of fatal transmissible spongiform encephalopathies (TSEs) . Pathological changes characteristic of the TSEs include vacuolation, gliosis, accumulation of a disease specific form of the normal cell-surface sialoglycoprotein PrP, and neuronal loss. Studies using experimental mouse models suggest that the neuronal cell death observed in TSEs is through an apoptotic mechanism, although the trigger for this event is unknown. In this study two scrapie mouse models showing a different sequence of pathological events were compared ; the 87V/VM model in which neurodegeneration is restricted to CA2, and the ME7/CV model where neurodegeneration is targeted to the CA1 of the hippocampus. Using immunocytochemical techniques the upregulation of proapototic markers caspase-3, Fas, Poly(ADP-ribose) polymerase(PARP) and Poly(ADP-ribose)(PAR) were analysed and compared.
In the 87V/VM model upregulation of caspase-3 and Fas was revealed early in the incubation period prior to abnormal PrP deposition. Dendritic damage of the CA2 neurons was observed prior to this upregulation.
In both mouse models PARP and PAR immunolabelling was targeted to specific brain areas and some upregulation was observed in both models at the terminal stages of disease. Early changes in the upregulation of both PARP and PAR are currently being investigated.

41.12

THE PATHWAYS OF TSE NEUROINVASION DIFFER BETWEEN NEUROTROPIC AND LYMPHOTROPIC TSE MODELS

McBride, P.A., Donaldson, M.I., Oxley, J. and Farquhar CF.
Institute for Animal Health, Neuropathogenesis Unit, Ogston Building, West Mains Road, Edinburgh EH9 3JF.

The oral route is the most relevant pathway for natural transmission of TSEs but the mechanisms involved require elucidation. The peripheral nervous system (PNS) and lymphoreticular system (LRS) are known to have a role in peripheral infection. Previous studies using hamsters orally-challenged with 263K scrapie showed that the infectious agent spread from the gastrointestinal tract to the CNS via the splanchnic and vagus nerves. Autonomic ganglia were early reservoirs of infection but there was little LRS involvement. However, after intraperitoneal infection with ME7, replication occurs initially in the LRS. We aimed to establish LRS involvement in the oral route and to identify the neuroanatomical pathways involved in spread of infection from the gut to the CNS. Immunocytochemistry and PET blotting were used to detect PrPsc, a marker for TSE disease, in tissues removed throughout the incubation period of mice orally challenged with ME7 scrapie. Results showed no evidence that ME7 reached the CNS via autonomic pathways of either vagus or splanchnic nerves. The gastrointestinal LRS was a key player in facilitating spread but the enteric nervous system was not. Therefore, the interaction and relative contribution of the PNS and LRS in establishing oral infection differs between TSE models.

41.13

GLYCINE RECEPTOR INTERACTIONS WITH SYNPHILIN-1

Kirsten Harvey1, Ian C. Duguid2, Julia H. White3, Brian R. Pearce1, Trevor G. Smart2 and Robert J. Harvey1
1Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London, 2Department of Pharmacology, University College London, Gower Street, London, and 3Pathway Discovery, Genomic and Proteomic Sciences, GlaxoSmithKline Medicines Research Center, Gunnels Wood Road, Stevenage.

The differential interaction of intracellular proteins with embryonic and adult glycine receptor (GlyR) isoforms may play a crucial role in the selective construction and maintenance of glycinergic synapses. To assess whether GlyR interacting proteins apart from gephyrin exist, we have used the GAL4 yeast two-hybrid system (YTH), a reliable method for assessing protein-protein interactions. We performed a YTH screen for the GlyR α3 subunit, using the large intracellular M3-M4 domain as bait, in conjunction with an adult human brain cDNA library. Over 100 `hits` were characterised, representing cDNAs encoding portions of three known proteins: importin α3, importin α4 and synphilin-1. In specificity tests, we found that the importin α subunits and synphilin-1 also interacted with the GlyR α1 subunit bait, but not baits for the GlyR α2 or β subunits. Synphilin-1 has been implicated in the pathogenesis of Parkinson`s disease, since it interacts with both α-synuclein and the E3 ubiquitin-protein ligase Parkin. Since GlyRs are also substrates for ubiquitination, and activation of GlyRs can cause cell death, we propose that GlyRs may be involved in the pathogenesis of PD.

This work is supported by the Royal Society and the Parkinson`s Disease Society.

42.01

OPTIMISING VIRAL VECTORS AS TOOLS TO STUDY NEURONAL FUNCTION

James Uney.
MRC Centre for Synaptic Plasticity and University Research Centre for Neuroendocrinology, University of Bristol, Bristol BS2 8HW, UK.

The efficient expression and targeting of transgenes to specific neuronal populations is a requirement for assessing the physiological function of neuronal genes. Study of the nervous system has, however, been particularly challenging because neurons are post-mitotic and exist as a myriad of discrete cell types that have an extremely heterogeneous structure and function. The historic difficulty of efficiently transfecting neurones has largely been overcome by the use of different types of attenuated virus including adenovirus (Ad), adeno-associated virus (AAV), lentivirus and herpes simplex virus. Generally, limitations of Ad systems (the advantages will also be briefly discussed) have been the inability to: (i) mediate long-term transgene expression; (ii) target expression to specific neuronal populations. To overcome these limitations a composite expression cassette, comprising of the weak human synapsin-I promoter and the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) were constructed. Studies in hippocampal cultures and organotypic cultures showed that the 3' addition of the WPRE to the synapsin-I element greatly increased EGFP expression levels with no loss of neuronal specificity. Furthermore, In-vivo studies also showed that long-term transgene expression was also enhanced with no loss of neuronal specificity in dentate-gyrus neurons for at least nine-months following transfection. In summary, data will be presented to show that non-integrating vectors can be used to mediate powerful, long-term episomal transgene expression in neurones.

42.02

STRATEGIES FOR GENE THERAPY: POTENTIAL AND PROBLEMS

Matthew Wood
Department of Human Anatomy and Genetics, South Parks Road, Oxford University, Oxford, OX1 3QX.

Important requirements of CNS gene therapy vectors are that they should allow the efficient and safe delivery of heterologous genes, as well as the potential to silence endogenous genes. The immunopathology associated with a range of adenovirus and herpes virus vectors has been studied, and strategies for minimizing it are being investigated. Additionally, the use of catalytic nucleic acids (ribozymes and DNAzymes) and RNA interference are two approaches currently being explored for endogenous gene silencing.

42.03

GENE THERAPY FOR INHERITED RETINAL DEGENERATION

Robin Ali
Department of Visual Science, Institute of Ophthalmology, University of London, 11-43 Bath Street, London, EC1V 9EL.

Inherited retinal disease, which includes conditions such as retinitis pigmentosa (RP), affects about 1/3000 of the population in the Western world. It is characterised by gradual loss of vision and results from mutations in any one of 60 or so different genes. There are currently no effective treatments, but many of the genes have now been identified and their functions elucidated, providing a major impetus to develop gene-based treatments. Many of the disease genes are photoreceptor- or RPE cell specific. Since AAV vectors are able efficient gene transfer to these two cell types, we are developing AAV-mediated gene therapy approaches for inherited retinal degeneration using a animal models that have defects in these cells. The retinal degeneration slow (rds or Prph2Rd2/Rd2) mouse, a model of recessive RP, lacks a functional gene encoding peripherin2, which is photoreceptor-specific protein required for the formation of outer segment discs. We have previously demonstrated restoration of photoreceptor ultrastructure and function by AAV-mediated gene transfer of peripherin2. We have now extended our assessment to central visual neuronal responses in order to show an improvement of central visual function.

42.04

TRANSFECTION OF AXOTOMISED BRAIN NEURONS BY A HERPES VIRUS VECTOR

John Rogers[1], Kate Rhodes[1,2], Mansoor Raza [1], Suzanne Roberts[1], Liz Muir[1], James Fawcett[1,2], Cinzia Scarpini[3], Stacey Efstathiou[3]
[1] Dept. of Physiology, University of Cambridge, CB2 3EG;[2] Centre for Brain Repair, Forvie Site, University of Cambridge;[3] Division of Virology, Dept. of Pathology, University of Cambridge.

For gene therapy to the injured CNS, replication-defective herpes simplex virus (HSV) vectors are promising candidates. We have examined whether they can express a transgene in injured neurons. In adult rats under anaesthesia, the medial forebrain bundle and nigrostriatal tract were transected unilaterally, and HSV vector was immediately injected just caudal to the incision. The vector carried the LacZ transgene controlled by the latency-associated transcript promoter. After 5-14 days, transgenic beta-galactosidase expression was seen in many brain regions, including neurons in the substantia nigra pars compacta (SNC) and parabrachial nuclei, whose projections had been cut by the lesion. Transfected neurons in both regions were confirmed as axotomised by double immunofluorescence for c-Jun. In SNC, at 5 days, up to 8% of dopaminergic neurons were transfected. By 12-14 days, many SNC neurons had disappeared but some transfected ones remained, up to 4% of the surviving neurons. In contrast there was no net loss of transfected neurons from the parabrachial nuclei. These results show that an HSV vector can transfect axotomised cells in the CNS and express a transgene in them for at least 2 weeks.
(Performed in accordance with the Animals (Scientific Procedures) Act 1986.

Supported by the Joan Holme Fund, International Spinal Research Trust, Wellcome Trust, MRC, and BBSRC.)

43.01

MOLECULAR AND CELLULAR MECHANISMS OF PAVLOVIAN FEAR MEMORY

Oliver Stork, Hans-Christian Pape
Institute for Physiology, School of Medicine, Otto-von-Guericke-University Magdeburg 39120, Germany.

The amygdala is a key structure for the integration of sensory and affective information and comprises a critical site of synaptic plasticity during Pavlovian fear conditioning. Changes of gene expression in the amygdala after fear conditioning were studied with polymerase chain reaction-based subtractive hybridization, revealing the differential regulation of several signal transduction (induction of aldehyde reductase, phosphodiesterase, somatostatin; decrease in glutamate decarboxylase (GAD)65, neuropeptideY) and structural reorganisation factors (induction of the neural cell adhesion molecules NCAM and neuroligin, E2 ubiquitin conjugating enzyme and E3 ubiquitin ligase Praja1). The analysis of null mutant and transgenic mice for GAD65 and for NCAM further confirmed the functional relevance of these factors for Pavlovian fear conditioning. GABAergic interneurons in the amygdala are thought to play an important role in the control and synchronisation of neural activity in the amygdala and the modification of GAD65 expression following fear conditioning training may strongly affect the neural activity patterns in this brain area. In fact we could show in freely behaving mice increases or rhythmic theta oscillations in the mouse amygdala and their synchronisation with hippocampal rhythms during fear memory retrieval. Supported by the Deutsche Forschungsgemeischaft (DFG).

43.02

FROM MOLECULAR GENETICS TO BEHAVIORAL ANALYSIS: COMPLEXITIES OF MEMORY RESEARCH

Robert Gerlai
Saegis, 60 Stone Pine Road, Half Moon Bay, California, USA 94019.

Bridging the gene–behavior gap requires building solid pillars on both ends. Molecular genetics allows manipulation of single components of the biochemical mechanisms underlying brain function. Similarly, behavioral analysis continues to become increasingly sophisticated in its ability to tease apart characteristic properties of the brain and to quantify induced functional changes. In this paper I focus on a particularly challenging trait, memory. Instead of reviewing the enormous amount of information, and the numerous "coherent" stories on how memory is encoded, I present isolated examples to illustrate the potential pitfalls of this field. We will learn, for example, that mGluR8 KOs are blind due to the wrong genetic background, the faster reversal learning of mGluR4 KO mice is explained by impaired long term memory, GluR2 KOs are impaired but this is not specific to memory, and that gene targeting is not easy for Eph receptors but an alternative strategy may work. What is the emerging pattern? The pillars are tough to build! The pros and cons of molecular and behavioral techniques must be carefully weighed with the particular molecular target and behavioral function in mind. We are at the data collecting phase. Filling databases with clean and correct results will lead us to the understanding of the core and modulating mechanisms of memory.

43.03

MORPHOLOGICAL BASIS OF SYNAPTIC PLASTICITY IN MAMMALIAN HIPPOCAMPUS

Michael G. Stewart
Department of Biological Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA.

.Alterations in neural circuitry are believed to occur as a result of memory formation. However, there is no consensus as to the precise nature of such changes in synapses and neurons, perhaps because of the differing nature and time scales involved in the various paradigms studied, coupled with different methodological approaches to measuring morphometric parameters. Even with a tightly controlled paradigm such as term potentiation (LTP) of the perforant path in hippocampus, which is believed to provide a model for memory formation (1), data on synaptic and neural morphological changes can vary from laboratory to laboratory. We have demonstrated that 24h following LTP in vivo there are increases in synaptic density in rat dentate gyrus, but not at earlier times post LTP induction (2). No major changes were found in synaptic morphometry in hippocampus 5 days after spatial training in a water maze. However, our previous studies relied upon 2-dimensional methods and unbiased stereology to estimate morphometric parameters. Here 3-D reconstruction methods at electron microscope level will be described which show how spine and synaptic morphology can be modified as early as 6h post LTP induction.

Supported by: BBSRC and The Leverhulme Trust.
(1) Abraham et al., [2002] J. Neurosci 22(21): 9626; (2)Stewart et al Neuroscience [2000].

43.04

PRESYNAPTIC CA^{2+} ENTRY IN INDIVIDUAL MOSSY FIBRE BOUTONS: BIPHASIC DEPENDENCE ON MEMBRANE POTENTIAL AND SHORT-TERM USE-DEPENDENT CHANGES

R. Scott, D. M. Kullmann, D. A. Rusakov
Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK.

Activity-dependent changes at hippocampal mossy fibre (MF) synapses provide important insights into the machinery of memory formation. It has recently been proposed that spike-driven Ca2+ entry into MFs is enhanced in a use-dependent manner, and that activation of presynaptic kainate receptors regulates the synaptic release probability at MFs by acting upstream of Ca2+ entry. To examine these hypotheses directly, we have applied fast two-photon excitation microscopy. In acute guinea pig slices, we held dentate gyrus granule cells in whole cell mode and filled them with the tracer Alexa Fluor 594 and the Ca2+ indicator Fluo-4. Both dyes were excited at 810 nm (MaiTai fs laser, SpectraPhysics) and their emission signals were recorded separately using a confocal imaging system (BioRad Radiance). We traced individual MF boutons (MFBs) and then recorded their Ca2+-dependent fluorescence in response to a train of spikes evoked ortho- or antidromically. We observed a biphasic relationship between the Ca2+ signal and the presynaptic membrane potential, with Ca2+ entry peaking at a holding voltage of -80-90 mV. Preliminary experiments have shown no evidence of use-dependent facilitation of Ca2+ signals. We are currently testing whether selectively blocking presynaptic kainate receptors affects Ca2+ signalling in individual MFBs.

43.05

EFFECTS OF NOVEL vs. FAMILIAR INESCAPABLE STRESS ON L-LTP IN THE AMYGDALA AND THE DENTATE GYRUS OF FREELY BEHAVING RATS

Vouimba Rose-Marie1, Yaniv Dan1, Diamond David2, Richter-Levin Gal1
1Department of Psychology, University of Haifa, Israel, and 2Department of Psychology and Pharmacology, Neuroscience Program, University of South Florida, USA.

Stress impairs hippocampal long-term potentiation (LTP), a model of synaptic plasticity that is assumed to underlie memory formation. In the amygdala, little is known about the effects of stress on LTP, or about its longevity. Here we assessed the ability of entorhinal cortex (EC) stimulation to induce late-phase LTP (L-LTP) simultaneously in the basal amygdaloid nucleus (B) and in the dentate gyrus (DG) of freely behaving rats. Once established, we investigated the effects of novel vs. familiar inescapable moderate stressful experiences on LTP in both structures. Results show that B, like DG, sustained LTP for 7 days. Furthermore, a single exposure to a moderate stress facilitated LTP in B but did not affect DG LTP. In contrast, stress re-exposure inhibited LTP both in B and DG. Behaviorally, animals exhibited a higher immobility when re-exposed to the moderate stressor as compared to a single/first exposure. These data support a role for B in memory storage. They also suggest that novel stress may be a learning-supporting situation whereas familiar stress may induce a depressive-like status in which learning and memory are hampered. Together, the results support a differential involvement of the amygdala and hippocampus in memory formation and storage depending on the emotional characteristics of the experience.

44.01

MECHANSIMS OF ADULT SENSORY NEURONE SURVIVAL AND GROWTH FOLLOWING AXOTOMY

Paul Fernyhough
School of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT.

The mechanism of sensory neuron survival subsequent to axonal damage is unknown but may involve autocrine or paracrine cell survival-promoting processes regulated by growth factors, such as brain-derived neurotrophic factor and tumour necrosis factor. In adult sensory neurones the role of the signal transduction pathways regulated by these critical growth factors are relatively unknown. The first objective of this presentation is to discuss the importance of the transcription factor NFkB in regulating survival of adult sensory neurones.
The second component of the talk will focus on the mechanism of action of neurotrophic factors in the maintenance of sensory neurone growth following axotomy. We have used real time fluorescence video microscopy to analyze mitochondrial membrane potential in cultured adult sensory neurons. The mechanism of insulin and neurotrophin-3 (NT-3)-dependent modulation of mitochondrial membrane potential involves the activation of the phosphoinositide 3-kinase (PI 3-kinase) pathway. Downstream targets of PI 3-kinase, such as AKT and the transcription factor CREB, are activated by insulin and NT-3 and regulate sensory neuron gene expression. These alterations in gene expression modulate critical components of metabolite pathways and the electron transport chain associated with the neuronal mitochondrion.

44.02

INTEGRIN/EXTRACELLULAR MATRIX INTERACTIONS IN PERIPHERAL NERVE REPAIR

Vogelezang, M, Forster U, M Ginsburg* and ffrench-Constant C
*Dept of Pathology and Medical Genetics, and Cambridge Centre for Brain Repair, University of Cambridge, UK and *Division of Vascular Biology, Scripps Research , Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037 USA.*

Peripheral nerve repair is associated with up regulation of the ECM molecule fibronectin (FN). Additionally, we have shown that the pattern of alternative splicing of FNmRNA changes in response to injury, with increased inclusion of an alpha4 integrin binding region. alpha4 is expressed on regenerating sensory axons, and experiments with PC12 cells and DRG neurones shows that alpha4 promotes neurite outgrowth in vitro. This requires the association of the adaptor protein paxillin with the alpha4 cytoplasmic domain, with the LD4 domain of paxillin involved in downstream signalling. The interaction of paxillin and alpha4 is inhibited by mutations that mimic phosphorylation of the alpha4 cytoplasmic domain, shown previously to prevent paxillin/alpha4 interactions in other cell types, and this mutation also prevents neurite outgrowth. These results point to a critical role for the alpha4 cytoplasmic domain in peripheral nerve regeneration and highlight the central role of paxillin in downstream signalling. Experiments using chimeric integrins have shown that the alpha4 cytoplasmic domain will promote neurite outgrowth when coupled to integrin extracellular domains conferring different ligand specificities. Chimeric integrins therefore provide a potential strategy for enhancing regeneration in those situations (such as the CNS) where normal regeneration does not occur.

44.03

REGENERATION IN THE SPINAL CORD

Stephen McMahon
Centre for Neuroscience, King's College London, UK.

The inability of axons to regenerate after a spinal cord injury in adult mammals is associated with permanent deficits in sensory and motor function. The reasons for this failure of regeneration are a weak cell body response to injury and the expression of multiple inhibitory molecules. One class of inhibitory factors are produced by oligodendrocytes and appear to act through a common binding receptor. These hare discussed at some length elsewhere in this meeting. Another class of inhibitor is produced at the site of CNS injury where a glial scar develops. This scar is made up of a number of extracellular matrix molecules including chondroitin sulphate proteoglycans (CSPGs). CSPGs are inhibitory to axon growth in vitro and regenerating axons stop at CSPG-rich regions in vivo. Removing CSPG glycosaminoglycan (GAG) chains attenuates CSPG inhibitory activity in vitro. In order to test the functional effects of degrading chondroitin sulphate (CS)-GAG after spinal cord injury, we delivered chondroitinase ABC (ChABC) to the lesioned dorsal columns of adult rats. We observed that intrathecal treatment with ChABC degraded CS-GAG at the injury site, up-regulated the regeneration-associated protein GAP43 in injured neurons, and promoted regeneration of both ascending sensory projections and descending corticospinal tract axons. ChABC treatment also restored post-synaptic activity below the lesion after electrical stimulation of corticospinal neurons, and promoted functional recovery of locomotor and proprioceptive behaviours. These results demonstrate that CSPGs are important inhibitory molecules in vivo and suggest that their manipulation will be useful for treatment of human spinal cord injuries.

44.04

GENE ARRAY PROFILING PROVIDES CANDIDATE GENES FOR CORRECTION OF EXPERIMENTAL DIABETIC NEUROPATHY BY SONIC HEDGEHOG

Rebecca C. Burnand1, Luke G. Eckersley1, Jonathan G. Moggs2, George Orphanides2 and David R. Tomlinson1
1School of Biological Sciences, University of Manchester and 2Syngenta CTL, Alderley Park, Macclesfield, Cheshire.

Hedgehog proteins are a family of morphogens with key roles in embryogenesis; effects in the adult are under explored. Treatment of STZ- diabetic rats with sonic hedgehog (Shh) reverses many indices of diabetic neuropathy by an unknown mechanism. Diabetic rats were treated with a sonic hedgehog-rat IgG fusion protein (Shh-IgG) and motor and sensory nerve conduction measured to verify a positive functional effect; both were normalised in the treated diabetic group. DNA array analysis was performed on reverse-transcribed mRNA from dorsal root ganglia (DRG) from the same rats and comparisons made between the gene expression of control, diabetic and Shh-IgG-treated diabetic animals. The expression of 738/8437 genes/ESTs was altered between diabetic and control animals, whereas 31/8437 genes/ESTs were altered between diabetic and Shh-IgG-treated diabetic groups. Of these, 12 were shifted by the Shh-IgG treatment to bring their expression close to that of non-diabetic control rats. Aldolase C was selected for future study on the basis of its role in glucose metabolism. Altered expression of aldolase C in DRG was confirmed using conventional and real-time PCR. A 50% down regulation in diabetic animals was demonstrated. This study demonstrates proof of principle for the identification of functionally-relevant target genes for correction of peripheral neuropathy by transcript profiling.

44.05

REGULATION AND MAINTENANCE OF THE MYELINATING PHENOTYPE OF SCHWANN CELLS BY KROX-20

D.B. Parkinson, A. Bhaskaran, S.Dickinson, K.R. Jessen and R. Mirsky
Department of Anatomy, University College London, Gower Street, London WC1E 6BT.

The zinc finger transcription factor Krox-20 (Egr2) is expressed in myelinating Schwann cells, and studies of Krox-20 null mice have shown that it is essential for myelination in the peripheral nervous system. During development, Schwann cells that myelinate large diameter axons withdraw from the cell cycle, strongly up-regulate myelin genes such as periaxin and P-zero and become less susceptible to apoptosis. Following nerve cut, Schwann cells in the distal stump show increased proliferation, down-regulate myelin genes and, in neonatal animals, show increased apoptosis. We find that expression of Krox-20 is sufficient to block the proliferation of Schwann cells in response to the axonal mitogen neuregulin. In addition Krox-20 strongly up-regulates the myelin specific genes periaxin and P-zero in Schwann cells, and inhibits the action of neuregulin and TGF-beta in suppressing myelin gene expression. We have also characterised a role for Krox-20 in positively regulating Schwann cell survival in both TGF-beta dependent and serum withdrawal models of apoptosis. Currently we are examining regulation of MAP kinase and other signalling cascades to determine how Krox-20 may alter cell signalling and promote the myelination of Schwann cells.

45.01

ASSESSING THE POTENTIAL FOR ISCHAEMIC RECOVERY IN MAN

J-C Baron
Dept of Neurology and Stroke Unit, University of Cambridge and Addenbrooke`s Hospital, Cambridge, UK.

Following stroke, an almost constant even though in most cases incomplete recovery of function is observed. In the first few days after stroke, rapid, and occasionally spectacular recovery can take place, but conversely clinical deterioration may also occur. Subsequently, recovery slows down but can span over months, and occasionally years. Mechanisms underlying recovery, such as reperfusion of the ischaemic penumbra, sprouting and neurogenesis, redistribution and reorganisation of functional maps and neural networks, and post-synaptic adaptation to deafferentation/diaschisis, likely operate at different, but probably overlapping, time intervals. Understanding these mechanisms would likely result in better-designed pharmacotherapy and rehabilitation procedures, and hence in better functional outcome. In the last two decades, functional brain imaging has immensely contributed to our understanding of the mechanisms underlying recovery after stroke directly in humans, including correlation to actual clinical recovery and monitoring of interventions. Studies have assessed the very acute phase of stroke, showing that rapid recovery or deterioration can occur due to the evolution of the infarct and its surroundings, but also the sub-acute and chronic stages, where plastic changes, repair phenomena and adaptive reorganisation, have been shown to take place. Manipulating recovery is now a realistic goal.

45.02

THE NEUROSCIENCE OF PSYCHOPATHOLOGY

RJ Dolan
Wellcome Department of Imaging Neuroscience, Institute of Neurology, London.

Understanding the neurobiology of dysfunctional psychological processes that underpin common mental disorders has proved elusive. In this presentation I will argue that considerable progress has been made in relation to one of the commonest symptom clusters, those involving emotional dysfunction. This will be illustrated by considering a common form of acquired psychopathology, namely post-traumatic stress disorder (PTSD. In this condition the key psychopathology involves recurrent intrusive memories of the trauma as well as recurrent episodes of intense fear triggered by cues related to the precipitating trauma. My aim in the presentation is to provide a brain based description of how emotional memories become indelible and how traumatic emotions are readily reactivated.

45.03

ADVANCES IN GENETIC DIAGNOSIS AND MANAGEMENT OF NEUROLOGICAL DISEASE

Nicholas W Wood
Institute of Neurology, Queen Square, London.

Such has been the progress in our understanding of the genetic basis of simple Mendelian neurological disorders that the investigation and practice of neurology has changed dramatically in the last 10 years. However these disorders tend to be rare and affect only a relatively small minority of the population. A major challenge for the clinical neurosciences is to use genetic techniques to identify the genetic substrates underlying the commoner forms of disease. There are 2 broad approaches to finding genetic factors. Firstly classical linkage can be used but this is generally insufficiently powerful to be definitive for many of the common disorders. The second approach uses linkage disequilibrium to help map common variants. Linkage disequilibrium mapping is powerful and allows fine localization. This approach can also be used to identify genetic factors underlying other complex traits such as disease heterogeneity and perhaps of most immediate potential the response to drugs.
This relatively new approach is already providing insights into these problems and examples from the field of neurology will be given. However, finding an associated haplotype within which is contained the causal variant is only part of the problem. Identifying the underlying mutation will involve a combination of genetic and functional approaches.

45.04

CHANNELS, MODULATORY TRANSMITTERS AND PAROXYSMAL NEUROLOGICAL DISORDERS

Dimitri M Kullmann
Institute of Neurology, UCL, Queen Square, London WC1N 3BG.

Several inherited paroxysmal neurological disorders have recently been found to be caused by mutations of ion channels. These include rare forms of migraine, episodic cerebellar ataxia, excessive startle disorder, epilepsy and periodic paralysis. In order to establish how mutations cause disease, we need to understand both the normal role of the ion channels, and the consequences of the mutations for their assembly, trafficking and kinetic properties. Although progress is being made in all of these areas, there is as yet no satisfactory explanation why CNS 'channelopathies' frequently cause intermittent symptoms. This work promises to shed light only on how derangements in membrane excitability gives rise to abnormal firing patterns in the brain, and possibly on the mechanisms of common neurological paroxysmal disorders such as migraine and epilepsy.

46.01

ASSEMBLY OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

Neil S. Millar
Department of Pharmacology, University College London, London, UK.

Nicotinic acetylcholine receptors (nAChRs) are pentameric neurotransmitter-gated ion channels which exhibit considerable subunit diversity. Our research is focussed upon a molecular and cell biological investigation of mammalian nAChRs and of nAChRs expressed in the fruit fly, Drosophila. In vertebrates, in addition to five nAChR subunits expressed in muscle (at the skeletal neuromuscular junction), twelve "neuronal" nAChR subunits have been identified within the central and peripheral nervous system. To date, ten neuronal nAChR subunits have been identified in insects. We are interested in establishing factors which influence the assembly of nAChR subunit into the pharmacologically and functionally diverse family of neuronal nAChRs. Current research is aimed at investigating the influence of a broad range of factors upon cell biological events such as subunit folding, receptor assembly, cell-surface expression and intracellular targeting. Factors being investigated include the importance of subunit composition and the contribution of discrete subunit sub-domains. We are also investigating the influence of phenomena such as chronic exposure to nicotine (as occurs during tobacco smoking and which appears to be responsible for changes in subunit conformation) and of the host cell intracellular environment.

46.02

PRESYNAPTIC LOCALISATION OF THE ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR IN THE RAT VENTRAL TEGMENTAL AREA: CONFOCAL AND ELECTRON MICROSCOPY EVIDENCE

Ian W Jones and Susan Wonnacott
Department of Biology & Biochemistry, University of Bath, Bath BA2 7AY, UK.

Neuronal nicotinic acetylcholine receptors (nAChR) are ligand-gated ion channels that mediate and modulate synaptic transmission. Numerous nAChR subtypes exist in the brain, one of the most common being the alpha 7 nAChR. These receptors are of particular interest due to their possible role in synaptic plasticity: within the rat ventral tegmental area (VTA) alpha 7 nAChR on glutamatergic axon terminals have been implicated in a form of long term potentiation which may contribute to mechanisms underlying addiction. This study uses confocal and electron microscopy to provide direct neuroanatomical evidence for presynaptic alpha 7 nAChR in the rat VTA. Alpha 7 nAChR are visualised either using fluorescent alpha bungarotoxin (an alpha7 nAChR specific antagonist) or antibodies to the alpha 7 subunit itself. Multiple labelling experiments, with antibodies to tyrosine hydroxylase and vesicular glutamate transporter, precisely define the cellular and subcellular distribution of alpha 7 nAChR with respect to the local neurochemical environment. Results demonstrate that, in the rat VTA, alpha 7 nAChR are indeed present on glutamatergic afferents synapsing onto dopaminergic neurones. The ability to discern presynaptic alpha 7 nAChR will facilitate analysis of any changes in density or localisation following chronic nicotine treatment.

46.03

PROTEIN COMPLEXES ASSOCIATED WITH THE P2X7 RECEPTOR

R.A. North
Institute of Molecular Physiology, University of Sheffield.

P2X receptors are ATP-gated ion channels in the plasma membrane, but activation of the P2X7 receptor couples cytoskeletal re arrangements such as membrane blebbing. We used affinity purification of the rat P2X7 receptor followed by mass spectroscopy and immunoblotting to identify proteins in human embryonic kidney cells that interact with the receptor. We found laminin a3, integrin b2, b-actin, a-actinin, supervillin, MAGuK, three heat shock proteins, phosphatidylinositol 4-kinase, and the receptor protein tyrosine phosphatase b. Activation of the P2X7 receptor with the agonist 2',3'-benzoyl-(4- benzoyl)ATP resulted in its dephosphorylation on tyrosine; by systematic mutagenesis we identified the residue involved as Tyr343 in the putative second transmembrane domain. Whole cell recordings from cells expressing P2X7 receptors showed that repeated applications of a high concentration of agonist led to a strong decline in the amplitude of the current; this was prevented by phosphatase inhibitors. Phosphatase inhibitors also accelerated membrane blebbing. The results indicate that activation of the P2X7 receptor results in the stimulation of an associated receptor protein tyrosine phosphatase. Dephosphorylation of the receptor on Tyr343 inhibits the flow of ionic current and impairs coupling to the downstream effectors leading to the cytoskeleton.

46.04

NEAR REAL TIME IMAGING OF AMPA RECEPTOR TRAFFICKING

Jeremy Henley
MRC Centre for Synaptic Plasticity, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK.

The mechanisms that direct the assembly, transport, targeting, localization and surface expression of AMPA receptors are of fundamental importance for neuronal function. It is now established that AMPA receptors are highly dynamic in both their transport and surface expression. For example, AMPA receptors constitutively recycle in the postsynaptic membrane with a surface expression half-life of minutes and, in addition, undergo activity dependent exo- and endocytosis during synaptic plasticity. We are interested in the mechanisms involved in these processes. I shall outline recent work in our laboratory aimed at determining the mechanisms that underlie these processes.

47.01

NEURAL STEMS: WHAT DO THEY TELL US ABOUT BRAIN REPAIR MECHANISMS?

Jack Price
Institute of Psychiatry, London, UK.

When certain populations of neural stem cells are transplanted into animal models of disease, they induce both structural and functional repair. They replace lost cells, and they elicit recovery of lost cognitive functions. This is a decidedly non-trivial property, since very few other agents could be said to show the same combination of properties. What is it about neural stem cells that makes them work, and what mechanisms are involved?
In my presentation, I shall consider two aspects of the interaction between engrafted neural stem cells and damaged brain. First, I shall consider the seminal property of neural stem cells—multipotentiality. We have identified a number of markers of multipotentiality in neural stem cell lines. Several of these are novel genes, and I will present evidence that they are controlled via a mechanism involving the Notch signaling cascade. Second, I shall consider markers of brain damage to which neural stem cells are responding. It is clear that neural stem cells respond to chronic damage. What is it that signals to the neural stem cells that the tissue is in need of repair? I shall present evidence that the transcription factor, Oct-6, might be part of that signaling mechanism.

47.02

ADULT NEURAL STEM CELLS: A NEW PARADIGM TO REPAIR MYELIN IN THE CNS

A.Baron-Van Evercooren
INSERM U546, Institut Fédératif des,Neurosciences, CHU Pitié-Salpêtrière, 105 bd de l'Hopital, 75 634 Pariscedex 13.

In the adult brain, the contribution of the subventricular zone (SVZ) tocell genesis is restricted to the renewal of the granular andperiglomerular neurons of the olfactory bulb. SVZ-derived neuralprogenitors elicit long-distance migration in a web-like pattern calledthe rostral migratory stream (RMS) as defined by the expression of thepolysialylated form of the neural cell adhesion molecule (PSA-NCAM). Wehave recently demonstrated that lysolecithin (LPC)-induced demyelinationcan trigger the SVZ cells to proliferate, deviate from their normalmigratory route to colonize the lesion and generate astrocytes andoligodendrocytes in due time for myelin repair (Nait-Oumesmar et al.1999). Proliferation, migration and differentiation of the adult SVZ inresponse to experimental autoimmune encephalomyelitis (EAE), a model ofMS(Picard-Riera et al., 2002). More recently, we found that PSA-NCAMprogenitors are present in chronique and acute lesions of multiplesclerosis (MS). Their potential for proliferation and differentiation inoligodendrocytes will be discussed. Our data suggest that the adult SVZcould be a source of oligodendrocytes and contribute witholigodendrocyte progenitors to the replacement of lost oligodendrocytesin animal models of CNS demyelination and possibly also in MS.

Supportedby INSERM-AFM, FRM and ARSEP.

47.03

THE USE OF OLFACTORY ENSHEATHING CELLS IN CNS REPAIR

Susan C Barnett and John S Riddell
Beatson Labs, Div of Clinical Neuroscience, University of Glasgow, G61 1BD; Div of Neuroscience and Biomedical Systems, IBLS, University of Glasgow, G12 8QQ.

The olfactory ensheathing cell (OEC) appears to have a remarkable ability to repair the damaged CNS after transplantation, by remyelinating demyelinated axons as well as promoting axonal regeneration. (Franklin and Barnett, 2000, Neuron 28:1-4). It has also been shown that transplanted OECs permit recovery of conduction properties in demyelinated axons that have been remyelinated after transplantation and recovery of function following spinal cord lesions. However, whether OEC grafts promote functional reconnection of transected fibres, is still a matter for debate. The advantages of OECs in transplant mediated repair will be considered, in particular their ability to mingle within an astrocyte environment without inducing markers of hypertrophy. The possible limitations of OECs will also be discussed. In recent work we have used OECs in an attempt to promote regeneration at the dorsal root entry zone following dorsal root lesions. Although limited growth of sensory fibres into the spinal cord was seen in a small proportion of experiments, no electrophysiological evidence of functional reconnection with spinal cord neurones could be obtained. Current evidence therefore suggests that OECs have the potential to promote limited CNS repair but additional strategies may be needed to promote functional regeneration.

47.04

COMBINATIONS OF NEUROTROPHIC FACTORS PROMOTE RETINAL GANGLION CELL AXON REGENERATION THROUGH THE GLIAL SCAR AFTER OPTIC NERVE TRANSECTION

A. Logan (1); A.-M. Gonzalez (2); A. Baird (2); M. Berry (1)
1. Centre for Neurodegeneration and Repair, Department of Medicine, University of Birmingham, Birmingham, B15 2TT, UK;2. GKT School of Biomedical Sciences, King`s College London, London SE1 1UL, UK.

We evaluated the effects of vitreal implants of fibroblasts transfected to express FGF-2, NT-3 or BDNF alone or a combination of FGF-2, NT-3 & BDNF on retinal ganglion cell (RGC) survival and optic nerve regeneration. Anterograde axon tracing with (rho-B) provided unequivocal qualitative evidence of regeneration in each group. Injection of rho-B at a site 2mm distal to the lesion gives a direct measure of the number of surviving axons growing across the lesion. By 20 days most RGC degenerate in control retina (5+/-3) and few if any GAP43 positive axons are present in the proximal nerve segment. No fibres crossed the lesion site and dense scar material was deposited in the wound. Twenty days after single NT implant, there was a marginal increase in the number of rho-B-positive RGC (21+/-5) and GAP43 positive axons in the proximal segment. Rats receiving all 3 NT showed the greatest number of rho-B-positive RGC (978+/-226), with significant number of GAP43 positive axons regenerating 3-5mm into the distal nerve segment. In this group a glial/mesenchymal scar was not formed at the wound site. Our results indicate that perisomatic cellular delivery of specific NT combinations is able to mobilise and maintain RGC axon regeneration for at least 20 days and that these regenerating fibres may regulate scar formation.

48.01

DUAL CODING IN HIPPOCAMPAL PLACE CELLS: SPIKE TIMING RELATIVE TO THE EEG THETA CYCLE CODES FOR POSITION, SPIKE RATE CODES FOR SPEED OF MOVEMENT

John O'Keefe, John Huxter and Neil Burgess
Department of Anatomy and Developmental Biology, and the Institute of Cognitive Neuroscience, University College LondonLondon WC1E 6BT.

Hippocampal pyramidal cells code for the animal's location by increasing their firing rates dramatically in one part of the environment (the Place Field). Different cells represent different patches of the environment. Within the place field a finer-grained spatial localisation is achieved by the temporal relationship between spike firing and the sinusoidal EEG theta activity. As the animal enters the place field, the first burst of spikes occurs late in the EEG cycle. As the animal moves through the field, successive bursts of spikes occur at earlier and earlier phases of the theta wave. The phase of firing correlates better with location within the field than with other variables such as time of entry into the field or time since the beginning of the run. In contrast, the firing rate correlates with the speed with which the animal moves through the field. We suggest that this dual coding mechanism may be a general principle in hippocampal pyramidal cells, with spike phase coding for the spatial variable and spike rate coding for the occurrence of objects and behaviours in that location.

Dedicated to the memory of Eberhard Buhl.

48.02

ASTROCYTIC AND NEURONAL OSCILLATIONS

V Crunelli
School of Bioscience, Museum Avenue, University College of Wales, Cardiff

I will describe two oscillatory activities in thalamic astrocytes and neurones, respectively, and discuss their significance for thalamocortical loop dynamics.
1. The 'window' component of the low voltage activated T-type Ca2+ current plays an essential role in the intrinsic slow (<1 Hz) sleep oscillation in neurones of sensory and reticular thalamic nuclei, indicating that the thalamic expression of this fundamental sleep rhythm is not a simple reflection of cortical network activity. The presence of gap junction potentials among GABAergic reticular and glutamatergic thalamic sensory neurones suggests that the slow oscillation may already be synchronized at the thalamic level.
2. Spontaneous [Ca2+]i waves propagate among thalamic astrocytes and appear not to involve gap junctions. These astrocytic waves elicit large and long-lasting NMDA-mediated currents in sensory thalamic neurones. The peculiar developmental profile of the astrocytic [Ca2+]i waves and the selective activation of neuronal NMDA receptors point to a possible role for this astrocyte-to-neurone signalling in the topographic wiring of the thalamocortical loop.As these novel cellular and intracellular properties are not restricted to thalamic astrocytes and neurones, the significance of these results may well apply to (patho)physiological functions of glial and neuronal elements in other brain areas.

48.03

SHAPING OF SYNAPTICALLY GATED NETWORK OSCILLATIONS BY GAP JUNCTIONS

Roger D. Traub, Miles A. Whittington, Andrea Bibbig, Fiona E.N. LeBeau, Hannah Monyer, Eberhard H. Buhl
SUNY Downstate Medical Center, Brooklyn, NY 11203, U.S.A.;School of Biomedical Sciences, University of Leeds, Leeds, U.K.;Dept. Clinical Neurobiology, University Hosptial of Neurology, Heidelberg, Germany.

Nanomolar concentrations of kainate are able to induce "persistent" gamma frequency (about 25-70 Hz) oscillations in hippocampus in vitro. Such oscillations depend upon chemically gated synaptic transmission, specifically via AMPA and GABA-A receptors. In addition, the oscillations depend upon electrical coupling between neuronal elements, of two fundamentally different sorts: between axons of pyramidal cells, and between the dendrites of interneurons. Experiments and simulations indicate that the roles played by the two sorts of electrical coupling are distinct. Axonal coupling is necessary for the oscillation to occur at all, unless the axons are spontaneously hyperactive; whereas, interneuronal dendritic coupling modulates the coherence of the oscillation.

48.04

THETA/GAMMA OSCILLATIONS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX

M.A.Whittington, M.O.Cunningham, R.D. Traub, M.Gillies, F.E.N.Lebeau, C.H.Davies, E.H.Buhl
SBMS, University of Leeds, The Worsley Building, Leeds, LS2 9NQ.

Oscillations within the EEG gamma (30 - 90 Hz) and theta (3 - 10 Hz) bands are a common feature of cortical activity. They have been associated with various cognitive processes at work during primary sensory processing and memory formation and recall. Gamma oscillations are generated by the concerted, rhythmic output of networks of fast-spiking inhibitory interneurons but the origin of the theta rhythm, and its relationship to gamma oscillations, is less well characterised. Here we demonstrate that activity within the fast theta band is also generated by networks of inhibitory interneurons in the archicortex. The pattern of intrinsic properties of interneurons responsible for gamma or theta rhythms, and the topography of their connections to principal cells, illustrates a functional consequence of the broad hererogeneity of this cell type. We provide evidence for the existence of subnetworks of interneurons, each capable of temporally controlling rhythmic output from cortical structures within discrete frequency bands.

49.01

ON THE SPATIAL RESOLUTION OF CORTICAL CODESS

Panzeri, F. Petroni, R.S. Petersen, M.E. Diamond
UMIST, Dept. of Optometry and Neuroscience, PO BOX 88, Manchester M60 1QD; SISSA, Via Beirut 2-4, 34014 Trieste, Italy.

Measuring haemodynamic responses to parametric stimulus variations in functional Magnetic Resonance Imaging (fMRI) experiments can elucidate how sensory information is represented in the brain. However, a potential problem with this approach is that haemodynamic responses reflect only a regional average of neuronal activity. Thus, measuring the spatial resolution used in the representation of sensory information by groups of cortical neurons could greatly help the interpretation of parametric fMRI experiments. We have characterized the spatial resolution of a cortical code by quantifying the extent to which information about a sensory stimulus is diminished when responses of neurons within a cortical region are pooled together. Analyzing the response of small groups of neurons in rat barrel cortex to whisker deflection, we found that pooling neurons within the same column caused a loss of only 1-5% of the information about whisker stimulation. Cross-column pooling led to larger information losses (25%-55%). Thus, if the spatial averaging spans more than one column, much of the information present in the neuronal signal is lost. Our findings suggest that parametric imaging experiments can unravel neuronal information processing only if their spatial resolution is at least of the order of the size of cortical columns.

49.02

FUNCTIONAL IMAGING AND INFORMATION ENCODING

Angel Nevado, Malcolm P. Young and Stefano Panzeri
The Henry Wellcome Building for Neuroecology. University of Newcastle, Framlington Place, Newcastle upon Tyne, NE2 4HH.

We studied the suitability of estimating the information conveyed bythe neuronal activity of different brain areas by using the signalmeasured in functional imaging experiments. The relationship betweenthe information conveyed by the activity of the neurons on the onehand, and both the signal change and the information conveyed by thesignal on the other hand, was analysed. For a model based on knowntuning properties of the neurons we found that the relationshipsbetween this quantities are highly nonlinear in general. It followsthat the brain area which experiences the largest signal change is notnecessarily the one which encodes more information about thestimuli. We suggest that a better interpretation of functional imagingresults in terms of information processing can be obtained if datafrom the experiments and knowledge about the tuning properties of theneurons are incorporated into a computational model. Imaging techniquesthemselves may be able to provide an estimation of the neuronal tuningproperties to some extent.

49.03

FUNCTIONAL MAPPING OF CAT AUDITORY CORTEX BY INTRINSIC OPTICAL IMAGING

Vassiliy Tsytsarev, Jérôme Ribot, Hisayuki Ojima and Shigeru TanakaVassiliy Tsytsarev, Ph.D.,
Laboratory for Visual Neurocomputing, Brain Science Institute of RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan.

Recently it is widely accepted that optical imaging of intrinsic signals is a useful tool for mapping functional organization in cortical areas with high spatial resolution. This technique is based on the change of light reflectance of a brain tissue associated with neuronal activities. We used this technique to examine the cortical representation of interaural time differences in cat primary auditory cortex (AI), which is expected to be related to the sound source localization. In the recording, sequences of pure tones or binaural clicks were presented to the left and right ears with interaural delays ranging from 0 to 250 μs. We found not only tonotopic organization of sound frequencies but also spatial representation of interaural time differences. Acoustic stimuli with different interaural time differences activated different localized domains in AI and the center of the activated domain shifted as the time difference was changed. We confirmed consistent observations between optical imaging and electrophysiological recording. Taken together with the idea that interaural time difference is an important cue for sound source localization, it is suggested that AI is involved in information transformation from interaural time difference to sound source location.

49.04

CORTICAL MAGNIFICATION AND THE LOCAL RETINOTOPIC ORGANISATION OF V1 IN THE MARMOSET MONKEY

I Schiessl and N McLoughlin
Dept of Optometry and Neuroscience, UMIST, Manchester M60 1QD, UK.

The common marmoset monkey is a small new world primate with excellent spatial vision. Previous electrophysiological studies have mapped the gross retinotopy of V1 and some of its response properties. In this study we present the first optical maps of the fine retinotopic organisation of V1 obtained by optical imaging of intrinsic signals.
Specifically, marmosets were anaesthetised and paralysed using standard procedures and focused onto a computer monitor. Preference for retinotopic space was computed by collecting camera frames when the marmoset viewed moving bars in one series of locations and subtracting these frames from camera frames collected during presentation of moving bars in a complementary series of locations. Images were then processed using a combination of first frame analysis and the extended spatial decorrelation algorithm. Our results demonstrate that V1 exhibits a smooth and regular retinotopic organisation with an isotropic cortical magnification factor that increases towards the fovea. This is similar to recent findings from the squirrel monkey.

49.05

5-HT2c RECEPTOR ACTIVATION DETECTED IN HUMANS BY fMRI: EFFECT OF PRE-TREATMENT WITH MIRTAZEPINE ON BRAIN ACTIVATIONS FOLLOWING MCPP INFUSION

Birgit Völlm 1), Paul Richardson 1), John Stirling 1), Rebecca Elliott 1), Shane McKie 2), Steve Williams 2) and Bill Deakin 1)
1) Neuroscience and Psychiatry Unit, University of Manchester, Oxford Road, Manchester, M13 9PT2) Imaging Science and Biomedical Engineering, University of Manchester, Oxford Road, Manchester, M13 9PT.

Background: – Serotonin (5-HT) has been shown to be implicated in the aetiology of psychiatric disorders and personality traits. We have previously shown BOLD signal changes in the hypothalamus and basal ganglia, areas rich in 5-HT2c-receptors, after administration of mCPP, a 5-HT2c-agonist. mCPP also resulted in enhanced region-specific neuronal responses to a go/no-go test of behavioural inhibition.
Methodology: – 40 healthy, right-handed male volunteers were randomly allocated to one of the following conditions in a double-blind fashion: mCPP infusion (0.08 mg /kg) with pre-treatment of the 5-HT2c-antagonist mirtazepine (30 mg), mCPP infusion without pre-treatment, placebo infusion with and without pre-treatment. A scan for each task (go/no-go, reward, punishment) was carried out prior to infusion and repeated after infusion. Functional images were acquired using a 1.5T Phillips Gyroscan. Data analysis was performed using SPM2 using random effects modelling.
Results and Conclusions: – Preliminary analysis confirms that mCPP has direct effects on subcortical and cortical regions with dense concentrations of 5-HT receptors, and significantly modulates the neuronal responses associated with performance of a behavioural inhibition task. Evidence from the first few subjects also suggests that reward and punishment sensitivity are significantly modulated by mCPP.

49.06

fMRI STUDIES OF SENSORY DRIVEN PLASTICITY IN HUMAN SWALLOWING MOTOR CORTEX

S.Williams3, C.Fraser1, M.Power1, J.Rothwell4, D.Hobday1, I.Hollander1, P.Tyrell2, A.Hobson1, D.Thompson1, S.Hamdy1
Departments of Gastroenterology1 and Stroke Medicine2 and Imaging Science and Biomedical Engineering3, University of Manchester; Institute of Neurology4, London.

Cortical plasticity is an important process involved in recovery from stroke. Somatosensory stimulation can promote cortical plasticity in man, but the underlying mechanisms are poorly explored. We have used electrical stimulation, transcranial magnetic stimulation (TMS) and fMRI to probe sensory-driven remodelling of the swallowing motor cortex in man. The pharynx was stimulated intraluminally by a train of electrical pulses. Following stimulation or sham, TMS was used to measure cortical excitability, by recording electromyelographic (EMG) responses from the pharyngeal electrodes. The TMS stimulation coil was moved over the scalp to map the cortical areas driving the EMG response. 8 healthy subjects were studied using fMRI after both pharyngeal and sham stimulation. The subjects were imaged during a swallowing task, employing a blocked design. Pharyngeal stimulation produced an increase in the cortical area excited by TMS and in the number of activated pixels detected by fMRI during the swallowing task. These studies provide evidence that cortical reorganization can be driven by external stimulation, and that this is associated both with an increased representation on TMS, and an increased BOLD signal. These approaches may have value in treating stroke patients and in monitoring the effectiveness of that therapy.

50.01

SUBTYPE-SELECTIVE MODULATORS OF G PROTEIN-COUPLED RECEPTORS BY ALLOSTERIC LIGANDS

Nigel J. M. Birdsall
Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

An increasing number of G protein-coupled receptors have recently been shown to possess ligand binding sites that are spatially and pharmacologically distinct from the site that binds competitive ligands (N), including the endogenous hormone or neurotransmitter. Ligands (L) binding to the new sites allosterically modulate the binding and functional properties of the receptor. The allosteric ternary complex model is the simplest to describe this behaviour, with the binding of an allosteric ligand being defined in terms of two parameters, its affinity for the unliganded receptor and its cooperativity with the binding of N. The cooperativity, the factor by which the affinity of L is changed by N when they simultaneously bind to the receptor, determines the very different pharmacological properties and novel selectivities of allosteric ligands. For example allosteric ligands, in contrast to competitive agonists and antagonists, act to tune up (or down) receptor function whilst maintaining the important temporal aspects of intercellular receptor signalling in the nervous system. In addition, some allosteric ligands exhibit 'absolute subtype selectivity'; action at one subtype but no action at any concentration at other subtypes. Examples of the novel pharmacological aspects of the actions of allosteric ligands, their possible uses and their therapeutic potential will be described.

50.02

GLUTAMATE SUBTYPE RECEPTORS: FROM SENSORY TRANSMISSION AND MODULATION TO CHRONIC PAIN

Min Zhuo
Washington University Pain Center, Depts of Anesthesiology, Anatomy & Neurobiology, Psychiatry, Washington University, St louis, MO 63110, USA.

Glutamate N-methyl-D-aspartate (NMDA) receptors play important roles in diverse brain functions, from memory formation to persistent pain. To ask whether forebrain NMDA receptors affect an organism's response to tissue injury and inflammation, we first studied transgenic mice with forebrain-targeted overexpression of the NMDA receptor subunit NR2B. NR2B transgenic mice exhibited enhanced NMDA receptor-mediated synaptic responses in the anterior cingulate cortex (ACC) and insular cortex, but not in the spinal cord dorsal horn. While transgenic and wild-type mice were indistinguishable in tests of acute nociception, transgenic mice exhibited enhanced behavioral responses after peripheral injection of formalin and complete Freund's adjuvant (CFA). Second, we studied changes of NR2B subunits in the ACC of mice after tissue inflammation. At day 3 and 7 after the injection of CFA, NR2B but not NR2A subunits were increased in the ACC, suggesting that inflammation causes the upregulation of NR2B subunit in the ACC. Finally, microinjection of selective NR2B receptor antagonists into the ACC produced inhibition of behavioral allodynia in freely moving mice. We conclude that genetic modification of forebrain NMDA receptor kinetics does influence nociceptive processing and NR2B receptor antagonists may be useful as new selective analgesics for persistent pain.

50.03

MORE GABA-A RECEPTOR SUBTYPES – MORE MEDICINES

John R. Atack
Neuroscience Research Centre, Merck Sharp & Dohme, Terlings Park, Eastwick Road, Harlow CM20 2QR.

The prototypic benzodiazepine diazepam (Valium), possesses a variety pharmacological effects, such as anxiolysis, anticonvulsant activity, myorelaxation, cognitive impairment and sedation, which it achieves via modulation of the effects of GABA at GABAA receptors containing an α1, α2, α3 or α5 subunit. The use of knock-out and knock-in mice has begun to identify which of these GABAA receptor subtypes may mediate which particular pharmacological action. Thus, GABAA receptors containing an α1 subunit play a role in sedation, those containing α5 play a role in cognition and α2 and/or α3 subtypes are involved in anxiety. The fact that different populations of GABAA receptors mediate distinct aspects of benzodiazepine pharmacology has opened up the possibility of generating compounds which selectively interact with these different subtypes to produce drugs with novel pharmacological profiles, such as α5-selective inverse agonist as cognition enhancers or agonists devoid of efficacy at the α1 subtype which are non-sedating anxiolytics.

51

GENOMIC IMPRINTING, BRAIN EVOLUTION AND BEHAVIOUR

E. Barry Keverne
Sub-Department of Animal Behaviour, University of Cambridge, Madingley, Cambridge, CB3 8AA.

Genomic imprinting confers functional differences on parental genomes such that certain autosomal alleles are only expressed according to parent of origin. Maternal and paternal genomes are not, therefore, transcriptionally equivalent. Hence, parthenogenetic and androgenetic embryos are developmentally lethal but can be rescued in chimeras that are parthenogenetic/normal (Pg) or androgenetic/normal (Ag). The distribution of these chimeric cells in the developing brain show a clear and distinct segregation. Ag cells contribute substantially to brain regions that are important for primary motivated behaviour (hypothalamus, MPOA, BNST and septum) and are excluded from the developing neocortex and striatum. By contrast parthenogenetic cells selectively accumulate in the neocortex and striatum. Interestingly, parthenogenetic chimeras have a larger forebrain while androgenetic chimeras have a smaller forebrain than normal mice. A phylogenetic analysis of brain areas to which Pg cells accumulate reveals an evolutionary size increase, while brain regions that accumulate Ag cells have decreased in size. The impact of this evolutionary remodelling of the brain is reflected in the behaviour. Interestingly, imprinted genes which are paternally expressed, map their expression to areas of the developing brain where Ag chimeric cells concentrate, and influence behaviour in a predictable way.

52

MEGA DATA FROM MICROARRAYS: CHIPPING AWAY AT PAIN MECHANISMS

Clifford J. Woolf
Neural Plasticity Research Group, Massachusetts General Hospital and Harvard Medical School, Boston MA, USA.

Neurons respond to alterations in activity, exposure to diverse synaptic and non-synaptic signaling molecules and axonal injury by multiple transcriptional and non-transcriptional changes. The sequencing of the human and mouse genome together with the development of high density oligonucleotide microarrays now enables determination of changes in the expression profiles of transcripts in situations as complex as those which produce pain. The reliability and sensitivity of the technique is contingent though on key technical and bioinformatic issues, while extracting useful data from the enormous data base requires careful experimental design and analysis. Multiple arrays are actually much better than fewer in identifying those genes that play specific functional roles. The technique is beginning to reveal new and unexpected players in the production of pain and has the advantage that its output is not biased by our preconceptions.

Griffin RS, Mills CD, Costigan M, Woolf CJ. Exploiting microarrays to reveal differential gene expression in the nervous system. Genome Biology 2003; 4:105
Costigan M, Befort K, Karchewski L, Griffin RS, D'Urso D, Allchorne A, Sitarski J, Mannion JW, Pratt RE, Woolf CJ.
Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury.
BMC Neurosci. 2002;3:16.
Scholz J, Woolf CJ. Can we conquer pain?
Nat Neurosci. 2002;5 Suppl:1062-7.

53.01

MATING CHANGES NEURONAL ACTIVITY AND RESPONSES EVOKED BY PHEROMONAL STIMULI IN THE VOMERONASAL SYSTEM OF FEMALE MICE

Peter A. Brennan & K. Esther Binns
Sub-Department of Animal Behaviour, University of Cambridge, High Street, Madingley, Cambridge, CB3 8AA.

During mating female mice learn to recognise their mate's urinary pheromones. Subsequently, her litter is aborted if she is exposed to male pheromones that differ from those of her mate. We have studied the formation of pheromonal memories in the accessory olfactory bulb (AOB) by recording urine-evoked activity in awake females. Local field potentials were obtained from the mitral and granule cell layers via pre-implanted electrodes (surgery under anaesthesia). The power spectrum analysed over 100sec periods indicated that the waveform was dominated by 8-12Hz oscillations. Presentation of urine soiled bedding from males (CBA or B6) led to in an increase in the power of these oscillations. In a second recording session after mating (with CBA male) the activity recorded in the subject's own bedding was greatly enhanced. Data obtained from the mitral layer showed a mating-dependent and mate-specific change in the waveform such that post-mating responses to mate bedding appeared normal while responses to strange bedding were greatly reduced. In the granule cell layer responses to both pheromonal stimuli were reduced.
These findings are consistent with the previously observed post-mating increase in transmitter release in AOB. Moreover, the data support the involvement of changes in neural oscillatory activity in pheromonal learning.

53.02

RESPONSES EVOKED BY PHEROMONAL STIMULI IN THE VOMERONASAL SYSTEM OF FEMALE MICE

K. Esther Binns & Peter A Brennan
Sub-department of Animal Behaviour, Dept Zoology, University of Cambridge, Madingley. UK CB3 8AA.

Male urinary pheromones carry information regarding identity. In mice, pheromones are detected by receptors in the vomeronasal organ that project to the Accessory Olfactory Bulb (AOB). The aim of this study was to record functional responses to naturally presented pheromonal stimuli in the AOB of awake behaving females. Local field potentials (LFPs) were obtained from the granule and mitral cell layers of the AOB using pre-implanted electrodes (surgery under general-anaesthesia). These contained Theta (4-12Hz) and Gamma (30-100Hz) oscillations. The direct application of urine (0.025ml/2 sec onto the nose) resulted in a short depolarisations in the waveform. The latency of the depolarisation varied between 1-5 seconds after stimulus onset. Thereafter the frequency of Theta oscillation was increased. In controls water was presented rather than urine and the waveform was unchanged. Single-units extracted from these signals only rarely fired action potentials but the firing rate was increased by urine presentation. Similar data were obtained when pheromones were presented for 1000sec in urine-soiled bedding (from CBA males).Oscillatory activity in AOB may result from the interaction of mitral and granule cells at dendro-dendritic synapses and could have a role in the transmission of the pheromonal information used by female to recognise individual males.

53.03

SEX DIFFERENCES IN ANXIETY-LIKE BEHAVIOUR IN FOUR ANIMAL MODELS OF ANXIETY

Bridges NJ and Starkey NJ
Department of Psychology, University of Central Lancashire, Preston, PR1 2HE.

Animal models for predominantly female disorders, such as anxiety, often use only male animals, with no investigation of sex differences. Therefore it is of interest to make comparisons of male and female behaviour within established animal models of anxiety. Behavioural measures were taken in male and female Mongolian gerbils tested in the elevated plus-maze, black-white box, social interaction test and open field. In the elevated plus-maze females spent proportionally less time in the centre square and proportionally greater time in the closed arms compared to males. In the black-white box females showed significantly greater entries into the black compartment in comparison to males. In the social interaction test, females showed more exploratory/cage-orientated behaviour and less active social and aggressive behaviour. In addition females terminated social contact more frequently. In the open field, females spent less time engaging in exploratory behaviours (sniffing and rearing) compared to males. In conclusion, the results show that these four animal models have the ability to detect sex differences in anxiety-like behaviour and in addition show that females exhibit greater anxiety-like behaviour within these four models compared to males.

53.04

INFLUENCE OF CLOSED ARM TRANSPARENCY ON ANXIETY-LIKE BEHAVIOUR IN THE GERBIL ELEVATED PLUS-MAZE

Bridges NJ, Hornby BF, Starkey NJ
Department of Psychology, University of Central Lancashire, Preston PR1 2HE

The elevated plus-maze is a widely used animal model of anxiety, and even though the basic design of the plus-maze is consistent between studies, there are many components of its construction (e.g. ledge height and arm width), which can vary from study to study. As a result of these variations, inconsistencies between the results obtained from plus-maze studies may arise. Behavioural measures in male and female Mongolian gerbils were made in two variations of the elevated plus-maze, one where closed arm walls were left transparent and one where the walls were black-opaque. It was found that black-opaque closed arms decreased % open arm time, rear frequency and duration, locomotor frequency and stretch attend frequency and increased % centre square time and immobile frequency and duration compared to transparent closed arm conditions. In conclusion, the results have indicated that black-opaque walls create greater aversion to the open areas of the plus-maze and decrease locomotion and exploratory behaviour. This highlights how simple design elements of the elevated plus-maze (e.g. wall transparency) can have dramatic effects on rodent anxiety-like behaviour.

53.05

THE EFFECTS OF INDIVIDUAL HOUSING ON 'ANXIOUS' BEHAVIOUR IN MALE AND FEMALE GERBILS

Starkey, NJ, Normington, G & Bridges, NJ
Department of Psychology, University of Central Lancashire, Preston, Lancs, PR1 2HE.

Gerbils live in family groups in the wild. Thus individual housing may be a psychosocial stressor. Gerbils were group housed for 4 weeks, then, either individually housed or remained with cage mates for 1 week. Gerbils were tested in the black/white box (BWB), elevated plus maze (EPM) and social interaction test. Results indicated no significant differences in behaviour in the BWB. In contrast, on the EPM, individually housed males showed increased anxiety (increased closed arm time) compared to other groups. Overall, females showed a less anxious profile than males (increased open arm time). However, individual housing increased anxiety-like behaviour in both sexes (decreased open arm time). In the social interaction test, individual housing decreased exploratory behaviour and partner investigation, and increased immobility in females. In males, individual housing increased partner orientated behaviour, indicating a decrease in anxiety. Overall, females showed increased defensive behaviour and less exploratory behaviour than males. Individual housing alone, decreased exploratory & locomotor behaviour and increased defensive behaviour in both sexes.Together, results indicate that individual housing leads to behavioural alterations in gerbils. However, whether this manipulation increases 'anxiety' depends upon the sex of the animal and the test used to assess behaviour.

53.06

HIGH RESPONSE RATES FOR LATERAL HYPOTHALAMUS STIMULATION ARE CORRELATED WITH A LOW ANXIETY LEVEL IN THE ELEVATED ZERO-MAZE IN RATS

F. D.C. Martin and H. M. Marston
CNS Section, LO Pharmacology, Organon Laboratories, Newhouse, Lanarkshire ML1 5SH, Scotland.

Little is known as to whether exposure to repeated stimulation in ICSS (intracranial self-stimulation) alters behaviour in other domains. Here the effects of ICSS on performance in the Zero-maze were examined. Fifty-five Sprague-Dawley rats implanted with an electrode in the lateral hypothalamus were allowed to self-stimulate for only 5 consecutive days, 30-min per session under a continuous reinforcement schedule. The animals were then divided into 3 groups of 10 animals according to response rate: non-responders (<20 responses/30-min), medium responders (300-1300 responses/30-min) and high responders (>1800 responses/30-min). Rats then received a 5-minute zero-maze test 72 hours post last ICSS session. The high responder group showed a trend towards an increased number of entries ($p=0.10$), distance travelled and velocity on the maze, a significant increase in the number of head dips ($p<0.001$) and a significant decrease in the number of stretch attend postures ($p<0.001$) compared to the non-responder group. This data shows that rats that self-stimulated at high rates in the early ICSS acquisition exhibited behaviour suggestive of lower levels of anxiety compared to poor responders. The question "does ICSS make the rats less anxious or are anxiolytic rats better responders to ICSS?" remains to be elucidated.

53.07

DOPAMINE RELEASE DURING UNCONDITIONED AND CONDITIONED AVERSIVE STIMULI: STUDIES USING ONE MINUTE MICRODIALYSIS SAMPLING IN THE RAT

Andrew M.J. Young
School of Psychology, University of Leicester, University Road, Leicester LE1 7RH U.K.

Previous studies have shown that aversive footshock, or neutral stimuli predictive of it, increase dopamine in nucleus accumbens (NAC). However the dialysis sample time was such that more than one stimulus occurred in each sample, precluding inference about responses to individual stimuli. This study used 1 min dialysis sampling in rats to measure responses to individual stimulus presentations.
Dialysis probes were placed in medial NAC through previously implanted guide cannulae. One hour later they were connected for dialysis in a behaviour box, and perfusion (5ul/min) started immediately. After 30 min equilibration consecutive 1 min dialysate samples were collected. After 5 basal samples footshock (4 x 1s train, 0.3mA at 5 min intervals), either alone or preceded by a tone (5s, 2.5kHz, 15dB above ambient) were presented.
In agreement with previous results, footshock caused increases in dopamine which were augmented when it was preceded by tone. Moreover, the profile of responses was different in the two cases. With footshock alone the dopamine was maximal on the second presentation, after which it declined. In contrast, with footshock preceded by tone there was a sustained augmentation of the response over the repeated presentations.
These results have implications for understanding the role of NAC dopamine in response to motivationally salient stimuli.

54.01

ACUTE PCP ADMINISTRATION PRODUCES ATTENTION SET SHIFTING DEFICITS IN THE RAT

.A. Egerton1, C.E. McKerchar1; B.J.Morris1,2 & J.A.Pratt1,3,
1Yoshitomi Research Institute of Neuroscience in Glasgow, University of Glasgow, Glasgow, G12 8QQ & 2Institute of Biomedical and Life Sciences, University of Glasgow, G12 8QQ & 3Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, G4 0NR.

Phencyclidine (PCP) has been used to provide a rodent model of schizophrenia. Acute administration of PCP produces neurochemical alterations in the rodent prefrontal cortex (Cochran et al., Synapse, 2002, 46 (3): 206 -214). The aim of this study was to investigate whether acute PCP administration produces deficits in prefrontal cortical-dependent cognitive behaviours similar to those that are reported in schizophrenia. 22 hours following acute administration of either vehicle or PCP (2.58 mg/kg i.p.), male hooded Long Evans rats were tested on performance in an attentional set–shifting task (Birrel and Brown, J.Neurosci., 2000; 20 (11), 4320-4). All animals were used in accordance with the Animals (Scientific Procedures) Act 1986. Animals were required to discriminate between different stimuli in order to obtain a food reward. The ability to switch attention either within (intradimensional shift (IDS)) or between (extradimensional shift (EDS)) perceptual dimensions was assessed by recording the number of trials required to reach criterion for each discrimination. Results showed that PCP administration was associated with selective deficits in EDS ability ($p = 0.0052$). As a selective impairment in EDS ability is also observed following lesions of the rat medial prefrontal cortex (see Birrel and Brown, 2000, as above), results suggest that acute PCP administration is associated with impaired prefrontal cortical function.

54.02

THE EFFECTS OF ROSE (ROSA CENTIFOLIA) AND LAVENDER (LAVENDULA ANGUSTIFOLIUM) OIL ODOURS IN TWO TESTS OF ANXIETY

Hornby,B.F. and Starkey,N.J.
Department of Psychology. UCLAN, Preston, Lancs, PR1 2HE.

Rose and lavender oil odours are popular in the treatment of mild anxiety. However, few formal tests of their efficacy have been conducted. Thus, these odours were tested in the elevated plus maze (EPM) and black white box (BWB) using individually housed male and female gerbils.
Lavender decreased locomotor activity in the BWB in both sexes. Whilst, in the EPM, exploratory behaviour and arm entries were decreased, mirrored by an increase in immobility.
In the BWB, rose oil increased exploratory behaviour (sniff & rear) at the crossover point in males. There was also a decrease in activity in the both sexes. In the EPM, rose oil increased risk assessment behaviour in males (head dips, stretch attend from centre). In addition, vertical exploration, open arm entries and some risk assessment behaviours (protected stretch attend) increased in both sexes. This was in contrast to an overall decrease in activity (total & closed entries).
These results indicate that lavender produced an overall sedative effect, reducing activity. In contrast, rose oil appeared to have anxiolytic properties in terms of traditional measures. Ethological measures reflect more of an anxiogenic profile particularly in males, which is worthy of further investigation.

54.03

THE IMPACT OF ANAESTHETIC, SURGERY AND SALINE ADMINISTRATION ON GUINEA PIG SPONTANEOUS HOME CAGE ACTIVITY

H Mumford and JR Wetherell
Dstl Porton Down, Salisbury, SP4 0JQ.

Mini-osmotic pumps are widely used in pharmacological studies for drug delivery at a constant rate over time, thus avoiding repeated dosing. Radiotelemetry allows continuous measurement of selected parameters from freely moving animals in their home cages, undisturbed by the measurement procedure. Understanding the impact of these procedures is important, and as part of a larger continuing study, telemetry transmitters (Data Sciences International, model TA10TA-F40) were used to record subcutaneous temperature and locomotor activity. Guinea pigs were singly housed from the day of transmitter implant. Alzet osmotic pumps containing saline (0.9%) were implanted at the time of transmitter implant and removed 6 days later under halothane anaesthesia. Home cage activity increased at dusk and dawn, with the largest peak at dusk. Activity peaks were generally accompanied by a slight rise in temperature. Following anaesthetic, the activity peak at dusk was greatly reduced and the rate of weight gain was reduced. On the day of pump explant, temperature did not return to normal values for 2-3 hours, but was subsequently slightly elevated during the light phase. The presence of a saline pump did not alter the pattern of activity or the daily temperature changes. This study provided valuable baseline data on the activity and temperature patterns of the guinea pig.

54.04

A ROLE FOR ADENOSINE IN REWARD MECHANISMS

L.K. Harper, S.R. Beckett, C.A. Marsden and S.P.H. Alexander
Neuroscience and Pharmacology, School of Biomedical Sciences, University of Nottingham, Nottingham, NG7 2UH

A2A adenosine receptors are co-localised with dopamine D2 receptors in the caudate, putamen and nucleus accumbens. A2A receptor antagonism leads to increased dopamine release in the nucleus accumbens of Lister hooded rats in vitro. We investigated the effect of a non-selective adenosine receptor antagonist CGS15943 in vivo using a conditioned place preference (CPP) paradigm. Male Lister hooded rats (n=24) weighing 250-350 g were used in the study. The CPP apparatus has been previously described by Cheer et al., (2000). Animals were assigned to four groups. Three groups received 1 mg kg-1, 2 mg kg-1 or 4 mg kg-1 CGS15943 i.p. paired with one compartment, alternated with saline paired with the other compartment. A control group received vehicle throughout. Forty-eight hours after the last pairing, the time spent in each compartment was determined in the trial period. The control group displayed no preference. CGS15943 elicited a preference for the drug-paired compartment at all concentrations used in a dose-dependent manner. Precisely which subtype of adenosine receptor mediates these effects will need to be examined using more selective ligands. All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986.

LKH is a BBSRC Research Committee Student
Cheer J.F., Kendall D.A. and Marsden C.A. (2000) Psychopharmacol. 151(1), 25-30.

54.05

MONOAMINE UPTAKE INHIBITION AND CIRCLING BEHAVIOUR IN 6-OHDA LESIONED RATS

E. L. Lane1, S. Rose1, S. Cheetham2 & P. Jenner1
NDRC, School of Biomedical Sciences, King's College London, London1, & RenaSci Consulting Ltd, BioCity Nottingham UK2.

The monoamine uptake inhibitor BTS 74 398 produces ipsilateral rotations in 6-OHDA lesioned rats that is attributed to the impairment of dopamine uptake. Studies in MPTP-treated marmosets also indicate the involvement of the dopamine transporter and that 5HT and noradrenaline uptake inhibition do not modulate the dopamine-mediated response. This study evaluates the roles of individual monoamine uptake inhibitors, (dopamine; GBR12909, noradrenaline; nisoxetine and 5-HT; fluvoxamine) on circling in the 6-OHDA rat.
Male Wistar rats unilaterally lesioned with 6-OHDA were given GBR12909, fluvoxamine or nisoxetine alone and in combination. Circling was recorded for 5hr post-dosing. GBR12909 produced dose dependent ipsilateral circling lasting for >5hr which was unchanged by nisoxetine co-administration. In contrast, fluvoxamine (with or without nisoxetine) co-administration significantly potentiated GBR12909-induced circling. Fluvoxamine and nisoxetine did not induce circling.
Thus the dopaminergic actions of BTS 74 398 are a pre-requisite for the induction of circling in this model. Circling was augmented by the inhibition of 5-HT, but not noradrenaline, uptake which could suggest increased dopamine release through 5HT receptor activation. This study supports work in the MPTP-treated marmoset, with the exception of the lack of potentiation by 5HT uptake inhibition.

54.06

EFFECT OF PROTEIN S100B ON CASPASE – 3 ACTIVATION AND SPECIFIC FRAGMENTATION DNA IN NERVOUS CELLS OF VARIOUS BRAIN STRUCTURES

Sherstnev V.V., Gruden M.A., Storogeva Z.I., Yurasov V.V., Jakovleva N.K., Proshin A.T. , P. K. Anokhin
Institute of Normal Physiology, RAMS, Moscow, Russia; Mohovaya st.11, Building 4, 103009 Moscow, Russia.

Application of S100b protein at the proapoptotic doses (500 ng and 50 ng) on the cerebellar vermis 1 or 4 hours before training caused the decreasing of acoustic startle reaction (ASR) amplitude, attenuated short- and long-term ASR habituation and conditioned contextual fear elaboration observed in 24 and 48 hours. Administration of S100b at the dose which is known to prevent apoptosis in vivo (5ng) resulted in stimulation of conditioned fear elaboration. In naive or saline rats caspase-3 activity was 2,07±1,3, 8,7±1,95 pmol/min*mg of protein in prefrontal cortex and hippocampus respectively. Caspase-3 activity increased in prefrontal cortex in up to 6,93±2,02 and 7,68± 2,02 pmol/min*mg of protein and in hippocampus up to 194,59 ±70,9 and 19,15±6,40 pmol/min*mg of protein through 24 or 48 hours, respectively after S100b injection.. In cerebellum caspase-3 activity was lower that level of determination at all examined points. Through 24 hours after S100b injection (500ng) process of DNA fragmentation was marked only in prefrontal cortex (2300; 861-758; 300, 189 thousands of nucleotide's pairs).and was comparable with control animals with saline. Through 48 hours after injection S100b - DNA fragmentation was observed as in prefrontal cortex (492; 369-300; 189 thousands pairs) as also at hippocampus (369, 300 thousands pairs of nucleotides).

Support by RFBR, grant _ 02-04-48847.

55.01

EFFECTS OF HOMOQUINOLINIC ACID IN THE RAT HIPPOCAMPAL SLICE

A. P. Mallon, T. W. Stone
#330, Neuroscience and Biomedical Systems, West Medical Building, Glasgow University, Glasgow, G12 8QQ.

There is evidence for the binding of homoquinolinic acid (HQA) to an NMDA-insensitive site in the brain. We have investigated the pharmacology of HQA on hippocampal slices taken from male Wistar rats (150-200g). Stimulation was delivered to the Schaffer collaterals and evoked excitatory postsynaptic potentials (EPSPs) were recorded extracellularly from the CA1 region. Both quinolinic acid (QA) 150uM and HQA 2.5uM cause an approximately 50% inhibition of EPSP slope. Paired-pulse studies indicate that there is a presynaptic component to this action. We were able to selectively block this inhibition using kynurenic acid (KYA) 100-300uM, a non-selective antagonist of NMDA receptors, 2AP5 50uM, a specific NMDA receptor antagonist and (+)-MK 801 maleate 10uM, a potent, selective and non-competitive NMDA receptor antagonist. None of these antagonists was able to reveal any NMDA-insensitive activity. We are undertaking further experiments to characterise this site using a wider range of antagonists including those with subunit selectivity.

55.02

BLOOD KYNURENINE LEVELS AFTER TRAUMATIC BRAIN INJURY

L G Darlington1, C M Forrest2, H Finch3, G M Mackay2, A J Sayer3, N Stoy3, T W Stone2
1Epsom General Hospital, Epsom, Surrey, UK; 2University of Glasgow, Glasgow, UK; 3Royal Hospital for Neuro-disability, Putney, London, UK.

The kynurenine pathway is activated in macrophages and glial cells following immune stimulation. One product, quinolinic acid, is an agonist at NMDA receptors, while kynurenic acid is a glutamate antagonist. We investigated whether the pathway is activated by brain injury, with activation persisting into the chronic state. Fifteen patients, clinically stable after brain injury, were recruited from a neurological hospital. Eighteen healthy controls were recruited from hospital staff without illness or medication which activated the kynurenine pathway. Subjects fasted overnight, then consumed a mixture of amino acids to deplete plasma tryptophan levels. Blood samples were taken before taking the mixture and after 5 and 7 hours. After 24 hours a further blood sample was taken, subjects were given a tryptophan load and blood samples were taken 5 and 7 hours later. Basal levels of tryptophan, kynurenine, kynurenic acid and quinolinic acid were no different between patients and controls, but after tryptophan loading, plasma tryptophan levels were greater in patients. The other kynurenines showed no differences between patients and controls. Neopterin and lipid peroxidation products were increased. The data suggest an inflammatory state associated with altered tryptophan metabolism.

55.03

NICOTINIC ACETYLCHOLINE RECEPTORS MODULATE A RANGE OF EPILEPTIFORM ACTIVITIES *IN VITRO*

Shiva Roshan-Milani, Mohammad Javad Khoshnood, Stuart Cobb
Division of Neuroscience and Biomedical Systems, IBLS, University of Glasgow, Glasgow, UK.

Nicotinic acetylcholine receptors (nAChRs) regulate neuronal excitability and may be relevant to several forms of idiopathic epilepsy. Our previous findings suggest that activation of nAChRs potentiate 4-aminopyridine induced epileptiform bursting. To assess whether nAChR activation has a modulatory influence over epileptiform activities more generally we examined the action of the selective nAChR agonist DMPP on two additional pharmacological paradigms. Bicuculline (reduced inhibition) and low magnesium (enhanced excitation) models were examined using hippocampal slices prepared from 2-5 week old rats. Extracellular recordings were obtained from stratum pyramidale of the area CA3. Following bath application of bicuculline (20 uM) or by incubating slices in a Mg++ free medium, spontaneous bursts occurred at a mean frequency of 0.14±0.02Hz and 0.16±0.02Hz, respectively. Subsequent co-application of DMPP (10-30 uM, n=22) produced a significant increase in both bicuculline and Mg++ free induced burst frequency with a maximal frequency potentiation of 348±76% and 210±37%, respectively ($P<0.03$). These effects were reversed upon subsequent washout of DMPP or upon co-application of selective nAChR antagonist dihydro-beta-erythroidine (20-40 uM, n=6/7). These results suggest that nAChRs exhibit a general excitatory influence over patterned neuronal activity within cortical circuits.

55.04

EFFECTS OF G-PROTEIN COUPLED RECEPTOR KINASE (GRK) KNOCKDOWN ON μ-OPIOID RECEPTOR DESENSITISATION ANDINTERNALISATION

C P Bailey, D Couch, E Kelly, G Henderson
Department of PharmacologyUniversity of BristolBristol BS8 1TD.

μ-opioid receptors exhibit rapid desensitisation and internalisation on exposure to agonists. This is thought to occur by receptor phosphorylation, recruitment of arrestin and subsequent internalisation. Many kinases have been implicated, but because these studies have been performed largely in expression systems, the mechanisms underlying desensitisation in native neurones are still unclear. In this study, we made patch-clamp recordings from rat locus coeruleus (LC) neurones in acute brain slices, whereby μ-receptor activation elicits a large, rapidly desensitising K+-current. By using protein kinase inhibitors we have excluded PKA, PKC, PKG, casein kinase I and II, PI 3-kinase, PI 4-kinase, phospholipase D, tyrosine kinase, CaM kinase II, MEK-1, MEK-2 and p38 MAP kinase from having pivotal roles in this rapid desensitisation. We are now examining the importance of GRKs. By transfection of dominant negative mutants for GRK2 and GRK6 in HEK cells stably transfected with the μ-receptor followed by ELISAs we can examine the effects of GRK knockdown on μ-receptor internalisation. Also, using adenoviral techniques, we are transfecting dominant negative mutant GRKs into the rat LC in vivo followed by patch clamp recordings in vitro. Initial experiments suggest that GRK6 has no role in μ-receptor internalisation.

55.05

EFFECT OF 4-AMINOPYRIDINE ON THE EFFLUX AND UPTAKE OF PRE-ACCUMULATED D-[^{3}H]ASPARTIC ACID IN RAT CEREBROCORTICAL MINISLICES

Abidali Fazal, *Alan M. Palmer, †Mike F. Snape and Martin J. Croucher
*Department of Neuroinflammation, Faculty of Medicine, Imperial College London, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, *Pharmidex, 72 Bond Street, London, W1S 1RR and †Vernalis Research Limited, Oakdene Court, Winnersh, Wokingham, RG41 5UA, UK.*

In this study, we compared the effect of 4-aminopyridine (4-AP) on the efflux of pre-accumulated D-[3H]aspartic acid (D-[3H]asp) from rat cerebrocortical minislices under static incubations with that observed with rapid superfusion. The uptake of D-[3H]asp in a static incubation system was also assessed. SUPERFUSION ASSAY: 4-AP failed to influence D-[3H]asp efflux at concentrations below 1 mM. With 1 min exposure, 4-AP (1-30 mM) evoked concentration-dependent increases in D-[3H]asp efflux. With 5 min exposure, these responses were maximal; 10 min exposure produced sub-maximal enhancement. When co-perfused for 1 min with 25, 50 or 75 mM K+ in Krebs Ringer Medium, 4-AP, below 1 mM, failed to influence the K+-evoked responses. Only 5, 10 and 30 mM 4-AP enhanced 25 mM K+-evoked efflux whilst only 30 mM 4-AP potentiated 50 mM K+-evoked efflux. STATIC SYSTEM: 5-15 min incubations with 4-AP (10 uM–10 mM) caused concentration-dependent increases in D-[3H]asp efflux. 4-AP (1–30 mM) significantly inhibited Na+-dependent D-[3H]asp uptake into cortical minislices. We conclude that the observed ability of 4-AP to inhibit EAA uptake is likely to complicate studies of EAA release where the influence of uptake is high. AF is a BBSRC Case Award student.

55.06

[^{3}H]-DPCPX BINDING IN RAT CEREBRAL CORTEX, HUMAN CEREBRAL CORTEX AND CHO-K1 CELLS XPRESSING THE HUMAN ADENOSINE A1 RECEPTOR

K Dhillon, A Misra, K Quirk, M Esiri, MJ Croucher, S Weiss, M Bickerdike and A R Knight
Vernalis Research Ltd., Oakdene Court, 613 Reading Road, Winnersh, Wokingham. RG41 5UA. M.J.Croucher: Imperial College School of Medicine, Charing Cross Hospital, London, W6 8RF. M. Esiri: University of Oxford, Dept. of Clinical Neurology, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE.

[3H]-DPCPX was used to determine the receptor density and pharmacological profiles of adenosine A1 receptors in rat cortex, human cortex and CHO-K1 cells transformed to express the human adenosine A1 receptor. It bound with high affinity to a single population of receptors in each preparation. Displacement assays confirmed that this population of binding sites represented the adenosine A1 receptor. In all preparations the most potent displacers were DPCPX and CGS 15943, followed by the three agonists R-PIA, NECA and CHA. The adenosine A2A receptor antagonists SCH 58261, KW 6002 and ZM 241385 lacked potency in this assay. The pharmacology of A1 receptors in rat and human tissue was closely similar. However, a number of compounds were more potent at displacing [3H]-DPCPX binding from the cloned human receptor. For example, CGS 15943 displaced [3H]-DPCPX from rat cortical and human cortical tissue with Ki values of 57.3 and 28.7 nM, respectively but displaced from human cloned receptors with a Ki value of 3.7 nM. Whilst the use of cloned, expressed receptors for biochemical characterization can be advantageous, the present results emphasise the need to relate data from cell lines to tissue assay data to assess relevance to physiological function.

55.07

ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTORS ARE ASSOCIATED WITH GLUTAMATERGIC TERMINALS IN THE RAT FRONTAL CORTEX: NEUROCHEMICAL AND ANATOMICAL EVIDENCE

Stephen J Rousseau, Ian W Jones, Ian A Pullar and Susan Wonnacott
Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY.

Electrophysiological studies indicate that presynaptic alpha7 nicotinic acetylcholine receptors (nAChR) modulate glutamate release in several brain regions. Here we provide direct evidence for this relationship in the rat frontal cortex using neurochemical and anatomical approaches. The alpha7-specific agonist, choline (1mM, 10mM), evoked the release of tritiated D-aspartate (a surrogate for glutamate) from frontal cortex synaptosomes, and this was blocked by alpha bungarotoxin (Bgt, 40nM). Nicotine and epibatidine also evoked D-aspartate release that was partially Bgt-sensitive. The beta2-preferring antagonist dihydro-beta-erythroidine also partially inhibited responses to these agonists. The additive inhibition by dihydro-beta-erythroidine and Bgt suggests heterogeneity of the nAChR subtypes governing this response. The physical presence of alpha7 nAChR on glutamatergic terminals in rat frontal cortex was revealed by confocal microscopy: Alexa fluor 488 Bgt co-localised with vesicular glutamate transporter-positive structures in synaptosome preparations and tissue sections. Bgt fluorescence was abolished in the presence of competing nicotinic ligands, demonstrating the specificity of labelling. The direct evidence for functional alpha7 nAChR at glutamatergic terminals supports their putative role in modulatory processes such as LTP.

55.08

DIFFERENTIAL EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) EXONS IN THE RAT BRAIN AFTER REPEATED ANTIDEPRESSANT DRUG ADMINISTRATION

1A .A. Khundakar, 2C. A. Marsden & 1T.S.C. Zetterström.
1School of Pharmacy, De Montfort University, Leicester. LE1 9BH and 2School of Biomedical Sciences, Nottingham University Medical School, Nottingham, NG7 2UH.

Chronic antidepressant drug treatment show a time dependent bi-phasic action on BDNF gene expression, down-regulation at 4 h and up-regulation at 24 h after the last injection (Coppel et al, 2003, Neuropharmacol. In press). The gene for BDNF gives rise to multiple transcript forms. The inhibitory action by antidepressant drugs on the BDNF gene at 4 h is accounted for by decreased expression of exon IV (ieg type gene) but not exon I which depends on protein synthesis (Khundakar and Zetterström, 2002, Brit. J of Pharmacol, 137, 122). This study investigated the effect of i.p. daily injections for two weeks with: fluoxetine, (flu, 10mg/kg), desipramine (des, 10mg/kg) and tranylcypromine (TCP, 5mg/kg) on hippocampal rat distribution of the complete BDNF gene as well as exon-specific mRNAs (exon I and IV) at 24 h after the last injection. A two week treatment with flu or TCP but not des significantly up-regulated mRNA for the complete BDNF gene and exon I: (flu +37%, TCP +49%, compared to saline controls) and (flu +64%; TCP +50%) respectively. In contrast, no significant changes were seen for exon IV after flu or TCP administration. We confirm here previous findings that chronic administration of antidepressant drugs increase total BDNF gene expression, an effect which appears to be mediated by exon I (protein synthesis dependent type) but not exon IV (ieg type gene).

55.09

EFFECTS OF CHRONIC PAROXETINE TREATMENT ON HIPPOCAMPAL DENTATE GYRUS CELL PROLIFERATION IN THE YOUNG ADULT RAT

Kendall. I, Bostock. S, Patil. P, Marsden. C, Wigmore. P, Bennett. G
School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham Medical School, Nottingham. NG7 2UH, UK.

Many recent studies have shown antidepressant induced neurogenesis in the adult rodent hippocampus. The aim of this study was to investigate the effects of chronic (21 day) paroxetine treatment on hippocampal dentate gyrus (DG) cell proliferation in the young adult rat. 20 young (24-27 post natal day) male Lister Hooded rats received either water or paroxetine (200mg/l) in drinking water for 21d. Brains were then hemispherically sectioned for Ki67 immunocytochemistry or homogenised for high performance liquid chromatography (HPLC) detection of 5HT turnover. Immunolabelling of Ki67 cell division cycle protein, failed to show a significant difference in Ki67 +ve cells per DG, between the paroxetine treated and control animals. HPLC determination of cortical 5HT and 5HIAA levels were also measured in 11 animals, the 5HIAA/5HT concentration was significantly reduced following paroxetine treatment ($p<0.001$, students unpaired t-test, n=6 control, n=5 paroxetine treated) indicating significantly reduced 5HT turnover. The effects of chronic paroxetine on 5-HT turnover may indicate an immature serotonergic system which may partly explain the lack of effect on DG cell proliferation in the young adult rat DG. Further studies are ongoing to investigate the effects of paroxetine treatment on hippocampal neurogenesis and in the developing serotonergic system.

55.10

FLUOXETINE INDUCED CHANGE IN RAT BRAIN BDNF MRNA AND PROTEIN EXPRESSION IS DEPENDENT ON LENGTH OF TREATMENT

1G. de Foubert, 2C.S. Robinson, 2T.K. Murray, 2M.J. O'Neill, and 1T.S.C. Zetterström
1DeMontfort University, LE1 9BH, Leicester UK and 2Eli Lilly & Co. Ltd, Erl Wood Manor, Surrey, UK.

Brain derived neurotrophic factor (BDNF) has recently been implicated in the clinical action of antidepressant drugs. Repeated (2-3 weeks) administration of antidepressant drugs increase BDNF gene expression. The onset of this response as well as concomitant effects on the corresponding BDNF protein are unclear. In this study we investigated the effects of daily treatment with fluoxetine (10 mg/kg p.o.) on BDNF mRNA (1, 4, 7 and 14 days) and protein levels (4, 7, 14 and 21 days). Regional hippocampal mRNA and protein levels were measured 24 h after the last administration by in situ hybridisation or immunocytochemistry respectively. Single injections did not change BDNF expression. Following 4 days of fluoxetine treatment, both the BDNF mRNA and protein levels were reduced by 15-27% in the hippocampal regions of the brain. At 7 days mRNA levels showed a non-significant increase, while protein levels were unchanged compared to saline controls. In contrast after 14 days mRNA levels were significantly increased by 47-61%. Protein levels started to increase at 14 days, reaching significance at 21 days. These results show that BDNF is expressed differentially depending on the length of fluoxetine administration and may help to explain the slow clinical onset of serotonin re-uptake inhibitors.

G. de Foubert is supported by a BBSRC industrial studentship (Eli Lilly & Co. Ltd.).

55.11

EFFECT OF NR3 NMDA RECEPTOR SUBUNIT EXPRESSION ON NMDA RECEPTOR PHARMACOLOGY

§ Sattelle B., § Bermudez, I., ¥ Wingrove, P., ¥ Whiting P.,¥ Wafford K., ¥ Clark S.
§ School of Biological & Molecular Science, Oxford Brooks University, Oxford. ¥ MSD, Neuroscience research centre, Terlings Park, Harlow, Essex.

Here we report on the effects of NR1/NR3 NMDA receptor expression and the effect of NR3 subunits on NR1/NR2B receptor current amplitude and pharmacology.
Xenopus oocytes were nuclear injected with cDNA for either hNR1/hNR2B(1:3), hNR1/hNR3A(1:10), hNR1/hNR3B(1:10) or hNR1/hNR2B/hNR3B(1:3:10). Currents were recorded using two electrode voltage clamp at Vh=-80mV. Cells were continuously perfused with Ba2+ ringer (mM: NaCl 1141, KCl 24.8, BaCl2 18, HEPES 100, pH7.5) and drugs were applied by bath perfusion. Both NR3A and NR3B subunits significantly reduced the amplitude of NR1/NR2B receptor mediated currents. However no significant currents were observed upon application of either 10 or 100μM glycine to NR1/NR3A or NR1/NR3B receptors, indicating that they do not form functional glycine receptors. The presence of NR3B altered the pharmacology of NR1/NR2B receptors. 0.1μM MK801 was significantly more effective at inhibiting NMDA receptors containing the NR3B subunit. Similarly Ifenprodil inhibited the NR3B containing receptors significantly more at both 0.3μM and 10μM. These data indicate that the NR3B subunit increases the sensitivity of NMDA receptors to certain antagonists. We have shown that hNR1/hNR3 receptors are not functional but that the presence of NR3 in addition to reducing current amplitude, alters the pharmacology of hNR1/hNR2B NMDA receptors.

55.12

PCP-INDUCED CHANGES IN THE LEVELS OF mRNA ENCODING COMPONENTS OF THE POSTSYNAPTIC DENSITY IN RAT BRAIN AFTER ACUTE AND CHRONIC TREATMEN.

S.M. Cochran1, C.E. McKerchar1; B.J. Morris1,2 & J.A. Pratt1,3
1Yoshitomi Research Institute of Neuroscience in Glasgow, University of Glasgow, Glasgow, G12 8QQ & 2Institute of Biomedical and Life Sciences, University of Glasgow, G12 8QQ & 3Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, G4 0NR.

Human postmortem studies have shown alterations in components of the post synaptic density (PSD) in the prefrontal cortex of schizophrenic patients. This study aimed to investigate mRNA levels of PSD95, GKAP and Shank 1, 2 and 3, in the rat brain after treatment with the psychotomimetic phencyclidine (PCP).
Adult male hooded Long Evans rats were given a single i.p. injection of PCP (2.58mg/kg; n=8) or vehicle (saline; n=8) (acute regime) or repeated i.p. injections of PCP (2.58mg/kg; n=10) or vehicle (saline; n=10) intermittently over 26 days (chronic regime) then sacrificed after 24 or 72 hours respectively. Animals were treated in accordance with the Animals (Scientific Procedures) Act 1986. In situ hybridisation was carried out according to the method of Simpson and Morris (1994).
No change was observed in Shank 1, 2 or 3 mRNA levels after either PCP treatments. PSD95 mRNA levels were significantly increased in the prelimbic cortex after chronic but not acute PCP treatment, whereas GKAP mRNA levels were significantly decreased in the thalamic reticular nucleus after acute but not chronic PCP treatment. These results suggest dysfunction of the NMDA receptor complex following PCP treatment and further implicate the prefrontal cortex and thalamus in the pathopsychology of schizophrenia.

56.01

EFFECTS OF ANTI-OXIDANT TREATMENT ON AXONS AND NEURONS AFTER BRAIN INJURY

DJ Cuthill, MJ O'Neill*, D Dewar
*Division of Cinical Neuroscience, University of Glasgow, Glasgow, UK; *Eli Lilly & Co. Ltd., Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK.*

Previous studies have demonstrated the ability of the glutamate agonist AMPA to cause axonal and neuronal damage when injected into the caudate nucleus of the mouse. Anti-oxidants can reduce neuronal damage in a variety of in vitro and in vivo models of injury. The aim of this study was to determine if the antioxidant LY341122 could reduce damage to both axons and neurons.
Anaesthetised mice received 100mg/kg LY341122 or vehicle i.p. 30 minutes prior to stereotactic AMPA injection into the caudate nucleus and allowed to recover for 24hrs. Coronal sections were taken through the brain. Histology with haematoxylin and eosin staining was used to determine neuronal damage. Neurofilament 200 immunohistochemistry was used to determine axonal damage. Anaesthetised Mongolian gerbils received 50mg/kg or 100mg/kg LY341122 30min before and 3.5hr after or 0 and 3hr after 5min global ischaemia, and allowed to survive for 5 days. Histology was used to assess neuronal damage in the hippocampus.
LY341122 did not reduce axonal or neuronal damage after AMPA injection, but significantly reduced neuronal damage after global ischaemia. The results suggest that lipid peroxidation does not participate in AMPA-mediated excitotoxicity, but does in global ischaemia.

56.02

GADD34 IS EXPRESSED IN THE PERI-INFARCT ZONE AFTER FOCAL CEREBRAL ISCHEMIA

D.McCaig, H.Imai, L.M.Gallagher, J.Harland, D.I.Graham, S.M.Brown, I.M.Macrae
Division of Clinical Neuroscience, University of Glasgow, Glasgow G61 1QH.

GADD34, a stress response protein associated with cell rescue, DNA repair & apoptosis, is expressed in the ischaemic brain (Imai et al, 2002, Eur J Neurosci, 15,1929-36). This study examines the distribution and time course of GADD34 expression after focal ischaemia. Permanent MCA occlusion was carried out under halothane anaesthesia in Sprague Dawley rats with survival times of 4,12, 24 hrs, 7 & 30 days. Brains were processed for histology and immunohistochemistry. Ischaemic damage was mapped onto line diagrams at the level of the caudate nucleus and GADD34 positive cells counted in selected regions. GADD34 immunopositive cells (mainly neurons) were present in ischaemic brains at 4 hrs (e.g. peri-infarct cortex 20±5; contralateral cortex 3±1 cells/mm2, $p<0.05$) reaching peak values at 24hrs (peri-infarct cortex 31±7, contralateral cortex 0.1±0.1, $p<0.05$). Immunopositive cells, following a similar time course, were identified at the infarct boundary in the caudate nucleus and in the ipsilateral cingulate cortex. Protein expression in key anatomical locations pertinent to the evolving ischaemic lesion indicates that GADD34 has the potential to influence cell survival.

Supported by: University of Glasgow and Proof of Concept Award from Scottish Enterprise.

56.03

DETRIMENTAL EFFECTS OF OESTROGEN REPLACEMENT IN AN EXPERIMENTAL MODEL OF STROKE

D Bingham, I.M Macrae, H.V.O. Carswell
Division of Clinical Neuroscience, Wellcome Surgical Institute, University of Glasgow, Glasgow G61 1QH.

Numerous animal studies have demonstrated 17beta-oestradiol mediated neuroprotection after middle cerebral artery occlusion (MCAO). However, a recent clinical trial reported that 17beta-oestradiol can worsen stroke outcome (Viscoli et al, 2001. N Engl. J Med, 345,17, 1243-49). This study is the first to use the proximal diathermy method of permanent MCAO to investigate the effects of 17beta-oestradiol on stroke. Rats were ovariectomised under halothane anaesthesia and a 21-day release pellet implanted (s.c.) in the neck. The pellets (Innovative Research of America) contained 0.025mg, 0.25mg 17beta-oestradiol or placebo. Two weeks later the MCA was electrocoagulated from the lenticulostriate arteries to the inferior cerebral vein under halothane anaesthesia with 24hr recovery. Coronal sections were stained with H&E and infarct volume quantified. 17beta-oestradiol significantly exacerbated ischemic damage by 20% (low dose, $p<0.05$) and 27% (high dose, $p<0.01$, one-way ANOVA and Dunnett's multiple comparison test). Mechanisms responsible for the exacerbation of damage remain to be elucidated but may involve promotion of excitotoxicity by potentiating the NMDA response or suppression of GABA-mediated inhibition.

Supported by the University of Glasgow and Research into Ageing.

56.04

THE INFLUENCE OF OESTROGEN ON BRAIN DAMAGE IN A RODENT MODEL OF EXPERIMENTAL STROKE

Wallace KB, Macrae IM, Carswell HVO
Division of Clinical Neuroscience, Wellcome Surgical Institute, University of Glasgow, Glasgow G61 1QH.

The neuroprotective effect of oestrogen was studied in a rodent stroke model. Mechanisms of action are currently unclear but it is hypothesised that oestrogen may act by scavenging free radicals and inhibiting lipid peroxidation.Sprague Dawley rats were ovariectomised and 17β-oestradiol (0.25mg, 21 day release) or placebo pellet implanted subcutaneously. Two weeks later permanent middle cerebral artery occlusion (MCAO) was induced. 24h post-MCAO neurological deficits were assessed. The brain was processed for histology and immunohistochemistry to measure infarct volume and tissue immunoreactive for 4-hydroxynonenol (a lipid peroxidation product). 17β-oestradiol increased infarct volume (17β-oestradiol, 124 ± 46; placebo, 57 ± 84 mm3, Mean ± SD, $p<0.05$). Neurological scores were similar between groups (oestradiol, 15 ± 5; placebo 14 ± 4, max score = 33). Weight gain after ovariectomy was inhibited by 17β-oestradiol ($p<0.0001$). Immunohistochemistry is in progress.
Chronic 17β-oestradiol pre-treatment significantly increased infarct size. Though contrary to our hypothesis, these data support recent clinical findings suggesting oestrogen can worsen stroke outcome. Since HRT is widely used in post-menopausal women, more research to clarify the influence of oestrogen on stroke is urgently required.

KBW & HVOC supported by Research into Ageing.

56.05

SIGNALLING PATHWAYS MEDIATING THE NEUROPROTECTIVE EFFECT OF CORTICOTROPIN-RELEASING HORMONE

Bayatti N., Zschocke J., and Behl C.
Institute of Physiological Chemistry and Pathobiochemistry, University of Mainz, Duesbergweg 6, Mainz, Germany.

Corticotropin-releasing hormone (CRH) is known to mediate the body`s stress response by regulating the HPA axis. CRH is also a potent neuroprotective agent, saving cultured neurones from a number of toxic insults. Although the participation of the CRHR1 receptor has been shown to be necessary in order to mediate neuroprotection, the downstream signalling pathways responsible have not been clearly characterised. Here we show a region-specific ability of CRH (10 nM) to protect neurones from amyloid beta (25-35) toxicity as measured in MTT and propidium iodide cell survival assays in primary neurones. CRH-mediated protection was observed in both hippocampal and cerebellar but not in cortical cultures. RT-PCR analysis for CRHR1 demonstrated that these differences are independent of receptor expression levels. As these effects may reflect differential activation of signalling pathways, we also investigated the involvement of cascades previously known to be activated by CRH, i. e. CREB and MAPK, by western blot analysis using phospho-specific antibodies. Increases in CRH (10 nM) -induced CREB phosphorylation mirrored the neuroprotective effects observed in both the hippocampus and cerebellum but was absent in the cortex, where no neuroprotection was observed. This study indicates that CRH-mediated neuroprotection predominantly occurs through the activation of the CREB pathway and that future investigations must concentrate on the identification of downstream targets of this pathway.

Supported by the Verum Foundation

56.06

ADENOVIRAL MEDIATED OVER-EXPRESSION OF THE TRANSCRIPTION FACTOR CREB PROTECTS HIPPOCAMPAL NEURONS FROM APOPTOTIC AND EXCITOTOXIC STRESS

Colin P.J Glover, Darren Heywood, Alison Bienemann, James N. Kew * and James B. Uney
MRC Centre for Synaptic Plasticity and University Research Centre for Neuroendocrinology, University of Bristol, Bristol BS2 8HW, UK CNS Research, F. Hoffmann-La Roche Ltd., Basel, Switzerland.*

The Cyclic-AMP Response Element Binding (CREB) protein is a post-transcriptionally regulated transcription factor that has been implicated in many aspects of neuronal functioning including memory, synaptic plasticity, learning, regulation of circadian rhythm, addiction and development of the nervous system. More recently, correlative evidence has implicated CREB in the molecular pathways that mediate neuronal cell death/survival. However, a direct relationship between CREB over-expression and neuroprotection has not been demonstrated. To investigate this, adenoviral vectors were employed to express CREB and a highly effective dominant negative known as A-CREB in cultured primary hippocampal neurons. The adenoviral-mediated expression of CREB was shown to protect neuronal cultures from apoptotic, ischaemic and excitotoxic stress. Furthermore, the protection observed was also demonstrated to be independent of the anti-apoptotic molecule Bcl-2. These results demonstrate that CREB mediated transcriptional pathways are involved in a neuroprotective response against cytotoxic stress.

56.07

TISSUE INHIBITOR OF METALLOPROTROTEINASE-1 INHIBITS GLUTAMATE MEDIATED CALCIUM ENTRY AND PREVENTS EXCITOTOXIC CELL DEATH IN NEURONES

Darren Heywood+ , Hiang Khoon Tan+, Alison Bienemann+, Andrew H. Baker*and James B. Uney+.
*+URCN, Bristol Royal Infirmary, Marlborough Street, Bristol, UK and *Department of Medicine and Therapeutics, University of Glasgow, Glasgow, UK.*

The up-regulation of TIMP-1 following an excitotoxic injury has recently been hypothesised to be part of a general neuronal response that mediates long-lasting changes involved in tissue reorganization and possibly neuroprotection. In this study we have shown for the first time that within hours of applying TIMP-1 in recombinant form or by adenoviral-mediated gene transfer, neurones are highly protected against excitotoxic injury. Neither TIMP-3 nor a non-secretable form of TIMP-1 protected neurones. TIMP-1 conferred highly significant protection to hippocampal cells exposed to a wide range of glutamic acid concentrations in both dissociated and organotypic hippocampal cultures. Mutant TIMP-1, which lacks matrix metalloproteinase (MMP) inhibitory activity, did not protect neurons, suggesting that TIMP-1 is exerting its neuroprotective effects via its MMP-binding domain. However, two broad-spectrum synthetic MMP inhibitors (BB-94 and MMP1-I) did not confer any neuroprotection to hippocampal cultures. TIMP-1 did not prevent apoptotic cell death or death mediated by chemical ischemia. The observed neuroprotection may be explained by a decrease in calcium influx into neurones following stimulation with glutamate. These findings have a fundamental implication for our understanding of the physiological role of secreted TIMP-1 in the CNS.

56.08

EFFECT OF THE FLAVONOIDS GENISTEIN, QUERCETIN AND DAIDZEIN AGAINST TBH INDUCED CELL DAMAGE IN A RAT OLIGODENDROCYTE CELL LINE

Asma Al-Shamahi and Rosemary H. Waring
School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT.

Multiple Sclerosis (MS) is an autoimmune disease in which there is an abnormal immune response to Central Nervous System myelin with development of oxidative stress. We have developed a model cell system, in which the myelinating cell line 33B was dosed with the oxidative stress inducer tert-butyl hydroperoxide (TBH). Caspase 3 activity was assayed to monitor apoptosis in the cells. Antioxidant flavonoids were introduced into the model cell system to protect the oligodendrocytes from TBH induced damage. Incubation of the cells with genistein prior to TBH (10uM) showed significant increase ($p<0.001$) in caspase 3 activity relative to TBH control at 50uM, with a 42 % activation of the enzyme. Quercetin pretreatment decreased caspase 3 activity most significantly at 50uM and 100uM ($p<0.05$) to give a 37% and 28% inhibition of the enzyme respectively. Daidzein pretreatment showed a significant decrease in caspase 3 activity ($p<0.05$) at 50uM (49% inhibition). Incubation with the flavonoids alone showed no effect on caspase 3 activity. Necrotic cell damage was minimal throughout as indicated by lactate dehydrogenase release. It was concluded that quercetin and daidzein at concentrations of 50uM were able to give partial protection to oligodendrocytes in this model system.

56.09

METABOLIC CONSEQUENCES OF EXPOSURE OF CAD CELLS TO C2-CERAMIDE: THE EFFECTS OF CASPASE INHIBITION AND GROWTH FACTORS

Gonzalo Arboleda, Rosemary Gibson, Catherine Waters
School of Biological Sciences, 1.124 Stopford Building, University of Manchester, M13 9PT.

Several lines of evidence have implicated apoptosis as the mechanism underlying catecholaminergic cell death in Parkinson's disease (PD) and in therapeutic grafts. Diverse endogenous and exogenous stimuli increase ceramide levels, which in turn induce apoptosis through activation and inhibition of different pathways. We have analysed the metabolic changes induced by C2-ceramide and explored potential neuroprotective strategies in the central mesencephalic catecholaminergic cell line, CAD.
C2-Ceramide causes cell death by a dose and time dependant mechanism. The cell death is accompanied by activation of caspases, JNK, ERK, NFkB and loss of phosphorylation of AKT. Pre-treatment with caspase inhibitors and/or growth factors (neurotrophin-3 and IGF-1) partially protect against C2-ceramide.
To evaluate the metabolic response we used the Microphysiometer system to measure real-time changes in extracellular pH related to alterations in cellular metabolism. The acute increases in metabolism induced by growth factors are inhibited by C2-ceramide. However, in response to exposure to C2-ceramide for 24 hours, cells treated with caspase inhibitors and/or growth factors preserve their metabolic response to receptor stimulation in contrast to un-treated cells. These results have important implications for the preservation of neuronal function in neurodegenerative diseases such as PD.

56.10

DIFFERENTIAL GENE EXPRESSION ANALYSIS FOR CORTICAL SPREADING DEPRESSION-INDUCED PRECONDITIONING

Obrenovitch TP, *Schepers C, Godukhin OV, *Wachtel A, Urenjak J, *Nietfeld W
*School of Pharmacy, University of Bradford, BD71DP Bradford, United Kingdom; and *Max-Planck Institute for Molecular Genetics, 14195 Berlin, Germany.*

Preconditioning refers to the natural adaptative cellular responses that can be induced by sublethal stress, and which increase the tissue tolerance to a subsequent, potentially lethal insult. This study aimed to identify the biological determinants of increased tolerance through the analysis of changes in gene expression at different time points after induction of preconditioning. All experiments complied with the Animal (Scientific Procedure) Act 1986. Preconditioning was induced by cortical spreading depression as this stimulus allows the preparation of large samples of mouse cortex that are preconditioned evenly and consistently. Gene expression analysis was carried out with high-density cDNA arrays prepared from the cDNA clones of the Mouse Unigene Set (24500 gene tags), with hemicortices (preconditioned or sham-treated) sampled at 1, 3 and 6 hours post-treatment. Significant changes in expression were found in a large number of genes, among which 178 had their expression changed by >100 folds during this period. The data suggest strongly that multiple, concomitant molecular changes are required for effective adaptative cytoprotection. They also indicate that, to unravel such a level of complexity, differential gene expression analysis must be carried out at several, carefully selected time points.

Supported by the European Commission, Contract QLG3-CT-2000-00934.

56.11

BRAIN AROMATASE EXPRESSION IS INCREASED AFTER EXPERIMENTAL STROKE

1 H.V.O. Carswell, 2 A.F. Dominiczak, 3 H. Johnston, 3 P. O'Shaughnessy, 4 J.B. Hutchison, 5 N. Harada, 1 I M Macrae
1 Division of Clinical Neuroscience, 2 Division of Cardiovascular and Medical Science, 3 Division of Veterinary Physiology & Pharmacology, University of Glasgow, G61 1QH, 4 Babraham Institute, Cambridge, 5 Fujita Health University, Toyoake, Japan.

17beta-oestradiol has been shown to exert neuroprotection after experimental stroke. 17beta-oestradiol is synthesised in the brain by the enzyme aromatase. The present study investigates if brain aromatase is increased after experimental stroke. Ovariectomised stroke prone spontaneously hypertensive rats (SHRSP) underwent permanent distal middle cerebral artery occlusion (MCAO) under halothane anaesthesia. At 2hours post-MCAO, brain aromatase gene expression was not increased in the ischaemic hemisphere versus non-ischaemic hemisphere as measured by real time polymerase chain reaction (rt PCR) (hippocampus 2.81±1.23 vs 4.23±0.54; caudate 0.66±0.21 vs 0.76±0.27; cortex 15.33±4.60 vs 11.42±1.98; relative to luciferase (external standard), respectively, n=3, mean±SEM). At 24 hours post-MCAO, pilot studies revealed increased aromatase immunoreactivity in cells within the peri-infarct cortex and within the hippocampus of the ischaemic hemisphere. Initial studies indicate that the immunopositive cells may be astrocytes. Thus, this is the first evidence that aromatase protein is increased after MCAO. Later time points are currently being investigated.This work was

Supported by a Research into Ageing Training Fellowship to HVOC.

56.12

LOSS OF VESL-1L/HOMER-1C IN THE RETINA INDICATIVE OF EARLY NEURONAL CHANGES FOLLOWING MILD NEURONAL DAMAGE AND PROTECTION BY ESTROGEN

S. Kaja*, S. Yang, R. Liu, A.M. Brun-Zinkernagel, J.W. Simpkins, P. Koulen
*Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107-2699, USA; * Present address: Leiden University Medical Centre, Depts. of Neurophysiology and Neurology, Wassenaarseweg 62, 2333 AL Leiden, The Netherlands.*

We determined the localization of Vesl-IL/Homer-1c proteins in the rat retina and investigated the effects of mild retinal ischemia and estrogen on Vesl-IL/Homer-1c expression. Using specific antibodies, we localized Vesl-IL/Homer-1c proteins at glutamatergic synapses. Ischemia/reperfusion injury was induced by temporary middle cerebral artery occlusion (MCAO). Effects of steroids were investigated by pre-treating ovariectomized rats with estrogen prior to ischemic injury. Staining for Vesl-IL/Homer-1c proteins follows the expression of postsynaptic group I mGluRs. Following ischemic injury, no significant decrease in the viability of ganglion cells could be observed. The number of Vesl-IL/Homer-1c-positive synapses in retinas of ovariectomized rats was significantly reduced as compared with control animals. Pre-treatment of the ovariectomized animals with estrogen prior to ischemic injury fully prevents the loss of Vesl-IL/Homer-1c-positive synapses. Vesl-IL/Homer-1c proteins are important signaling components in synaptic processing in the retina that can serve as indicators for the detection of early changes in the neuronal circuit following mild neuronal damage. Estrogen exerts a neuroprotective effect preventing damage following neurotoxic insults. This may be relevant for the treatment of neurodegenerative diseases of the retina.

56.13

SPREADING DEPRESSION-INDUCED PRECONDITIONING IN THE MOUSE BRAIN ELICITS UPREGULATION OF nAChR α7 IN OUTER LAMINAE OF THE CORTEX

Paul L Chazot(1), Oleg V Gogukhin(2) and Tio P Obrenovitch(2)
1. Sunderland Pharmacy School, University of Sunderland, Sunderland, UK 2. School of Pharmacy, University of Bradford, Bradford, UK.

Cortical Spreading Depression (CSD) is a robust method for the induction of brain preconditioning, i.e., the adaptive cytoprotection that protects against subsequent, potentially lethal insults. We are interested in the mechanisms underlying brain preconditioning with a view to identify novel neuroprotection strategies. Previously, we have reported a profound 12-fold increase in α7 nAChR subunit expression following CSD-induced preconditioning [1]. In this present study, we investigated the topology of these changes in α7 protein, using semi-quantitative immunohistochemistry [2]. Up-regulation of α7 was clearly observed in layers II and III of the cortex, while cortical α4 expression appeared largely unchanged. This confirmed and expanded on the observed nAChR subtype selective up-regulation demonstrated previously by quantitative immunoblotting [1]. The expression pattern of α7 appeared to be mainly neuronal in appearance. We are currently addressing this issue using double labelling techniques. We propose that this marked change in nAChR α7 expression may underlie, in part, the adaptive cytoprotection afforded by CSD.

1. Chazot, PL et al. (2002) J. Neurochem. 83 1235-1238.
2. Thompson, CL et al. (2002) Molecular Brain Res. 102, 55-61.
Work supported by the European Commission, Contract No QLG3-CT-2000-00934 and the Wellcome Trust (UK).

56.14

PROBING NEUROPROTECTION WITH LENTIVIRAL VECTORS

Liang-Fong Wong, Scott Ralph, Lucy Walmsley, Steve Parham, *James Uney, Susan M. Kingsman and Nicholas Mazarakis
*Oxford Biomedica (UK) Ltd, Medawar Centre, The Oxford Science Park, Oxford OX4 4GA, *University Research Centre for Neuroendocrinology, University of Bristol, Bristol BS2 8HW.*

Lentiviral vectors based on the equine infectious anaemia virus (EIAV) have been previously demonstrated to transduce and express exogenous proteins efficiently in neuronal cultures and in rat nervous system with minimal toxicity. In order to validate the utility of these genetic tools to deliver therapeutic proteins for the treatment of neurodegenerative diseases, EIAV vectors expressing neuroprotective targets were tested in in vitro and in vivo neurotoxicity models. In primary hippocampal and cortical cultures, EIAV vectors encoding the anti-apoptotic molecule Bcl-2 prevented neurones from undergoing cell death under neurotoxic conditions. In in vivo models, injection of glutamate receptor agonists such as NMDA induced a large lesion in the CA1 layer of the hippocampus which could not be protected by prior transduction of a control vector expressing LacZ. In contrast, EIAV-mediated expression of Bcl-2 in the hippocampus significantly protected the CA1 layer from this excitotoxic lesion. Using a similar model, the neuroprotective function of glial-derived neurotrophic factor (GDNF) will also be investigated. Together, these studies illustrate the efficacy of EIAV vectors for quick and reliable validation of putative neuroprotective targets that arise from gene discovery programmes, leading to novel therapeutic strategies for neurodegenerative diseases such as stroke.

56.15

IDENTIFICATION OF NOVEL HYPOXICALLY REGULATED GENES IN PRIMARY CORTICAL NEURONS

S.Ralph, D.Krige, S. Lee, M.Craigon, G.Beard, L-F. Wong, S.Parham, S.Kingsman, C.Mundy and N.Mazarakis
Oxford Biomedica (UK) Ltd., The Medawar Center, Robert Robinson Avenue, Oxford Science Park, Oxford, OX4 4GA, UK.

The identification of genes differentially regulated by ischemia will lead to an improved understanding of cell death pathways such as those involved in the neuronal loss following a stroke. Furthermore, the characterization of such pathways could facilitate the identification of novel targets for stroke therapy. Previous attempts to elucidate differential expression patterns in stroke models have proven difficult since the changes in expression observed in neurons are relatively small.
Hypoxia is a cardinal feature of ischemic stroke. We have dissected and amplified differential gene expression patterns in an in vitro model of stroke by employing lentiviral vector systems to specifically bias the transcriptional activation of hypoxically regulated genes. Primary rat cortical neurons were transduced with minimal EIAV-based vectors encoding the transcription factors Hif-1a and EPAS. These proteins which are stabilized under conditions of low oxygen are responsible for mediating the hypoxic response. Transduced neurons were subjected to a hypoxic challenge and transcriptional profiling was performed using the Affymetrix system.
From these studies we have identified genes that were previously unrecognised to be hypoxically regulated. Further investigation will reveal the importance of these genes in understanding ischemic cell death and their potential as therapeutic targets.

56.16

THE ROLE OF c-JUN NH2-TERMINAL KINASE IN 1-METHYL-4-PHENYL-1, 2,3,6-TETRAHYDROPYRIDINE-INDUCED ABERRANT NEUROFILAMENT PHOSPHORYLATION AND CELL DEATH

Luigi A. De Girolamo, E. Ellen Billett
School of Science, The Nottingham Trent University, Clifton Lane, Clifton, Nottingham NG11 8NS.

MPTP causes the selective degeneration of dopaminergic neurones in animal models, in which the c-Jun NH2-terminal kinase (JNK) signalling cascade is implicated. We have used differentiated mouse neuroblastoma N2a cells to investigate the role of JNK in MPTP-induced neurotoxicity and cell death. Treatment of N2a cells with cytotoxic MPTP concentrations (5 mM) caused rapid and sustained JNK 1/2 phosphorylation, accompanied by cell death. In contrast, exposure of N2a cells to sub-cytotoxic concentrations of 10 uM MPTP resulted in much lower JNK activation, which was transient in nature. Using sub-cytotoxic MPTP levels we have previously observed an aberrant increase in neurofilament heavy chain (NF-H) phosphorylation associated with perikaryal accumulation and inhibition of axonal outgrowth, prior to cell death. Inhibition of the JNK pathway with a specific mixed lineage kinase inhibitor (CEP-11004) blocked MPTP-induced aberrant NF-H phosphorylation, protected against cell death and maintained the differentiated phenotype. These findings support a role for JNK in MPTP-induced neurotoxicity and enhance our understanding of the mechanisms causing aberrant NF-H phosphorylation. Moreover, our data support the suggestion that modulation of JNK activity could have therapeutic value in slowing or preventing the progression of Parkinson's disease.

56.17

MUTATING c-JUN N-TERMINAL KINASE-3 (JNK3) ACTIVE SITE RESIDUES TO THEIR p38 COUNTERPARTS AFFECTS BINDING OF JNK- AND p38-SELECTIVE INHIBITORS

Fricker, M., Ellis, S., Wilkie, N., Hunt, P. & Pollack, S
Merck Sharp & Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

Activation of c-Jun N-terminal kinase (JNK) has been linked to aberrant cell death in neurodegenerative disorders. The JNK3 isoform is selectively expressed in the brain, making it a potential candidate for therapeutic intervention. However, sequence similarity among the JNK isoforms and another MAP kinase, p38, have rendered the challenge of producing JNK3-specific inhibitors difficult. Using the crystal structure of JNK3 complexed with several JNK inhibitors, potential compound interacting amino acid residues were identified and mutated to the corresponding residues in p38. The effects of these residue changes on the kinetic parameters with three compounds were examined: a JNK3-selective inhibitor (Merck X); a p38-selective inhibitor (Merck Z) and a potent combined JNK3 and p38 inhibitor (Merck Y). The data confirm the role of the JNK3 residues I70 and V196 in both inhibitor and ATP binding. Remarkably, I70V and V196A mutations caused an increase and decrease, respectively, in the binding affinity of the p38-specific compound, Merck Z, of up to ten-fold. The I70V effect can be rationalized from the increased capacity of the active site to accommodate Merck Z, whereas the V196A mutant may induce an unfavourable conformational change.

56.18

IMMUNOREACTIVITY OF pMSK-1 AND INFLAMMATORY MARKERS FOLLOWING A TIME COURSE OF FOCAL ISCHAEMIC INJURY IN THE RAT

J.C.Roberts, C.A.Campbell, A.J.Lawman, A.A.Parsons and E.A.Irving.
Neurology and GI CEDD,GlaxoSmithKline, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AW.UK.

Msk1 (Mitogen and stress-activated protein kinase 1) is an in vivo substrate of the p38 and Erk MAP kinases.Selective inhibitors of p38 and MEK are protective in a variety of in vitro excitotoxic injury models.p38 and MEK inhibition has also proved efficacious in in vivo stroke models.
An Msk 1 inhibitor could provide an approach for the treatment of stroke combining effects at these protective pathways with acute neuroprotection.
We have used phospho specific antibodies to demonstrate activation of pMsk1 and pCREB 24hrs,1, 2, 4 and 8 weeks following transient ischaemia in the rat.In parallel,we have examined the distribution of T-ymphocytes,microglia and macrophages during the same ischaemic time course. In sham animals levels of pMsk 1 and pCREB immunoreactivity were minimal.Both pMsk1 and pCREB immunostaining increased markedly in the lesion area from 1 week post ischaemia compared to control.This increased pMsk1 immunoreactivity co-localised with ED-1 staining(macrophage and microglia marker)but not with CD43(T-lymphocyte marker).This study shows that Msk1 is expressed by a specific inflammatory cell population post ischaemia.The development of novel selective Msk1 inhibitors would allow the role of this activation to be investigated in the pathogenesis of stroke.

All experiments were performed in accordance with the Animal(Scientific Procedures)Act 1986.

57.01

CONTINUOUS ADMINISTRATION OF PYRIDOSTIGMINE IN GUINEA-PIGS SENSITISES FAST MUSCLE BUT NOT BRAIN ACETYLCHOLINESTERASE TO SUBSEQUENT ACUTE TREATMENT WITH PYRIDOSTIGMINE

MC Lintern, L Cooke, J Wetherell* C Taylor* and ME Smith
*Department of Physiology, Medical School, University of Birmingham, Birmingham B15 2TT, UK and * Dstl Chemical and Biological Sciences, Porton Down, Salisbury, UK.*

Pyridostigmine is used as a pretreatment for nerve agent poisoning. In previous studies 6 days continuous administration of pyridostigmine to guinea-pigs caused delayed changes in acetylcholinesterase (AChE) activity in skeletal muscles and brain, which were manifest after the inhibitory effect of the drug had worn off. Here we investigated whether this treatment affected the response of the tissues to a subsequent 'booster' dose of the drug. Pyridostigmine (5.1µg/h) or saline (0.9%, controls) was administered continuously for 6 days. A single 'booster' injection (20µg/kg) was given 14 days later when the AChE activity was previously shown to be normal. The booster injection produced a red cell AChE inhibition of ~30%. The animals were killed 24h later. The diaphragm, extensor digitorum (EDL) muscles, and cerebellum and striatum were dissected and the molecular forms of AChE were separated. The continuous pyridostigmine administration resulted in a significant reduction in activity in all major molecular forms in the EDL muscle compared to the saline-treated controls but had no effect on the other tissues. These decreases were probably due to downregulation of the enzyme protein indicating that the pretreatment sensitised EDL to subsequent treatment with pyridostigmine.

Supported by Dstl, Porton Down. British Crown Copyright 2003.Animals (Scientific Procedures) Act 1986.

57.02

SENSITISATION OF MUSCLE AND BRAIN ACETYLCHOLINESTERASE ACTIVITY TO PYRIDOSTIGMINE FOLLOWING SURGICAL PROCEDURES WITH HALOTHANE ANAESTHESIA IN GUINEA PIGS

MC Lintern, L Cooke, JR Wetherell* C Taylor* and ME Smith
*Department of Physiology, Medical School, University of Birmingham, Birmingham B15 2TT, UK and * Dstl Chemical and Biological Sciences, Porton Down, Salisbury, UK.*

Implantation of mino-osmotic pumps under halothane anaesthesia in guinea-pigs causes changes in acetylcholinesterase (AChE) activity in skeletal muscles and brain . Treatment with pyridostigmine also caused changes which were manifest when the inhibitory action of the drug had worn off. Here we investigated whether saline-pump implantation and anaesthesia affects the response of muscles and brain to pyridostigmine treatment. Mini-osmotic pumps which released saline (0.9%) were implanted under halothane anaesthesia and removed after 6 days. After 14 days a single injection of pyridostigmine (20µg/kg) was given. Control animals were given the drug injection alone. After 24h the major molecular forms of AChE were determined in extensor digitorum longus (EDL) and diaphragm, and cerebellum and striatum. The animals, which had been implanted with saline pumps exhibited a significantly higher activity of all three major forms of AChE in EDL (but not diaphragm) than control animals. They also exhibited a higher G4 activity in cerebellum and a lower G1 activity in striatum. Thus acute administration of pyridostigmine following anaesthesia and the surgical procedures caused more marked changes in muscle and brain AChE than pyridostigmine alone.

Supported by Dstl, Porton Down C British Crown Copyright 2002. Animals (Scientific procedures)Act 1986.

57.03

POLYCHLORINATED BIPHENYL-INDUCED DEPLETION OF INTRACELLULAR Ca^{2+} STORES AND CATECHOLAMINERGIC CELL DEATH: VIEWPOINTS OF PROTECTION

In-Sook Park, Ju-Hee Kang, Hwa-Kyung Lim, Woo-Yong Oh, So-Young Wang, Ki Hwan Choi, Joo-Il Kim, Dong Sup Kim
Department of Pharmacology, NITR, KFDA, Seoul, Korea.

The studies were designed to evaluate effects of A1254 on receptor-mediated depletion of endoplasmic reticulum-stored calcium, on the antioxidant enzyme and the subsequent cell death in catecholaminergic cells, and effects of A1254 on striatal dopamine content in SD rat. The exposure to the A1254(10mg/ml) decreased in cell viability and produced intracellular free calcium elevation in the presence or absence of extracellular calcium. From the experiment using Ca2+-ATPase inhibitor, the A1254-induced [Ca2+]i elevation was likely to result from the release of ER-stored calcium. Treatment with IP3 receptor antagonist dramatically inhibited the A1254-induced [Ca2+]i elevation. IP3 receptor blockers gave substantial protection against A1254-induced cell death, but not calcium chelators or NMDA blockers. Also, the treatment of A1254 significantly increased in glutathione reductase activity over 2 folds. Interestingly, micromolar concentrations of vitamine E not only protected the cells against A1254 but also inhibited the A1254-induced dopamine depletion. Consistent with the cell culture experiment, the intrastriatal administration of A1254 using osmotic pump significantly reduced the striatal dopamine content. We suggest that A1254-induced depletion of intracellular calcium storage is an important cause of subsequent cell death, and which is protected by IP3 receptor blockers.

57.04

MDMA INDUCES APOPTOSIS VIA A MITOCHONDRIAL CELL DEATH PATHWAY IN HUMAN DOPAMINERGIC CELLS AND SEROTONERGIC CELLS

Soo Kyung Suh, Kyu Bong Kim, Bo Kyung Lee, Chang Won Park, Jong Won Kim, Kwang Jin Kim, Jae Hee Kim, Chan Woong Park1, Joo Il Kim and Sun Hee Lee
Pharmacology Department, National Institute of Toxicological Research, Korea Food & Drug Administration, 5-Nokbun-dong, Eunpyung-gu, Seoul 122-704, Korea; 1College of Medicine, Seoul National University, Seoul, Korea.

3,4-Methylenedioxymethamphetamine(MDMA) is a psychostimulant and has become a popular recreational drug of abuse in many countries. Although the neurotoxic damage caused by MDMA is characterized by degeneration of the dopaminergic and serotonergic systems in brain, the molecular and celluar mechanisms remain to be clarified. The purpose of this study was to find out whether MDMA-induced neurotoxicity in human serotonergic JAR cells and dopaminergic SK-N-SH cells is related to apoptosis. In the results, MDMA caused dose-dependent loss of cellular viability in both cell types. Using Giemsa staining, TUNEL assay, and DNA fragmentation, we show that MDMA exposure can cause DNA strand breaks, chromatin condensation, nuclear fragmentation and DNA laddering. MDMA-induced apoptosis correlated with decreased level of bcl-2, increased level of BAX and activation of caspase-3, 9. These data suggest that a mitochondrial cell death pathway may play a role in MDMA neurotoxicity.

57.05

CLOSTRIDIUM PERFRINGENS EPSILON TOXIN IS NOT DIRECTLY NEUROTOXIC *IN VITRO*

A. C. Green and R. D. Heal1
Dept of Biomedical Sciences, Dstl, Porton Down, Salisbury Wiltshire SP4 0JQ. 1Sensors and Electronics, QinetiQ, Winfrith Technology Centre, Dorset.

Epsilon toxin, a potent and rapidly lethal bacterial toxin, has been suggested to have direct neurotoxic effects. Here we have used cultured rat cortical neurones to investigate both the neurotoxicity of epsilon toxin and its effect on network electrical activity in neurones grown on multielectrode arrays (MEAs). In cultures treated with epsilon toxin (0.1-30 µg/ml, 24 h), no overt neurotoxicity was observed using phase contrast microscopy. However, viability staining with fluorescein diacetate and propidium iodide revealed some cell death at the highest concentrations of toxin (10 and 30 µg/ml) that was not apparent by phase contrast observation. This appeared to be due to a loss of the background non-neuronal cells and of normal glial cell morphology. To further assess the effects of epsilon toxin, MEA recordings were made. No effect of epsilon toxin on network electrical activity was observed at concentrations up 10 µg/ml. The activity of epsilon toxin was confirmed using MDCK cells. Major morphological changes were seen after ~1-2 h at concentrations >2 µg/ml and the LC50 for MDCK cell death at 24 h was 1-2 µg/ml. These results suggest that epsilon toxin is not directly neurotoxic in vitro and that in vivo neurotoxicity may be a secondary consequence of endothelial and/or glial cell damage.

This work was carried out as part of the MoD Corporate Research Programme.

57.06

CHARACTERISATION OF NON-ACETYLCHOLINESTERASE ACTIONS OF ORGANOPHOSPHATES BY IDENTIFICATION OF NOVEL PROTEIN TARGETS OF ADDUCTION

A Murray, AJ Rathbone & DE Ray
MRC Applied Neuroscience Group, School of Biomedical Sciences, University of Nottingham Medical School, Queen`s Medical Centre, Nottingham, NG7 2UH, UK.

There are some indications that low-level exposure to some organophosphates (OPs) might lead to human health problems. We are using proteomic techniques to identify novel protein targets in the nervous and immune systems that are more sensitive to OP adduction than is AChE. Brain proteins are allowed to react with azamethiphos, diazoxon, chlorfenvinphos, chlorpyrifos oxon or maloxon in vitro or primiphos methyl in vivo at levels below those producing conventional toxicity. Any remaining covalent binding sites are then labelled in vitro with an excess of tritiated diisopropylflurophosphate. The proteins are then separated by gel electrophoresis and target proteins are visualised using a novel high sensitivity digital autoradiography system based on micro channel plate technology. Potential target proteins appear as tritiated spots in controls that are absent or reduced in organophosphate treated tissues. Initial studies on proteins significantly adducted at 30% AChE inhibition have identified acylpeptide hydrolase (1) as a sensitive target for chlorpyrifos and dichlorvos. Other proteins of around 27-30 KDa (as yet unidentified) are significant targets for chlorfenvinphos, diazoxon, dichlorvos, maloxon and paroxon. We are in the process of mass spectrometric sequencing of these proteins.

(1) Richards, P.G., Johnson, M.K. & Ray, D.E. (2000) Mol. pharmacol. 58:577-583.

57.07

ENERGY FAILURE OR EXCITOTOXICITY LEAD TO DISTINCT AXONAL PATHOLOGIES IN THE MOUSE

DJ Cuthill, *J McCulloch, JH Fowler, D Dewar
*Division of Clinical Neuroscience, Univeristy of Glasgow, Scotland; *University of Edinburgh, Division of Neuroscience, Edinburgh, Scotland.*

Excitotoxicity and energy failure are implicated in acute brain damage and chronic neurodegeneration. This study determined whether axonal damage was induced by intrastriatal injection of malonate (a succinate dehydrogenase inhibitor) or AMPA (a glutmate receptor antagonist). Anaesthetised adult mice received malonate, S-AMPA or vehicle, stereotactically injected into the caudate nucleus. Sections were immunostained for markers of disrupted axonal transport (amyloid precursor protein; APP) or disrupted axonal structure (neurofilament 200; NF200).
Malonate caused a dose-dependent increase in axonal damage, revealed by both NF200 and APP immunostaining. AMPA also caused a dose-dependent increase in axonal damage detected by NF200 immunostaining only; APP immunostaining was minimal at every AMPA dose. Western blotting analysis revealed significant reductions of both NF200 and kinesin levels in the caudate nucleus following the highest dose of either malonate or AMPA.
The study demonstrates that either metabolic insults elicit axonal damage in the mouse brain. The difference in APP immunostaining patterns could suggest differences in the severity of insults or a diversity of underlying mechanisms of axonal damage.

57.08

THE NEUROTOXIC POTENCY OF NOVEL ALUMINIUM COMPOUNDS

A.J. Drysdale1, E.v.L. Roloff1, T. Salifoglou2 & B. Platt1
1 Biomedical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK. 2 Chemistry Dept., University of Crete, PO Box 1470, 71409, Heraklion, Greece.

Aluminium (Al) is a neurotoxic element that has been controversially hypothesised to be involved in Alzheimer's disease (AD). Contradictory experimental results may have been brought about by the complex chemistry of Al in solution and in biological systems. Hence, the present study aimed to compare the neurotoxicity of three different Al salts.Primary hippocampal cultures were exposed to two novel organic Al citrate salts that are stable in physiological solutions (S1: $AlCit_2$ & S2: Al_3Cit_3) at 10, 100 and 500 μM for 3 hrs and compared to an inorganic Al compound (Al standard solution, in 1% HCl). Cell viability (evaluated with propidium iodide stain and confocal/fluorescent microscopy) was quantified for both neurones and glia.
All three compounds induced dose-dependent cell death, but showed significant differences in their profile. S2 had the steepest concentration-response curve, i.e. 10 μM had the least and 500 μM the strongest neurotoxic effect (survival: 89 and 24% of controls, respectively). Glia were generally less affected and showed higher resistance to S2 but not to S1 toxicity. Inorganic Al was less toxic at 500 μM and caused similar death rates in neurones and glia (~50%).
Our results prove the impact of the Al species in toxicity assessments, i.e. organic Al compounds appear particularly potent with neurones being more susceptible than glia.

57.09

AMPA-INDUCED AXONAL DAMAGE IS REDUCED IN PROTEOLIPID PROTEIN DEFICIENT MICE

J.H. Fowler (1), D. Dewar (1), J. McCulloch (2), M.McLaughlin (3), I.R. Griffiths (3), J.Y. Garbern (4), J.M. Edgar (3).
(1) Division of Clinical Neuroscience, (3) Applied Neurobiology Group, University of Glasgow, UK(2) Division of Neuroscience, University of Edinburgh, UK(4) Department of Neurology and Center for Molecular Medicine and Genetics, University School of Medicine, Detroit, USA.

AMPA receptors are present on oligodendrocytes and the myelin sheath, however there is no evidence that they are present on axons. We have recently shown that intracerebral injection of AMPA causes axonal damage in vivo. Proteolipid protein (PLP), the most abundant CNS myelin protein, is important for normal glia-axonal interactions. To examine the role of glia-axonal interactions in AMPA-induced axonal damage, we injected AMPA (1.5nmol) into the caudate of anaesthetised PLP deficient and wild type mice (n=13). Twenty-four hours later, mice were perfuse fixed, then axonal damage was detected using neurofilament 200 (NF 200) immunohistochemistry, and neuronal damage detected with histological techniques. In addition, we determined if PLP deficient mice contained similar levels of the AMPA receptor subunits GluR1-4 compared with wild type mice using western blotting. AMPA-induced axonal damage was significantly reduced in PLP deficient mice compared to wild-type mice (P=0.015). Neuronal damage was also reduced in PLP knockout mice compared to wild-type (P=0.06). There was no significant difference in the levels of GluR1-4 in PLP knockout compared to wild-type littermates. The present study suggests that excitotoxic events at the oligodendrocyte and myelin sheath may contribute to AMPA mediated axonal damage via PLP-mediated interactions.

58.01

ALTERED GABA-A RECEPTOR REGULATION AND FUNCTION IN THE ENTORHINAL CORTEX IN A CHRONIC MODEL OF EPILEPSY

Bailey SJ, Woodhall GL and Jones RSG
Departments of Biochemistry and Physiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD.

A shift in balance between excitatory and inhibitory influences is thought to contribute to the generation of seizures. Here we report an apparently paradoxical increase in synaptic inhibition associated with an epileptic phenotype. We have examined spontaneous inhibitory synaptic transmission and the expression of GABAA receptor subunits in the entorhinal cortex (EC) in a chronic model of epilepsy in the rat. The EC has been implicated in the pathogenesis of temporal lobe epilepsy.
In a low-dose Li+-pilocarpine model of epilepsy, acute epileptogenesis is followed 6-8 weeks later by the development of spontaneous recurrent seizures. At this time we prepared EC slices for whole-cell patch clamp recording of miniature inhibitory postsynaptic currents (mIPSCs), or micro-dissected the EC for RNA and protein isolation. The frequency of mIPSCs was increased (211%) in the superficial layers of the EC, in epileptic animals compared to controls, reflecting an increase in GABA release. Concurrently, the decay time of mIPSCs was reduced. Real-time RT-PCR and semi-quantitative Western blotting showed a significant decrease (40-60%) in the expression of mRNA and protein for GABAA receptor alpha1, alpha2, beta2 and gamma2 subunits in superficial EC neurons.

58.02

LIMBIC ENCEPHALITIS WITH AUTOANTIBODIES TO POTASSIUM CHANNELS: EVIDENCE FOR AN ANTIBODY-MEDIATED CNS CONDITION

Angela Vincent, Jessica Keal, Linda Clover, Robert Deacon
Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Department of Experimental Psychology, University of Oxford.

Antibodies are not thought to get through the blood brain barrier, yet there is growing circumstantial evidence that CNS diseases can be antibody-mediatd. Limbic encephalitis (LE) includes memory loss, confusion, hallucinations and seizures. We detected antibodies to voltage-gated potassium channels (VGKC) in a proportion of LE patients. The antibodies immunoprecipitate rabbit brain VGKCs labelled with 125I-dendrotoxin, and bind to the hippocampus in frozen sections of rat brain, with a distribution similar to VGKC Kv1.1 and 1.2. Many of these LE patients respond to immunotherapies with a fall in VGKC antibodies, suggesting a causal relationship between the antibodies and the CNS symptoms. Purified IgG from one VGKC antibody positive patient was injected intracerebroventricularly into adult mice. Subsequently, the treated mice showed an increased latency to rear, both in an open field and a holeboard, with an accompanying decrease in the number of rears (all P values <.02). Head dipping in the holeboard was not affected, nor was performance in a range of motor ability tests, indicating a highly selective effect. Since reduced rearing is found with hippocampal lesions, it is possible that the patient`s IgG antibodies acted on the hippocampus. This approach can now be used for further studies of this and other potential antibody-mediated disorders.

58.03

EXPRESSION OF CEREBELLAR GRANULE CELL-SPECIFIC GABA-A RECEPTORS IS IMPAIRED IN THE EPILEPTIC AND ATAXIC MOUSE, *TOTTERING*

S. Kaja1, A. Wilton, M. Bingley, P. Tiwari, C.L. Thompson
School of Biological and Biomedical Sciences, University of Durham, Science Research Laboratories, South Road, Durham, DH1 3LE, UK.1Present address: Leiden University Medical Centre, Department of Physiology, 2300 RC Leiden, The Netherlands.

Tottering (tg) is a mouse model of human absence epilepsy and cerebellar ataxia. A single mis-sense mutation (P601L) in the gene encoding the a1A subunit of P/Q-type voltage-gated calcium channels (VGCCs) is the genetic basis for this phenotype. This leads to a decrease of approximately 40% in the number of functional P/Q type VGCCs in the cerebellar cortex. Calcium signalling has been shown to affect GABAA receptor (GABAR) subunit expression during development. We have, therefore, investigated whether GABAR expression is impaired in the cerebellum of tg mice.By semi-quantitative autoradiography we determined a 25 ± 3% reduction in [3H] muscimol binding to the cerebellar granule cell layer of tg. By [3H] muscimol binding to cerebellar membrane preparations we determined that the KD was unaffected whilst BMAX was reduced by 40%. Total specific [3H] Ro15-4513 binding sites were reduced by 27 ± 5% relative to control. This comprised a 24 ± 6% reduction in the BZ-S subtype and 35 ± 3% reduction in the BZ-IS subtype. The KD for [3H] Ro15-4513 was unaffected. Immunohistochemical analysis suggested that GABAA receptor a6 but not a1 nor d expression was reduced in the cerebellar granule cell layer of tg. The molecular mechanisms involved are under study.

Funded by AstraZeneca UK Ltd.

58.04

NITRIC OXIDE FORMATION DURING CORTICAL SPREADING DEPRESSION MAY CONTROL MULTIPLE MECHANISMS

Minyan Wang, Jens P. Dreier*, Jutta Urenjak, Tihomir P. Obrenovitch
*School of pharmacy, University of Bradford, Bradford BD7 1DP, United Kingdom; and *Experimental Neurology, Charité Hospital, 10098 Berlin, Germany.*

Cortical spreading depression (CSD) is a transient disruption of transmembrane ionic gradients, and nitric oxide (NO) formation was previously found critical for rapid ionic recovery after CSD. Here we examine whether this effect may be associated with NO-mediated changes in regional cerebral blood flow (rCBF). All experiments complied with the Animal (Scientific Procedure) Act 1986. Microdialysis electrodes allowed to elicit and record CSD in the cortex of anaesthetized rats, and to apply the NO synthase (NOS) inhibitor, L-NAME either alone or with exogenous NO (SIN-1) or the cyclic GMP analogue, 8-pCPT-cGMP. In controls, laser Doppler flowmetry revealed a brief hypoperfusion synchronous of CSD, followed by hyperaemia and secondary hypoperfusion. L-NAME delayed the ionic recovery, reduced rCBF overall, and increased the magnitude of the brief hypoperfusion. Both SIN-1 and 8-pCPT-cGMP reversed completely the effect of NOS inhibition on CSD ionic recovery but only partially those on rCBF. In conclusion, endogenous NO formation during CSD is necessary for rapid initiation of the subsequent ionic recovery, and it also contributes to some of the rCBF changes that are associated with CSD. Whether or not these two actions are independent remains to be determined.

Work supported by the Migraine Trust (Grant No 127).

58.05

GABA-A RECEPTOR EXPRESSION IN THE GRANULE CELLS OF THE DENTATE GYRUS IS IMPAIRED IN THE EPILEPTIC MUTANT MOUSE, *STARGAZER*

P. Tiwari1, J. H. Ives1, W. Sieghart2, H. Payne1, Mark Wright1, C. L. Thompson1.
1School of Biological and Biomedical Sciences, Science Research Laboratories, University of Durham, South Road, Durham, DH1 3LE, UK.2Brain Research Institute, Division of Biochemistry and Molecular Biology, Spitalgasse 4, A-1090, Vienna, Austria.

Stargazer (stg) mice experience frequent episodes of absence-like epilepsy. A mutation in the CACNgamma2 gene results in complete ablation of its translated product, stargazin. Stargazin is normally heavily expressed in the hippocampal formation, especially the granule cells of the dentate gyrus (DG). Using a combination of receptor ligand autoradiography and immunohistochemistry we have shown that NMDA receptor expression was normal. Expression of AMPA receptor subunits GluR1, 2, 3 and 4 were also unaffected in stg. GABA-A receptor (GABAR) expression, however, was dramatically altered. [3H]Ro15-4513 binding, was up-regulated in the DG. Interestingly, it was the flunitrazepam-insensitive subtype of Ro15-4513 binding sites, most likely conferred by alpha4/beta/gamma2 GABARs, that was up-regulated. These GABARs are not normally a major component of the GABAR population expressed in the DG. Dentate granule cells are normally rich in alpha4/beta/delta-containing GABARs, which are Ro15-413-insensitive. Absence of the stargazin protein prompts a switch from alpha4/beta/delta to alpha4/beta/gamma2 receptors which we predict would result in dentate granules enriched in synaptic receptors at the expense of extrasynaptic, tonic inhibition-conferring GABARs. The molecular mechanisms involved are under study. These affects possibly contribute to, or are a consequence of, seizure activity.

58.06

HIPPOCAMPAL N-ACETYLASPARTATE AND N-ACETYLASPARTYLGLUTAMATE IN SCHIZOPHRENIA AND AFFECTIVE DISORDERS

Lindsay M Reynolds and Gavin P Reynolds
Department of Biomedical Science, The University of Sheffield, Sheffield, S10 2TN.

There is increasing evidence both from magnetic resonance spectroscopy and post mortem studies that brain N-acetylaspartate (NAA) concentrations are decreased in schizophrenia. While NAA is a marker for neuronal integrity, there are indications that depletion of this amino acid may also reflect reversible neuronal dysfunction. The precursor of NAA is the dipeptide N-acetylaspartylglutamate (NAAG), which is localized to discrete neurons throughout the nervous system and is thought to be involved in glutamatergic transmission. In this study, NAA and NAAG were analysed by HPLC in frozen sections of hippocampus and parahippocampal gyrus taken post mortem from patients with schizophrenia, bipolar illness and depression and matched control subjects. Deficits in NAA were found in the hippocampus, significant in bipolar illness ($p<0.05$) and particularly in schizophrenia ($p<0.01$). In the parahippocampal gyrus NAA was significantly reduced only in the schizophrenic group ($p<0.05$). Hippocampal NAAG concentrations were decreased, but not significantly, in the schizophrenia and depression groups. This work presents a novel technique for the analysis of NAA and NAAG in post mortem tissue and provides further indications of neuronal dysfunction in the medial temporal lobe in schizophrenia that is also apparent in bipolar disorder.

58.07

A STUDY OF THE SUBCELLULAR LOCALIZATION AND TARGETING OF DISRUPTED IN SCHIZOPHRENIA 1 (DISC1)

N.J.Brandon, I.L.Schurov, L.M.Camargo, E.Handford, M.S.Shearman and P.J.Whiting.
Merck Sharp & Dohme, The Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, UK.

Disrupted In Schizophrenia 1 (DISC1) was originally discovered as the putative gene disrupted on chromosome 1 by a balanced translocation t(1;11)(q42.1;q14.3) shown to co-segregate with major psychiatric illness in a Scottish pedigree. Recently a study has linked genetic markers within the DISC1 gene to schizophrenia in a Finnish cohort suggesting that DISC1 may be a more general risk-factor for schizophrenia. Our aim is to understand the function of the DISC1 protein to provide potentially novel insights into the disease mechanism.
We analyzed the DISC1 protein sequence by extensive bioinformatic methods and have shown that DISC1 contains a number of classical protein motifs/domains including 5 putative coiled coils. We have used our results to design a number of DISC1-GFP fusion chimeras for overexpression studies. We also raised several specific antibodies for studying expression of endogenous DISC1 in recombinant and neuronal cell lines as well as in primary neurons. We established a strong biochemical association between DISC1 and NUDEL, a protein that is thought to play an important role in neuronal migration and nucleokinesis, and determined the molecular details of this interaction. We hope that such knowledge of the basic cell biology of DISC1 will contribute to our understanding of it's role in the etiology of the disease.

59.01

POTENTIATION OF SOLUBLE APP PRODUCTION IN THE RAT RETINAL-VITREAL MODEL *IN VIVO* BY METABOTROPIC GLUTAMATE RECEPTOR STIMULATION

H. Patel, D.T. Walsh, J. A. Moncaster, S. M. Gentleman, L-S. Jen and M. J. Croucher
Department of Neuroinflammation, Imperial College London, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK.

Amyloid precursor protein (APP) is abnormally processed in Alzheimer's disease (AD) to produce the neurotoxic b-amyloid peptide (Ab), a core component of the senile plaques which are a hallmark of this disorder. The present study investigated the actions of the broad-spectrum mGluR agonist (1S,3R)-ACPD on APP processing in vivo, utilising the rat retinal-vitreal model. Sprague-Dawley rats were intravitreally injected under Hypnorm/Hypnovel anaesthesia with the agonist or with phosphate-buffered saline. Western blot analysis revealed a marked increase in APP processing with a clear shift towards the production of neuroprotective, soluble APP fragments (APPs) of lower molecular weight than the holoprotein. These changes were both concentration (10-1000uM) and time (2-24hr) dependent and were observed principally in the non-cellular vitreous compartment. The responses were abrogated by the selective, competitive mGluR antagonist, (S)-MCPG, indicating mGluR mediation. Immunohistochemical studies confirmed the morphological and biochemical integrity of the retinal neurones following mGluR stimulation. We conclude that mGluR activation modulates APP processing towards the formation of APPs in vivo, suggesting that mGluRs may provide future novel therapeutic targets for the treatment of AD.

We thank the Hammersmith Hosptials Trust Research Committee for their financial support.

59.02

MODULATION OF CALCIUM SIGNALLING BY CHRONIC HYPOXIA AND AMYLOID-BETA PEPTIDE IN HUMAN ASTROCYTOMA D384 CELLS

Z. Downes, C. Peers & E. Carpenter *
ZD - Pfizer Limited, Sandwich, Kent, CT13 9NJ, UK.CP - Academic Unit of Cardiovascular Medicine, University of Leeds, Leeds, LS2 9JT, UK.EC - School of Life Sciences, University of Bradford, Bradford, BD7 1DP, UK (presenting author).

Periods of prolonged hypoxia are deleterious to higher brain functions and increase the likelihood of developing dementias. Furthermore, the accumulation of fibrillar deposits consisting of amyloid-beta peptides is associated with dementia. Here, the effects of chronic hypoxia (CH, 2.5 percent oxygen for 24h) and 1 microM amyloid-beta peptide (ABP[1-40], for 24h) on intracellular calcium signalling in D384 human astrocytoma cells were investigated using fura-2 microfluorimetry. CH, but not ABP[1-40], caused significant elevation of basal calcium levels. Neither CH nor ABP[1-40] could modulate bradykinin (BK)-evoked rises in intracellular calcium, nor BK-mediated capacitative calcium entry (CCE). However, ABP[1-40] significantly augmented the CCE seen when effecting store depletion using 1 microM thapsigargin, which was not mimicked by ABP analogues nor CH. In fact, ABP analogues and CH caused a reduction in CCE. Inhibition of mitochondrial function using FCCP (10 microM) and oligomycin (2.5 microg/ml) significantly reduced the CCE responses seen in ABP[1-40] treated and control cells, but not in cells exposed to CH. Thus, CH and ABP[1-40] exert distinct effects on the calcium signalling in D384 human astrocytoma cells. The augmentation of CCE by ABP[1-40] is dependent upon functioning mitochondria and may be an important step in amyloid-beta peptide-mediated neurotoxicity.

59.03

THE CIRCADIAN RHYTHM AND SLEEP-WAKE PROFILE OF THE AMYLOID PRECURSOR PROTEIN (APP) OVEREXPRESSING TAS10 MOUSE

Smith M.I., Ahmed S., Read S., & Upton N.
Neurology & Gastroenterology Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW.

Alzheimer`s Disease (AD) is characterised by progressive memory loss with cognitive impairment. Patients also display a variety of other behavioural changes such as alterations in circadian rhythm, sleep-wake and electroencephalogram. In the AD brain abnormal APP processing leads to deposition of insoluble amyloid-beta. Recently biochemical and cognitive changes arising from amyloid-beta deposition have been studied in APP overexpressing transgenic mice. Transgenic mice with the Swedish double mutation, overexpressing human APP (Transgenic Amyloid Swedish, Line 10 (TAS10)), display age-related amyloid-beta deposition and learning deficits. Here we investigate the circadian rhythm and sleep-wake profile of TAS10 mice. Male wildtype and TAS10 mice (aged 22-24mths) were housed singly with free access to a running wheel for circadian rhythm, or prepared for frontal-occipital cortex EEG for sleep-wake, analysis. No alteration was observed in the TAS10 behavioural circadian rhythm compared to wildtype mice. TAS10 mice exhibited a significant redistribution of sleep stages with increased wake (45.1%), reduced slow wave sleep (21.5%) across the 24hr light-dark period and increased paradoxical sleep (42.8%) over the last 4hrs of the light period. From this study it appears that amyloid deposition in mice induces some additional behavioural alteration in addition to cognitive decline.

59.04

A MAGNETIC RESONANCE INVESTIGATION OF SCRAPIE PATHOGENESIS

J Barr1, Y-L Chung2,3, A Williams4, S Rebus4, K K Changani1, E L Thomas2,
1Institute for Animal Health, Edinburgh,BBSRC Neuropathogenesis Unit,2MR Unit, MRC CSC, Imperial College Faculty of Medicine, London, 3CR UK MR Research Group, St George's Hospital Medical School, London,4Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow.

Transmissable spongiform enchalopathies (TSE) are a group of fatal neurodegenerative diseases. Their neurohistopathological features include neuronal loss, gliosis, vacuolation and abnormal prion protein (PrPsc) accumulation.
We studied the incubation period of a scrapie model (ME7 via intercerebal inoculation) by magnetic resonance (MR) imaging and spectroscopy. We have determined the: a) earliest time when MR changes could be observed, b) time and extent of BBB disruption, and c) relationship between severity of scrapie pathology (by histology and western blots) and detectable MR changes.
Hyper-intense MR changes correlated with onset of detectable gliosis (from 90 d.p.i.). Vacuolation (from 120 d.p.i.) and gliosis were found to give contrasting MR image signals that can combine to give apparently normal MR images. The effect of PrPsc deposition on MR image intensity remains unclear. N-acetyl-aspartate (NAA) appeared to show regional and temporal specificity. Changes in NAA levels were preceded by PrPSC deposition. Earliest time of detectable BBB disruption occurred just before the onset of clinical signals. Areas of BBB disruption were those with the most intense gliosis and PrPsc deposition. Results suggest that MR can be used to detect anatomical and biochemical changes in the TSE-infected brain well before clinical symptoms become apparent (150 d.p.i.)

59.05

NOVEL LIGANDS (R,R)-I-QNB AND (R,S)-I-QNB BINDING TO HUMAN MUSCARINIC RECEPTORS M1, M2 AND M4

Margaret Piggott, Sally Pimlott, Jonathan Owens, George Wake, Elaine Perry
MRC Building,Joint MRC/University of Newcastle Centre Development for Clinical Brain Ageing,Newcastle General Hospital, Westgate Road, Newcastle NE4 6BE.

Derivatives of the muscarinic antagonist 3-quinuclidinyl-4-iodobenzilate (QNB), [123I]-(R,R)-I-QNB and [123I]-(R,S)-I-QNB, are currently being assessed as in vivo ligands to monitor muscarinic receptors in Alzheimer's disease (AD) and dementia with Lewy bodies (DLB), relating changes to disease symptoms and to treatment response with cholinergic medication. Autoradiographic distribution studies of [125I]-(R,R) and (R,S)-QNB in normal human brain showed a pattern consistent with binding to M1 and M4 subtypes.
Binding of [125I]-(R,R)-I-QNB and [125I]-(R,S)-I-QNB to cloned human muscarinic receptor subtypes M1, M2 and M4 showed both ligands to have high affinity for all three receptors. [125I]-(R,S)-I-QNB had marginally higher affinity to the M4 receptor (Kd = 0.2-0.3nM), compared to the M1 and M2 receptor (0.3-1.6 nM). The [125I]-(R,R)-I-QNB derivative similarly had highest affinity for M4 (0.2-1.0nM), with equivalent affinities for M1 and M2 (0.4-1.3nM). Non-specific binding was defined with atropine and was very low in all cases except (R,R)-I-QNB binding to M4 which had nsb 50%.
Autoradiographically (R,R)-I-QNB also showed high nsb which was not apparent in vivo SPECT imaging. In AD and DLB in vitro autoradiography (R,R)-I-QNB binding was raised in insula and cingulate cortex and correlated positively with increased Lewy body pathology.

59.06

DOPAMINERGIC AND CHOLINERGIC PARAMETERS IN PROGRESSIVE SUPRANUCLEAR PALSY, DEMENTIA WITH LEWY BODIES, PARKINSON'S AND ALZHEIMER'S DISEASE

Elizabeth Greally, Michelle Lake, Margaret Piggott, David Burn.
MRC/University of Newcastle Joint Centre Development for Clinical Brain Ageing,Newcastle General Hospital, Westgate Road, Newcastle NE4 6BE.

Progressive supranuclear palsy (PSP), Parkinson's disease (PD), dementia with Lewy bodies (DLB) and Alzheimer's disease (AD) are neurodegenerative diseases characterised to different extents by movement disorder and cognitive decline. Dopaminergic and cholinergic changes probably underlie these symptoms but while PD responds to dopamine replacement, PSP patients gain little benefit, and the response of DLB and AD to levodopa is not known. Treatment with cholinesterase inhibitors is efficacious in DLB and AD, and PD with dementia, but the response in PSP is generally negative with worsening of motor function. Novel therapeutic approaches are needed PSP.
We have compared dopaminergic loss by [125I]PE2I autoradiography to dopamine reuptake sites in basal ganglia in these patient groups. Changes in nicotinic cholinergic receptors (alpha4beta2 type), muscarinic M1 and dopamine D2 receptors have also been investigated by autoradiography using [125I]5IA85380, [3H]pirenzepine and [125I]epidepride respectively.
Extensive reductions in dopamine reuptake sites have been found in PSP, PD and DLB but not in AD, while nicotinic receptors are reduced in striatum in all groups, especially PSP and PD. Unexpectedly striatal M1 and D2 receptors showed little change in PSP but were reduced in DLB. These results provide a plausible explanation for observed therapeutic responses.

59.07

INTERSPECIES COMPARISON OF SOLUBLE Aβ1-40 LEVELS EXTRACTED FROM MAMMALIAN BRAIN TISSUE.

R. Marwood, T. S. Samji, T.W. Rosahl, J. Peachey, J. Atack and J.D. Best.
Merck, Sharp and Dohme, Harlow, Essex.

Alzheimers's disease (AD) is an illness characterised by severe impairment of memory and cognitive function. A distinctive cortical pathological feature of AD is the presence of extracellular neuritic plaques whose cores comprise beta-amyloid (A[beta]), various forms of which are yielded from amyloid precursor protein (APP) by the proteolytic action of secretases. We developed an immunoassay method to measure soluble levels of one particular form, A[beta]1-40, extracted from rodent brain tissue homogenised in diethylamine (DEA) buffer. Using this protocol we assessed A[beta]1-40 levels in the brains of Tg2576 mice expressing the Swedish mutation of APP, APP-YAC mice expressing the human APP sequence in a yeast artificial chromosome and non-transgenic C57BL/6J mice, determining approximate levels of 15, 1.5 and 0.5 nM in these strains respectively. Soluble A[beta]1-40 levels were also measured in rats and guinea-pigs, with both species yielding levels of approximately 0.4 nM. Regional analyses showed a widespread and relatively homogeneous distribution of A[beta]1-40 in all three mouse strains evaluated. Studies will be conducted to determine whether A[beta]1-40 levels in Tg2576, APP-YAC and non-transgenic mice are proportional to the expression of APP, or rather whether there are regional differences in [gamma]-secretase activity.

59.08

EXCLUSIVELY TARGETING BACE TO LIPID RAFT DOMAINS BY ADDITION OF A GPI ANCHOR SUBSTANTIALLY UPREGULATES BETA-SITE APP PROCESSING

Jo M. Cordy, Ishrut Hussain#, Colin Dingwall#, Nigel M. Hooper & Anthony J. Turner
School of Biochemistry & Molecular Biology, University of Leeds, Leeds, LS2 9JT, U.K.# Neurology CEDD, GlaxoSmithKline, Harlow, Essex, CM19 5AW, UK.

Beta-secretase (BACE, Asp2) is a transmembrane aspartic protease responsible for cleaving the amyloid precursor protein (APP) to generate soluble, secreted APPbeta and the C-terminal fragment CTFbeta. CTFbeta is subsequently cleaved by gamma-secretase to produce the neurotoxic Abeta peptide that accumulates in Alzheimer`s disease. BACE has been shown to be present in lipid rafts where amyloidogenic APP processing may preferentially occur. However, processing within other membrane domains cannot be excluded. Here, we confirm that a fraction of wildtype BACE is targeted to rafts prepared using Lubrol as detergent, whereas a much smaller fraction is present in Triton-isolated rafts. In order to investigate further the significance of lipid rafts in APP processing, a GPI anchor has been added to BACE, replacing the transmembrane and C-terminal domains. This targets the enzyme exclusively to lipid raft domains, as shown by standard raft isolation methods using either Triton or Lubrol. Expression of GPI-BACE in an SHSY5Y cell line substantially upregulates both the secretion of sAPPbeta into the medium and the production of Abeta, implying that processing of APP to the Abeta peptide occurs predominantly in lipid rafts and that BACE is the rate-limiting enzyme in this process.

59.09

ACAT INHIBITORS AND THEIR EFFECT ON REDUCING A-BETA IN STABLY TRANSFECTED CELL LINES

S. Ellis, S. J. Pollack
Merck Sharp & Dohme, Harlow, Essex, UK.

Epidemiological studies have suggested a link between high levels of plasma cholesterol and Alzheimer`s disease (1). Research suggests that elevated plasma cholesterol is responsible for an increased synthesis and deposition of the A-beta peptide (2). It is conversely reported that cholesterol-lowering treatments decrease levels of A-beta (3). Early work in this field focused on the cholesterol binding properties of statins, however, a recent report suggests that inhibitors of the enzyme Acyl-coenzyme A: cholesterol acyltransferase (ACAT) may also be a novel target for the treatment of Alzheimer`s disease (4). The role of ACAT in cholesterol metabolism is to catalyse the formation of cholesterol-esters (CE) from free-cholesterol (FC). It is proposed that ACATs ability to tightly control this equilibrium between FC and CE is responsible for its role in modulating levels of A-beta. In particular an increase in CE is sufficient to up-regulate the generation of A-beta. The mechanism of action is not clear, but is thought to be related to the subcellular localisation of cholesterol and the affect of this on the processing of the amyloid precursor protein (5). We have examined the effects of several of ACAT inhibitors and report their effects on a number of stably transfected cell lines.

59.10

EXPRESSION AND SECRETION OF APP SUB-TYPES FROM Ntera2/D1 CLONAL CELLS INDUCED TO DIFFERENTIATE TO THE NEURONAL PHENOTYPE BY TREATMENT WITH RETINOIC ACID

S.G.Boyce and D.Parkinson
Biomedical Research Centre, Sheffield Hallam University, Sheffield S1 1WB.

Human neurons are difficult to culture yet would be important to study neurochemistry in diseases such as Alzheimer's Disease (AD). The Ntera2/D1 cell line has potential because treatment with retinoic acid (RA) induces NTera2 cells to differentiate to a neuronal phenotype. Here we report on APP subtype expression and processing to assess their utility for investigation of the role a neuronal phenotype on the molecular events that underly AD pathogenesis.NTera2 cells were cultured with RA up to 35 days and expression of APP subtypes was studied by immunoblotting with APP antibodies that resolve APP695, APP751 and APP770. Untreated NTera2/D1 cells expressed predominantly APP751/770. RA treatment led to a rapid increase of APP695 after eight days followed by sustained production, suggesting these cells commit to neuronal differentiation at an early stage. APP770 and 751 also showed a dramatic increase which was sustained over the time-course, probably originating from the non-neuronal cells. Expression of neuronal markers was also investigated to determine the correlation with APP expression. Purified neuronal cultures expressed predominantly APP695. Studies are in progress to determine the effects of RA on expression of alpha- and beta-secretase in NT2/D1 cells to assess the utility of these cells for investigation of amyloid peptide production in a neuronal phenotype.

59.11

ALZHEIMER'S LINKED UBB+1 CAUSES A HEAT-SHOCK RESPONSE

Andrew D. Hope*, Rohan de Silva*, Rina Bandopadhyay*, David F. Fischerº, Elly M. Holº, Fred W. van Leeuwenº, Andrew J. Lees*
**Reta Lila Institute of Neurological Studies, University College London, 46 Cleveland Street, London W1T 4JF. ºNetherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.*

Tau positive inclusions in Alzheimer's disease and progressive supranuclear palsy contain a variant ubiquitin, UBB+1, the result of mRNA slippage of the UBB gene. UBB+1 is known to inhibit the 26S proteasome. Proteasome inhibition has previously been shown to induce expression of molecular chaperones. We developed a stable, Tet-On expression model of UBB+1 in human neuroblastoma cells. UBB+1 induction caused proteasome inhibition as was confirmed by reduced ability to process misfolded canavanyl proteins, and accumulation of GFPu, a 26S proteasome substrate. We show that expression of UBB+1 induces expression of heat-shock proteins, priming the chaperone system. These cells were subsequently resistant to tert-butyl hydroperoxide, an oxidative stressor. We conclude that although UBB+1 expressing cells have a compromised ubiquitin-proteasome system, they are protected against oxidative stress conditions.

59.12

CHANGES IN KINASE ACTIVITY ASSOCIATED WITH CELL SURVIVAL CORRELATE WITH COGNITIVE DECLINE IN AN ALZHEIMER'S MOUSE MODEL EXPRESSING CT100 HUMAN APP

Shewly K. Choudhury, Lynda Sellers, Piers Emson.
The Laboratory of Cognitive and Developmental Neuroscience, Neurobiology Programme, The Babraham Institute, Babraham, Cambridge, CB2 4AT, UK.

In this study we investigated the phosphorylation status of key signalling effectors implicated in cell survival mechanisms in our thy1CT100 (V717I) mouse model. We examined 4 brain regions (cerebellum, cortex, hippocampus and subcortical, fore and midbrain) of wildtype, hemizygous, and homozygous male mice, which displayed cognitive deficits at 12 but not 3 months. In the 3 month homozygotes we found a decrease in CREB, ERK1/2 and Akt phosphorylation primarily in the cortex and to a lesser extent in the hippocampus, which was not apparent at 12 months. Conversely, there was no change in SAPK at 3 months, but there was at 12 months. This suggests an age- and region-dependent effect of CT100 expression on cell survival signalling.

59.13

STRONG ASSOCIATION OF THE SAITOHIN GENE Q7 VARIANT WITH PROGRESSIVE SUPRANUCLEAR PALSY

Alan Pittman(1), Andrew Hope(1), Michael E. Weale(3), Huw R. Morris(1), Nicholas W. Wood, (2), Andrew J. Lees (1) and Rohan de Silva(1,2).
(1) Reta Lila Weston Institute of Neurological Studies, (2) Dept of Molecular Pathogenesis, Institute of Neurology and (3) The Centre for Genetic Anthropology, Dept of Biology, University College London, London.

The tau gene (MAPT) on chromosome 17 is frequently mutated in cases of familial frontotemporal dementia with parkinsonism linked to chromosome 17. Furthermore, H1, an extended haplotype block encompassing a region of linkage disequilibrium (LD) containing not only the MAPT but also at least 500kb of flanking sequences, is robustly associated with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). The pathogenic basis of this association is not clear although genetic and neuropathological precedents indicate strongly that it involves MAPT function. However, it could also involve another locus within the region of LD. In any case, it is important that we identify the pathogenic culprit. Recently, a novel nested gene, Saitohin (STH) was identified within intron 9 of MAPT. We show that the Q variant (chi^2=17.79, p=2.5x10^{-5}) and QQ genotype (chi^2=19.55, p=5.7x10^{-5}) of a Q7R polymorphism of STH are strongly associated with PSP with complete LD between the extended MAPT H1/H2 haplotype and STH Q7R (chi^2=94.08, p=3x10^{-22}). This implicates the STH polymorphism as a potential pathogenic candidate. The nested STH may play an important role in tau regulation, and the Q7 polymorphism may cause a defect in this role, causing a predisposition to pathogenesis of PSP and, possibly, CBD.

60.01

SERUM LEVELS OF sICAM-1 AND sVCAM-1 AS MARKERS OF CEREBROVASCULAR DISEASE IN LATE-LIFE DEPRESSION

Alan Thomas, Sue Davis, Elizabeth Jackson, Nicky Barnett, Richard Harrison, Chris Morris and John O`Brien
Institute for Ageing and Health,Wolfson Research Centre, Newcastle General Hospital, Newcastle NE4 6BE.

Clinical investigations have demonstrated a bidirectional relationship between vascular disease and depression. We reported an increase in ICAM-1 and VCAM-1, markers of vascular disease, in prefrontal cerebral microvessels in late-life depression. Soluble ICAM-1 and VCAM-1 are recognised serum markers of vascular disease so we hypothesised they would be increased in late-life depression. We measured the levels of sICAM-1 and sVCAM-1 using ELISAs in serum from 24 subjects with current major depression (MajD), 25 with minor depression (MinD) and 24 recovered from major depression (RecD) and compared each group with 26 controls (never had psychiatric illness), matched for age and sex. Subjects with any inflammatory or infectious illnesses were excluded, as were those with CRP levels exceeding normal levels for healthy elderly (>18). Results: depressed subjects in each group were significantly more cognitively impaired than controls (P<0.01) but sICAM-1 and sVCAM-1 levels did not differ in any of the depressed groups from the controls. There were no differences in white cell count or CRP (P>0.34) between the groups. Subjects with a late onset of depression also did not show higher levels than controls. This study provides no evidence that sICAM-1 and sVCAM-1 are either state or trait markers of vascular disease in late-life depression.

60.02

ANALYSIS OF CHANGES IN GENE REGULATION AND PROTEIN ACTIVATION FOLLOWING ACUTE ISCHAEMIC STROKE

Mohamad Saka, John Gaffney, Jurek Krupinski, Mark Slevin
Manchester Metropolitan University, Department of Biological Science, John Dalton Building, Chester Street, M1 5GD.

Ischaemic stroke is a leading cause of death and disability worldwide. Usually it results from a transient or permanent reduction in cerebral blood flow. 2/3 of patients survive but are left with significant degrees of sensorimotor, cognitive, or other impairment. Previous studies have described the importance of individual signalling proteins on infarct development and tissue regeneration after stroke. Here we have performed a global study employing a cDNA micro-array (containing 1200 genes) to examine the level of expression of apoptotic, angiogenic, neuroprotective and inflammatory/excitotoxic genes in a rat model of Middle Cerebral Artery Occlusion (MCAO). Tissue samples from infarct, penumbra (the region of tissue surrounding the ischaemic core) and normal contralateral hemisphere were examined at time points 1h, 4h, 12h, 24h, 3, 7 and 21 days after stroke. We have found differences in gene regulation of multiple genes encoding angiogenesis, neuroprotective and apoptotic molecules in the stroke hemisphere. The number of genes up regulated greater than 2 fold increased up to 3 days (350 genes) decreasing again by 7 days (14 genes). Expression of key regulatory genes has been confirmed by RT-PCR. The important of these genes in the pathophysiology of stroke development will be investigated in more detail.

60.03

CHANGES IN GENE REGULATION AND SUBSEQUENT SIGNAL TRANSDUCTION ACTIVATION FOLLOWING ACUTE ISCHAEMIC STROKE IN MAN

Nick Mitsios, John Gaffney, Pat Kumar, Shant Kumar and Mark Slevin
Department of Biological Sciences, Manchester Metropolitan University, Manchester M1 5GD.

The status of angiogenic and apoptotic factors was investigated in brain samples taken less than 24 hours after death, from 18 patients with acute ischaemic stroke. Both grey and white matter samples were examined, in areas of infarct and penumbra, with association to normal contralateral hemisphere, as control.
cDNA micro-array technology was used to screen for changes in gene expression at time points 2-6, 9-17, and 26-37 days after ischaemia. There was an increased expression in both penumbra and infarct, compared to contralateral hemisphere, in a significant number of genes which are now under investigation, including PAX3, TGF[alpha], IL-6, -8 and -15, Bcl-6, Caspase-3 and -10. PAX3 and TGF[alpha] differential expression was confirmed by semi-quantitative RT-PCR.
Western blotting was used to study expression of TNF[alpha], Il-1[beta], c-myc, p53 and p-p53, Bcl-2 and Caspase-3. Increased expression of p53, Bcl-2 and Caspase-3 was demonstrated in infarct and penumbra. Of the signalling molecules studied -PKC[zeta], p-JNK, Ksr-1, p-p38 and p-c-jun- PKC[zeta] and p-JNK were elevated in infarct and penumbra. However, there was no correlation between patient survival time and upregulation of these proteins.

60.04

AN INVESTIGATION INTO THE ROLE OF HYALURONATE IN ANGIOGENESIS FOLLOWING ISCHEMIC STROKE

Ahmed Al Qteishat, John Gaffney, Jurek Krupinski, Mark Slevin
The Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester, M1 5GD.

Ischemic Stroke is a major cause of morbidity and mortality. Evidence suggests that angiogenesis in penumbra tissue may aide reperfusion and improve neuronal survival. However, angiogenesis only occurs 3-4 days after stroke, which may be beyond the time-window, which allows reversible changes in the ischemic penumbra. Many angiogenic molecules are over-expressed in damaged tissue. Hyaluronan (HA) has differing roles in angiogenesis depending on its size. High molecular mass HA (n-HA) is antiangiogenic whereas o-HA (4-20 disaccharides) stimulates EC proliferation, migration, and is angiogenic in vivo. N-HA is found in significant amounts in brain but the effects of infarction on HA production and metabolisms have not been investigated. Our results showed a significant increase in total HA expression in the serum of patients suffering from acute ischemic stroke (20) (191.15±113.6ng/ml) and ICH (14) (90.16±56.66ng/ml), compared to normal healthy age-matched controls (24) (21,6±18.726ng/ml). Low molecular weight fragments were also detected in stroke (54±32.56ng/ml), ICH (120.3±90.46ng/ml), Control (21.4±16.76ng/ml). Hyaluronidase activities for stroke (57) (4±2.36U), ICH (19) (3.1±2.13U), control (21) (1.6±0.97U) from the time of admission within 24 hours then at day 1,3,7, and 14. The important of HA in tissue remodeling after stroke is the subject of our continuing study.

60.05

FUNCTIONAL RECOVERY FOLLOWING STROKE IN THE STROKE-PRONE SPONTANEOUSLY HYPERSTENSIVE RAT (SHRSP) AND WISTAR KYOTO RAT (WKY)

McGill JK(1)1, Gallagher L(1), Carswell H(1), Irving EA(3), Dominiczak AF(2), Macrae IM(1)
1).Wellcome Surgical Institute, Division of Clinical Neurosciences; 2)Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK. 3) Department of Neuroregeneration, Neurology & GI Centre of Excellence for Drug Discovery, GlaxoSmithKline Research & Development Limited, UK.

This is the first study to investigate functional recovery following middle cerebral artery occlusion (MCAO) in SHRSP and its reference strain, WKY using a 33 point neurological score and has both Home Office and University Ethical Review Panel approval. After 2mm distal diathermy MCA occlusion SHRSP displayed larger infarcts than WKY after MCAO. We therefore performed a second study increasing infarct size in WKY to comparable levels with SHRSP by extending length of vessel occlusion in WKY. Infarct volumes and initial post-MCAO neurological scores, respectively (Mean±SD) were: Study 1, SHRSP 138±2.9mm3, 22±3, and WKY 62±46.5mm3, 25±2; Study 2, SHRSP 158±9.6mm3, 22±4 and WKY 131±23.7mm3, 23±4, respectively. Repeated measures ANOVA revealed a significant effect of time (Study 1,p=0.0002; Study 2, p=0.0001) and strain (Study 1,p<0.0001; Study 2, p=0.0116) on neurological score with SHRSP scoring significantly lower at all time points from 48 hours to 14 days post-occlusion even when both strains sustained the same initial deficit in Study 2. Therefore, following MCAO in which infarct sizes and initial neurological deficit in both strains are comparable, SHRSP have an impaired ability to recover compared to WKY.

JM is supported by an MRC CASE studentship with GlaxoSmithKline.

60.06

CELLULAR ALTERATIONS IN METALLOPROTEINASE-9 IN RESPONSE TO GLOBAL ISCHEMIA IN MICE

Sandra Magnoni1, Andrew Baker2, James McCulloch1, Karen Horsburgh1
1Division of Neuroscience, University of Edinburgh; 1 George SquareEdinburgh EH8 9JZ; 2 Department of Medicine and Therapeutics, University of Glasgow.

An abnormal activity of the matrix metalloproteinases (MMP) is suggested to contribute to the pathophysiology of cerebral ischemia. This study examined temporal and topographical alterations of MMP-9 after transient forebrain ischemia to investigate a potential role for MMP in selective neuronal damage. Ischemia was induced in anaesthetised male C57Bl/6J mice by bilateral carotid occlusion for 17 minutes followed by 24 hr (n=4) and 72 hr reperfusion (n=5). Sham-operated animals (n=5) were used as controls. Neuronal damage and MMP immunostaining was assessed in adjacent sections. Cerebral ischemia was associated with selective and delayed neuronal damage in the caudate nucleus and CA2 hippocampal region at 72 hr reperfusion. In sham-operated animals, there was minimal immunostaining for MMP-9 in the caudate nucleus but neuronal immunoreactivity could be detected in cortex and CA2 and CA3 hippocampal regions. After 24 hr reperfusion, intense cellular MMP-9 staining was observed in the caudate nucleus, and the immunoreactive cells predominantly had the morphological appearance of neurons. After 72 hr, MMP-9 immunoreactivity was less intense and confined to microglial cells. The study demonstrated that MMP-9 is expressed at low levels in normal mouse brain and suggests a possible early up regulation of MMP-9 in regions which are likely to develop neuronal damage after ischemia.

60.07

THE DURATION AND SEVERITY OF CEREBRAL HYPOPERFUSION INFLUENCES THE EXTENT AND DISTRIBUTION OF NEURONAL DAMAGE AFTER TRANSIENT MCAo IN C57BL/6J MICE

McColl BW (1), McCulloch J (2,3), Horsburgh K (3)
(1)Wellcome Surgical Institute, Division of Clinical Neuroscience, University of Glasgow, UK(2) Fujisawa Institute of Neuroscience, University of Edinburgh, UK(3)Division of Neuroscience, University of Edinburgh, UK.

The intraluminal filament method of middle cerebral artery occlusion (MCAo) is an extensively used model of transient cerebral ischaemia. We investigated the mechanism(s) underlying the variation in extent and distribution of neuronal damage with increasing duration of MCAo. 30g male C57Bl/6J mice were assigned to three groups: 15, 30 or 60min MCAo with 24h reperfusion. A heat-blunted nylon filament was positioned to occlude the MCA 8-10mm distal to the carotid bifurcation. The volume and anatomical distribution of neuronal damage was assessed. Local cerebral blood flow was assessed and compared in further groups of mice subjected to 15 or 60min MCAo using the 14C-iodoantipyrine technique.
The volume of neuronal damage was highly reproducible: 15min MCAo (9+2mm3); 30min (56+6mm3) and 60min (70+2mm3). Increasing the duration of MCAo recruits regions outside classic MCA territory. Cerebral hypoperfusion was observed within (e.g. striatum) and outside (e.g. hippocampus) MCA territory after 15min and 60min MCAo compared to the contralateral hemisphere (sham animals showed no asymmetry). Hypoperfusion was more severe after 60min compared to 15min MCAo.
Our results demonstrate that the duration and severity of the ischaemic insult influences the extent and distribution of neuronal damage after intraluminal MCAo in C57Bl/6L mice.

60.08

APOE EPSILON 4 MICE SHOW POORER OUTCOME AFTER TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION COMPARED TO EPSILON 3 MICE

McColl BW (1), Roses AD (2), Horsburgh K (3)
(1)Wellcome Surgical Institute,Division of Clinical Neuroscience, University of Glasgow, UK; (2) Glaxo SmithKline Research & Development, North Carolina, USA; (3) Division of Neuroscience, University of Edinburgh.

The APOE e4 allele is the major genetic risk factor for development of Alzheimer's disease and is associated with a poor outcome after head injury. We investigated if the APOE e4 allele is associated with a poorer outcome after transient MCAo compared to the e3 allele.
We used 30g male APOE transgenic mice expressing the human APOE e3 or e4 alleles. 15min MCAo by intraluminal filament was performed followed by 24h reperfusion. Animals were assigned a neurological deficit score after 24h. Brains were perfusion-fixed, paraffin-embedded and 6um sections were cut at eight anatomically defined coronal levels. The volume of neuronal damage and apoE staining was assessed.
The volume of neuronal perikaryal necrosis was significantly larger in the APOE e4 group compared to the e3 group (9+1mm3 vs 7+1mm3, p=0.006). Similarly, the neurological deficit was significantly higher in the APOE e4 group compared to the e3 group (2.00+1.29 vs 0.71+0.49, p=0.038). The volume of apoE staining was larger in the APOE e4 group compared to the e3 group, although this did not reach statistical significance (5+1mm3 vs 4+1mm3, p=0.176). The volume of apoE staining correlated with the volume of neuronal damage (p=0.019).
Our results show that possession of the APOE e4 allele is associated with a poorer outcome, both pathologically and neurologically, after transient MCAo.

60.09

SPHINGOSINE-1-PHOSPHATE (SIP) INHIBITS VOLTAGE-GATED K^+ CURRENTS AND IS LINKED TO INCREASED CREB PHOSPHORYLATION IN CEREBRAL VASCULAR SMOOTH MUSCLE

Frederic Coussin, Roderick Scott & Graeme Nixon
Dept. of Biomedical Sciences, IMS, The University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD.

SIP activates G-protein coupled EDG receptors to evoke vasoconstriction in cerebral arteries. This constriction was dependent on IP3-evoked Ca2+ mobilisation from stores and activation of Rho-kinase, which maintained the contraction after Ca2+ declined. In this study, whole cell patch clamp recording was used to show that 5 minutes application of 1μM SIP inhibited voltage-gated K+ currents. This inhibitory action was maintained after SIP was removed but was attenuated by intracellular GDP-ß-S (2mM) or extracellular application of the protein kinase C inhibitor calphostin C (250nM). SIP, 50mM K+ and the K+ channel inhibitor 4-AP (5mM) all evoked increases in intracellular Ca2+ and cyclicAMP response element binding protein (CREB) phosphorylation. The underlying common mechanism linking these treatments appears to be depolarisation and activation of L-type voltage-gated Ca2+ channels because the 1,4-dihydropyridine L-type channel antagonist, nifedipine, reduced rises in intracellular Ca2+ and CREB phosphorylation in response to SIP, 50mM K+ and 4-AP. CREB is a potentially important transcription factor in regulating smooth muscle cell proliferation. Our results demonstrate that SIP, released from platelets, produces maintained constriction of cerebral arteries and may activate transcription events associated with proliferation and a long-term decrease in cerebral blood flow.

60.10

SUBCORTICAL DAMAGE AFFECTS CORTICAL PERI-INFARCT FUNCTION AFTER FOCAL CEREBRAL ISCHAEMIA IN RATS

J. Pyne; H. Imai; I.M. Macrae; D. Dewar
Division of Clinical Neuroscience, Faculty of Medicine, University of Glasgow, Glasgow G61 1QH.

Recovery after stroke involves plasticity and reorganisation in cortical regions surrounding the infarct. This may be influenced by subcortical damage. We examined the extent of subcortical tissue loss and cortical peri-infarct function-related glucose use after focal cerebral ischemia in rats. Anaesthetised rats received either permanent or transient middle cerebral artery occlusion. One month later function-related glucose use was determined by in vivo 14C-2-deoxyglucose autoradiography. The amount of tissue loss from the cerebral cortex and subcortex was determined postmortem. Subcortical tissue loss was greater after transient (24+2%) compared to permanent (15+1%) ischemia. Cortical tissue loss was similar after transient (27+5%) or permanent (30+5%) ischemia. There was an association between subcortical tissue loss and glucose use in peri-infarct cortex after transient but not permanent ischemia. The neuroanatomical distribution of subcortical tissue loss was different after transient or permanent ischemia. The data indicate that amount and location of subcortical damage may influence post-stroke recovery.

Supported by the University of Glasgow.

61.01

MOLECULAR MECHANISMS UNDERLYING CONGENITAL MYASTHENIC SYNDROMES

R.G. Webster, S. Brownlow, A. Vincent, D. Beeson
Neurosciences Group, Weatherall Institute of Molecular Medicine, University of Oxford, OX3 9DS, UK.

Congenital myasthenic syndromes (CMS) can be caused by missense mutations of the acetylcholine receptor (AChR) at the neuromuscular junction. Here we use electrophysiological techniques to investigate 4 missense mutations that underlie different CMS, and show the different molecular mechanisms that can cause synaptic dysfunction. Two missense mutations studied were αF256L and αS226F which showed a kinetic abnormality. The former results in shortened receptor activations (duration {occurrence} of longest burst population (t3) reduced from 3.62±0.61 {28%} to 1.87±0.49 {2%} ms at low [ACh], n=5) whilst the latter, conversely, produces prolonged receptor activations. These mutations underlie "fast" and "slow" channel syndromes, respectively. Two other missense mutations βR220C and εR217L (both 3 residues prior to TMD1) result in severely reduced surface expression (10-20 % of wild type). Those channels which reach the surface appear kinetically unaffected (t3 for βR220C 3.98±0.81 ms and 4.01±0.50 ms for wild type, n=5). In these cases dysfunction in the assembly of subunits into the AChR pentamer probably underlies the CMS. Here we have shown that missense mutations may cause a CMS through reduced surface expression, or kinetic abnormality. This information can help define appropriate treatment and could lead to novel therapeutic approaches.

61.02

RAPSYN MUTATIONS IN CONGENITAL MYASTHENIA

Judith Cossins, Susan Maxwell, Gregory Owens, Georgina Burke, Angela Vincent and David Beeson
Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, OX3 9DS.

Rapsyn is a 43kDa protein that is involved in clustering of nicotinic acetylcholine receptors (AChRs) at the endplate of the neuromuscular junction. We have identified ten rapsyn mutations in patients with congenital myasthenic syndrome. Two distinct phenotypes have been identified: early and late onset. In early onset, the disease is characterised by weakness at birth, respiratory crisis, and arthrogryposis multiplex congenita is usually evident. These patients spontaneously improve with age and have minimum disability later in life. The late onset phenotype develops in adolescence or adulthood, is progressive and could be mistaken for seronegative myasthenia gravis. Interestingly, patients with the same mutation can exhibit either phenotype.
Here we have examined the pathogenicity of two missense mutations in rapsyn, A25V and R91L. Constructs containing mutated EGFP-tagged human rapsyn were co-expressed with the AChR in TE671 cells. We demonstrate that both mutants alter the ability of rapsyn to form clusters with itself and with the AChR. The constructs have also been expressed in C2C12 myotubes using ecotropic recombinant retroviral technology, to investigate the effects of the rapsyn mutations on agrin-induced clustering of the AChR.

61.03

A MYASTHENIA GRAVIS NON-IgG PLASMA FACTOR THAT INHIBITS MUSCLE ACETYLCHOLINE RECEPTOR (ACHR) FUNCTION BY PROMOTING DESENSITISATION

Ian Spreadbury, Uday Kishore, Susan Maxwell, David Beeson, Angela Vincent
Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DS.

10-20% of myasthenia gravis (MG) patients are `seronegative`, having no detectable anti-AChR IgG antibodies. Many of these patients have a non-IgG factor that inhibits AChR function (Plested et al 2002). The mechanism of this inhibitory action was studied by whole-cell patch clamping of cell lines. Dialysed 1:20 whole plasma and non-IgG fraction from a SNMG patient reduced AChR current amplitude by 44 % in the TE671 subline CN21, which expresses muscle AChR, and in transiently transfected HEK 293 cells. This effect was not prevented by internal 10 mM BAPTA, 40 nM staurosporine, or inhibitors of various phosphatases, excluding some intracellular pathways. Surprisingly, inhibition in either cell type varied greatly between individual cells but, within each cell, the inhibition closely resembled the desensitisation produced by 100 micromolar nicotine. Prolonged co-application of the plasma and nicotine together did not affect the peak-response, but accelerated desensitisation. These data are consistent with the non-IgG factor binding to the AChR itself and acting allosterically to promote desensitisation. Further studies are needed to see whether the non-IgG factor binds to one of the previously defined allosteric sites on the AChR.

61.04

A DELETION MUTANT IN THE *C. ELEGANS* GENE SMN-1 PROVIDES A MODEL FOR INVESTIGATING SPINAL MUSCULAR ATROPHY

Stefanos Christodoulou, Behrooz Esmaeili, Emma Burt and David B Sattelle
MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

A deletion mutant (ok355) of the nematode Caenorhabditis elegans smn-1 gene, the orthologue of the human SMN gene encoding the survival of motorneurons protein, SMN, a deficit of which is linked to spinal muscular atrophy (SMA), provides a simple model for investigating this human disorder. Worms with the smn-1 deletion are sterile and show arrested larval development (L2-L3 stage). A pan-neuronal GFP marker shows neurogenesis to be perturbed with anomalies in neuronal spacing and nerve bundle organization. Striated and smooth muscle functions are both compromised in the worm, as reflected by defects in locomotion, pharyngeal pumping and defecation. A defect in the reverse movement observed in response to head-touch, is seen in late L1 larvae at which time forward locomotion in response to tail-touch is unaffected. This finding indicates nerve/muscle control of reverse movement may be particularly susceptible to the loss of SMN protein. As lower motorneuron and proximal muscle defects are observed in SMA patients, the C. elegans smn-1 mutant may enable genetic analysis of differential susceptibility to SMN loss of particular motorneurons and muscle cells.

62.01

ACTIVATION OF JNK IN SENSORY NEURONES IS PROTECTIVE IN DIABETES AND ON EXPOSURE TO GLUCOSE/OXIDATIVE STRESS *IN VITRO*

Sally Price, Tertia Purves, Luke Hounsom, Paul Fernyhough and David Tomlinson
Division of Neuroscience, School of Biological Sciences, University of Manchester, Stopford Building 1.124, Oxford Road, Manchester M13 9PT, UK.

Diabetes activates MAP kinases in sensory ganglia and inhibition of ERK and p38 prevents nerve damage. Agents that improve neuronal function in diabetic rats - antioxidants and aldose reductase inhibitors - also inhibit activation of ERK and p38 in dorsal root ganglia (DRG). However, these same treatments consistently increase activation of JNK. Thus, in DRG from rats with streptozotocin(STZ)-induced diabetes of 8 weeks duration JNK was activated by 2.75 compared to controls ($p<0.05$). In DRG from diabetic rats treated with the diester of gamma-linolenic acid and alpha-lipoic acid (GLA^^LA), the activity was increased to 3.75 that of controls ($p<0.05$ compared to both controls and untreated diabetics). We therefore tested the hypothesis that JNK activation is protective. Exposure of rats to diabetes or to oxidative stress, by avitaminosis E, increased activation of JNK in DRG; treatment with GLA^^LA increased this effect ($p<0.05$). Inhibition of JNK (JNKI1, 159-600-R100, Alexis Biochemicals) in cultured DRG cells increased the release of LDH and reduced MTT staining; indicating increased neuronal damage. These findings indicate that activation of JNK in sensory neurones of diabetic rats serves to protect the neurones from damage.

62.02

MAP KINASE p38 IS A GLUCOSE DAMAGE TRANSDUCER IN SENSORY NEURONES OF DIABETIC RATS

David Tomlinson, Sithiporn Agthong, Alicia Middlemas and Sally Price
Division of Neuroscience, School of Biological Sciences, University of Manchester, Stopford Building 1.124, Oxford Road, Manchester M13 9PT, UK.

MAP kinase p38 is activated in neurons exposed to raised glucose; this may be a transduction mechanism for diabetes-induced damage. We examined potential mechanisms for p38 activation and downstream consequences in diabetic rats. In rats with streptozotocin-induced diabetes of 8-10 weeks duration, activation of p38 was coincident with reduced sensory nerve conduction velocity and both were blocked in diabetic rats by the p38 inhibitor SB239063 or by the aldose reductase inhibitor, fidarestat. This indicates that exaggerated flux through the sorbitol pathway via aldose reductase is responsible for p38 activation and that its consequence is linked in some way to impaired action potential propagation.

62.03

DETECTION OF THE p75 NGF RECEPTOR IN PLASMA IN DIABETIC NEUROPATHY

Lucy Chilton and David Tomlinson
Division of Neuroscience, School of Biological Sciences, University of Manchester, Stopford Building 1.124, Oxford Road, Manchester M13 9PT, UK.

Receptor shedding is a recognised symptom of certain disease states. Western blot analysis has been used to show low-affinity NGF receptor (p75NTR) immunoreactivity in plasma. To investigate this further streptozotocin (STZ) was used to induce type 1 diabetes in male Wistar rats. All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986. Time-course studies showed that the amount of shed p75NTR is positively correlated to the duration of diabetes suffered becoming significantly elevated ($p<0.05$) compared to control animals after 12 weeks. The titre of p75NTR detected was affected by treatment with insulin and the neurotrophins NT-3 and NGF in vivo; resulting in the amelioration of increase immunoreactivity ($p<0.05$) in a dose dependent manner. Glycated haemoglobin was used as a measure of hyperglycaemia, this was unaffected by neurotrophin treatment. p75NTR is expressed by Schwann cells and is upregulated after injury. A similar response is observed when examining the damage caused by diabetic neuropathy. These cells may be the source of the circulating p75NTR after nerve damage.

63.01

HETEROMULTIMERIZATION AND COLOCALIZATION OF TrpV1 AND TrpV2 IN MAMMALIAN CELL LINES AND RAT DORAL ROOT GANGLIA

A Richard Rutter, Qing-Ping Ma, Matthew Leveridge, Kevin Oliver and Timothy P Bonnert
Merck Sharp and Dohme, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

TrpV1, formerly known as the vanilloid receptor 1 (VR1), is the founder member of the TrpV subfamily of the Trp channel superfamily. It is localized to the small sensory neurons of the dorsal root ganglion (DRG) responsible for transducing multiple pain-inducing stimuli and is activated by capsaicin, acidic pH and noxious heat. The related ion channel, TrpV2 has also been implicated in mechanisms of pain and is thought to detect noxious heat with an elevated activation threshold. While TrpV2 shows a more widespread tissue distribution compared to TrpV1, both channels can be found in a population of cells in the DRG where they may colocalize and potentially coassemble to form novel heteromeric complexes. We have shown by co-immunoprecipitation both an in vitro and in vivo coassociation of TrpV1 and TrpV2. Co-labeling of DRG sections also revealed colocalization to selected sensory neurons. This association may have important implications on the specific responses of individual sensory neurons to pain-inducing stimuli where the TrpV1/TrpV2 heteromer may be one of a multitude of distinct TrpV1 ion channel complexes.

63.02

COMPARISON OF DISTRIBUTION OF PROTEASE ACTIVATED RECEPTOR (PAR) mRNAs IN ADULT MOUSE DORSAL ROOT GANGLIA (DRGs)

Anna M. Kinsey1, Kevin R. Oliver2 and Peter A. McNaughton1
1. Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1PD2. Merck Sharp and Dohme Neuroscience Research Centre, Harlow, Essex, CM20 2QR.

Protease activated receptors (PARs) are G-protein-coupled receptors with a novel mechanism of activation, in which a tethered ligand results from protease-mediated cleavage of the extracellular amino terminal domain of the receptor. Of the four known members of this recently discovered family of receptors, PAR1, PAR2 and PAR4 can also be activated by synthetic peptides corresponding to the exposed neo-N-terminus. PARs play an important role in responses to injury and there is growing evidence to suggest a role for PAR1 and PAR2 in pain and inflammation.
We have sought to localise expression of mRNAs for PAR1-4 in adult mouse DRGs. Neuronal distribution was determined by grain-counting emulsion dipped sections hybridised with radiolabelled oligonucleotide probes specific for each receptor. PAR2 mRNA was localised exclusively in small diameter neurones. PAR1 and PAR4 mRNAs were expressed in a small percentage of neurones across the whole size range. Notably, PAR3 mRNA was the most intensely and widely expressed of the receptors with expression being predominantly in small diameter neurones. These studies aim to assist in the elucidation of the physiological (and perhaps pathophysiological) function(s) of these receptors in vivo.

63.03

ACID-INDUCED PAIN AND ITS MODULATION IN HUMANS

N.G.Jones1; R. Slater1; H.Cadiou2; P.A.McNaughton2; S.B.McMahon1
1. Sensory Function Group, King's College London, UK; 2. Dept. of Pharmacology, University of Cambridge, Cambridge, UK.

Acid can induce pain under both normal and pathophysiological conditions. However, the details of how they do this has been relatively little studied. Here we have used psychophysiological techniques to study acid mediated pain induced by iontophoresis of acid across the skin of volunteers. Weak acid iontophoresed across the volar forearm skin of healthy volunteers causes significantly more pain compared to saline ($n = 12$, $p < 0.01$). The perceived pain increases in a dose dependent manner (current 0.1-0.4mA) reaches a peak at one minute, and desensitises rapidly over the subsequent time period (three minutes).Changing the experimental skin temperature over the range 30-43oC did not significantly alter the degree of acid-induced pain, at least for the moderate currents used (0.1-0.4mA). Additionally, topical application of NSAIDs (non-steroidal anti-inflammatory drugs) which can directly inhibit ASIC channels in vitro specifically decreases acid mediated pain, without affecting the perceived pain caused by other noxious stimuli. Since the proton-gated current at the VR1 (vanilloid receptor 1) receptor shows a strong modulation with temperature both of these results suggest the involvement of ASICs (acid sensing ion channel), in mediating acid-induced pain.

63.04

ACTIVITY-DEPENDENT PHOSPHORYLATION OF AKT IN ADULT RAT DRG NEURONES

Pezet Sophie, Williams Robert J and McMahon Stephen B
Center for Neuroscience, Hodgkin Building, Guy`s Campus, SE1 1UL, London.

The serine/threonine protein kinase AKT has been implicated in cell survival signalling in many cells types, including the dorsal root ganglion (DRG). However, little is known about its role in physiological or pathophysiological conditions in the adult sensory and nociceptive system. We show in this study that in naïve animals almost all cells in the DRG express AKT but only a subset of small diameter neurons expresses a high level of phospho-AKT (pAkt/Ser473). Thermal or chemical (capsaicin or ab met ATP) stimulation of peripheral nociceptors in anaesthetized rats induced a rapid onset but time-dependent increase in phosphorylation of AKT in small diameter DRG neurons. Increased phosphorylation induced by capsaicin occurred in VR1 positive cells, whereas ab met ATP selectively activated DRG cells expressing P2X3. In addition, electrical stimulation of A or A and C fibers in the sciatic nerve induced an increased activation of AKT in a appropriate subsets of DRG neurons. A quantitative analysis suggested that most of the stimulated neurones exhibited phosphorylation of AKT. These results suggest that in the small nociceptive neurons in the DRG, activity regulates the phosphorylation state of AKT.

63.05

METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5 IN SPINAL CORD MEDIATES ACUTE INFLAMMATORY MECHANICAL HYPERALGESIA

Dolan S. & Nolan A.M.
Division of Veterinary Physiology and Pharmacology, Institute of Comparative Medicine, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK.

A role for group I metabotropic glutamate receptors (mGluR1 and 5) in pain processing has been identified. This study was designed to study the role of mGluR5 in mediating inflammatory hyperalgesia and to characterise associated expression of mGluR1 and 5 in spinal cord. The effect of central (0.02 - 20 micromole, i.t.) or systemic (0.1, 1 mg/kg, i.v.) administration of the mGluR5 antagonist: 2-Methyl-6-(phenylethynyl) pyridine (MPEP) (Novartis Pharma AG) or vehicle on limb mechanical withdrawal thresholds was measured for 6 h following intradermal carrageenan injection (0.5mg, 0.5 ml into a forelimb) in 6 adult sheep. Cervical spinal cord tissues were recovered from control and carrageenan animals and processed for mGluR1 and 5 mRNA expression using 'Taqman' real-time PCR. Carrageenan induced significant bilateral hyperalgesia 3 h post-injection. Spinal administration of MPEP pre-carrageenan significantly attenuated hyperalgesia on the ipsilateral but not contralateral limb. Systemic administration of MPEP had no effect on hyperalgesia. No alteration was detected in spinal mGluR1 or 5 mRNA 3 h after carrageenan (time of maximum hyperalgesia). The anti-hyperalgesic action of MPEP demonstrates a significant role for spinal mGluR5 in acute inflammatory hyperalgesia, independent of lasting transcriptional changes.

63.06

ANALYSIS OF BDNF EXPRESSION AFTER SCIATIC NERVE AXOTOMY IN RAT DORSAL ROOT GANGLIA USING EXON SPECIFIC PROBES

R.C. Leavesley1, C. Magoulas2, G.J. Michael1 and J.V. Priestley1
Departments of Neuroscience1 and Neurosurgery2, Queen Mary University of London, London.

BDNF is synthesised by certain dorsal root ganglion (DRG) cells and is thought to play a role in modulating spinal cord excitability. Peripheral axotomy results in an upregulation of BDNF in large, trkB and trkC, expressing cells. In contrast inflammation, or exogenous NGF treatment leads to a prominent expression in small, trkA expressing DRG cells. The molecular basis for this is unknown, but may occur due to differential regulation of different BDNF transcripts. Eight BDNF transcripts have been described, formed from one of four regulatory exons (I-IV) together with exon V (which codes for the mature protein) and alternative polyA sites. We have previously shown that exons II and III are upregulated following NGF treatment. We have therefore now examined the effects of axotomy using in situ hybridization.
Sciatic nerve axotomy was performed and animals were perfused at four different timepoints. All procedures were performed in accordance with Home Office regulations. Lumbar ganglia were cut on a cryostat, hybridised with exon specific riboprobes and processed for liquid emulsion autoradiography. Exon II showed a prominent upregulation in axotomised DRG cells. Exons I and III exhibited weak expression. In contrast no signal was seen for exon IV.

Supported by the Wellcome Trust.

63.07

EFFECTS OF THE SPECIFIC INHIBITORS OF PROTEIN KINASE A (PKA), Myr-PKI AND Rp-cAMPS ON NOCICEPTIVE BEHAVIOURS AFTER CHRONIC CONSTRICTIVE NERVE INJURY

Andrew Moss, Emer M. Garry, Rory Mitchell & Susan M. Fleetwood-Walker
1. Dept Preclinical Veterinary Sciences, R(D)SVS, University of Edinburgh, EH9 1QH, 2. MRC Membrane and Adapter Proteins Co-op, Dept. Biomedical Sciences, University of Edinburgh.

The aetiology of neuropathic pain is little understood and the condition is poorly treated by currently available analgesics. Following nerve injury, changes in membrane excitability, changes in protein phosphorylation and the resultant post-synaptic activation of the NMDA receptor within the spinal dorsal horn may lead to a state of central sensitisation. It has been demonstrated that central sensitisation is accompanied by alterations in signal transduction cascades, and some of these may contribute to bringing about the sensitised state. This study sought to investigate the potential role of the second messenger-regulated kinase (PKA) during the peak sensitisation following an experimental peripheral nerve injury (CCI). The effects of intrathecal injection of the specific PKA inhibitors Myr-PKI (5-22) and Rp-cAMPS were investigated. The results show that inhibition of PKA catalytic activity or PKA activation by cAMP at the peak of neuropathy result in marked reduction of the hyperalgesia and allodynia that characterises neuropathic pain. These results suggest that PKA catalytic activity may be a key event in the generation of central sensitisation following nerve injury.

63.08

RNAi MEDIATED DOWNREGULATION OF MOB-5 (IL-24/MDA-7) REVERSES HYPERALGESIA IN A RAT MODEL OF CHRONIC NEUROPATHIC PAIN

Sarah Moffatt, Jane Barclay, Gabriele Dorn, Alyson Fox, Sadhana Patel, Viral Patel, Chuanzheng Song, Robert Terry, Janet Winter, William Wishart, Glen Wotherspoon and Pam Ganju
Novartis Institute of Medical Sciences, 5 Gower Place, London, WC1E 6BS.

Mob-5(C49a) is the rat homologue of human IL-24 (Mda-7), a recent addition to the IL-10 superfamily of cytokines. It was originally identified as a cytokine-like protein activated by oncogenic ras and is up-regulated during wound-healing. Here, we present differential display and RT-PCR data demonstrating that Mob-5 mRNA is up-regulated in dorsal root ganglia in the several rat models of chronic neuropathic pain. Furthermore, in situ hybridisation and immunolocalisation studies in the Chung model of neuropathic pain confirm that Mob-5 mRNA and protein are present in the majority of DRG neuronal cells. We also show that intrathecal administration of Mob-5 specific anti-sense RNAi to rats with established neuropathic pain results in significant reversal of mechanical hyperalgesia and tactile allodynia associated with downregulation of Mob-5. This work provides the first evidence for Mob-5 involvement in neuropathic pain and provides further insight into the biological role of this cytokine.

63.09

RNA INTERFERENCE BLOCKS CHRONIC PAIN *IN VIVO*

Gabriele Dorn, Sadhana Patel, Glen Wotherspoon, Maja Hemmings-Mieszczak, Jane Barclay, Francois Natt, Pierre Martin, Stuart Bevan, Alyson Fox, Pam Ganju, William Wishart & Jonathan Hall.
Novartis Institute for Medical Sciences, 5 Gower Place, London, WC1E 6BS, UK; Functional Genomics, Novartis Pharma AG, Lichtstrasse 35, 4002 Basel, Switzerland.

Double stranded, short interfering RNAs (siRNA) of 21-22-nt length initiate a sequence-specific, post-trancriptional gene silencing in animals and plants known as RNA interference (RNAi). Previously, RNAi has been applied in plants, in invertebrates, in mammalian cell culture, in early mouse development, and to inhibit an exogenous luciferase gene expressed in adult mice. Here we show for the first time that RNAi can block a pathophysiological pain response in an animal disease model by downregulating an endogenous, neuronally expressed gene. Rats, intrathecally infused with a 21-nt perfectly matched siRNA against the pain-related cation-channel P2X3, showed diminished pain-responses compared to missense-treated (MS) siRNA and untreated controls in an agonist-evoked pain model, as well as in a chronic neuropathic pain model. No adverse effects were observed in any animal receiving P2X3 siRNA, MS siRNA or vehicle. At a molecular level, P2X3 mRNA expressed in DRG, and P2X3 protein trafficked into the dorsal horn of the spinal cord, were significantly diminished. These observations open a path toward use of siRNA as a genetic tool in mammals and ultimately as an in vivo therapeutic agent in man.

63.10

IDENTIFICATION OF STAC LIKE-1: A NOVEL GENE REGULATED IN NEUROPATHIC PAIN.

J. Egerton, D. Gale, T. Testa, R.E. Macdonald, J. Biggs, S.A. Bates, N.C. Day, R.E. Kelsell, I.S. Gloger, J.B. Davis
Neurology & GI - CEDD, GlaxoSmithkline, NFSP-North, Third Avenue, Harlow, essex CM19 5AW.

Partial ligation of the sciatic nerve is an established model of neuropathic pain that results in sensory hypersensitivity. In this study we used cDNA representational difference analysis (RDA) to identify differentially regulated transcripts in the dorsal root ganglion (DRG) of hypersensitive animals. SYBR Green and Taqman RT-PCR were used to confirm regulation of transcript levels in a second rat partial ligation study.Several genes known to be upregulated following nerve ligation were identified (eg. galanin, NPY). A novel EST, 20D, was confirmed by secondary validation. 20D expression was found to be low in the DRG of sham or naïve animals, whereas expression in DRG increased to a maximum at 7 days post-ligation, decreasing somewhat by days 14 and 28. 20D expression was also identified in isolated DRG neurones. A full-length cDNA for 20D was obtained and the novel gene termed STACL-1. Rat STACL-1 is 93% identical to its human orthologue and 50% identical to mouse STAC. Human STACL-1 mRNA was detected at high levels in DRG and cerebellum and at lower levels across all other human brain and tissue regions. STACL-1 may be an important component in signal transduction critical to the development of neuropathic pain.

63.11

IDENTIFICATION OF ENDOGENOUS PROSTANOID RECEPTOR IN HUMAN ASTROCYTOMA CCF-STTG1

D.E. Owen*, B. Whitehead, D. Smart
Neurology and GI Centre of Drug Discovery, GlaxoSmithKline Research and Development, NFSP(N), 3rd Avenue, Harlow, Essex, CM19 5AW

Prostaglandins include a varied family of autocoids whose synthesis is initiated by cyclooxygenase-mediated metabolism of arachadonic acid generating five primary bioactive prostanoids: PGE2, PGF2a, PGD2, PGI2, and TXA2. Prostanoid receptor-mediated sensitisation of sensory nerve fibres is a key contributor to the generation of hyperalgesia. Compounds acting on specific receptors may offer advantages over upstream inhibitors of Cox-2 or more traditional non-steroidal anti-inflammatory drugs. Pharmacological screening of potential inhibitors in cells expressing cloned receptors is a valuable tool for identifying highly selective antagonists but comparisons with endogenously expressed receptors gives rise to potencies that have more physiological relevance. A number of human cell lines were screened for responses to PGE2 in a FLIPR™ based assay. The human astrocytoma cell line CCF-STTG1 gave a robust signal to PGE2 (pEC50 6.36 + 0.03). Further pharmacological characterisation to a number of known agonists of prostanoid receptors gave a rank order of potency PGF2a > PGE2 > Iloprost > Sulprostone (pEC50 7.3+0.06, 6.36+0.03, 5.85+0.05, 5.96+0.06 respectively). This data indicates the presence of FP receptors. Further data will be presented on more extensive characterisation of these endogenous receptors including antagonist studies.

64.01

OXYGEN FLUCTUATIONS INDUCE LOSS OF MYELIN EXPRESSION IN THE NEONATAL RAT BRAIN

Sedowofia K, Giles D, Cunningham S, McColm J, Minns R, McIntosh N.
Child Life and Health and Neonatology Sections, University of Edinburgh.

Objective: Premature infants receiving oxygen treatment after birth, experience frequent fluctuations in their blood oxygen levels, the severity of which have been associated with the incidence of Retinopathy of Prematurity (ROP) (Cunningham et al, 1995, Lancet 346:1464-65). The objectives of the study were to investigate whether these oxygen fluctuations affected the development of the brain.
Methods: Newborn rats were reared in either room air (controls), or in a chamber in which a computer replicated the fluctuations in oxygen recorded from a preterm infant for 14 days (Cunningham et al, 2000, IOVS 41:4275-80). Rats were sacrificed on day 14, and the brains examined for the expression of active caspase-3, myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP), using western blotting and immunohistochemistry.
Results: Active caspase-3 expression was increased by between 30-100% in different regions of oxygen exposed brains compared to controls, whilst MBP expression was reduced by between 20-60% and GFAP expression increased by between 20-85%.
Conclusions: Fluctuations in blood oxygen concentrations similar to those experienced by many preterm infants receiving oxygen therapy induce a reduction in myelin and an increase in GFAP expression in the neonatal rat brain. These fluctuations might constitute an as yet unidentified source of injury to the developing immature brain.

64.02

PROFILE OF NEUROTROPHIC FACTOR RECEPTOR EXPRESSION FOLLOWING CORTICOSPINAL RACT (CST) TRANSECTION

E. Brown, M. Berry and A. Logan
Department of Medicine, University of Birmingham, B15 2TH.

Abortive attempts of axons to regenerate following injury is proposed to result from limited supplies of essential trophic molecules. Various neurotrophic factors (NTF) have shown positive effects upon cell survival and neurite outgrowth in vitro. Effects of these molecules in vivo has proven disappointing, receptor availability being a potential limiting factor. We have described the post-axotomy expression profile of several neurotrophic factor receptors (NTFR) by the corticospinal tract neurons (CSTN) following unilateral transection of the CST. The CSTN are immunoreactive for a number of NTFR. Expression levels of the neurotrophin receptors, TrkB and TrkC, and the GDNF receptor, GFRa1, altering significantly after injury, decreasing transiently (5-10 days post lesion; dpl) to coincide with the abortion of initial regenerative responses, eventually returning to control levels by 20dpl. Down-regulation of these receptors may reduce the neurotrophic capability of corresponding ligands by limiting the activity of relevant trophic signalling cascades. The observed post-injury modulation of these receptors indicates that their associated ligands, the neurotrophins, BDNF and NT-3, and also GDNF may be the most relevant injury-responsive NTF for the axotomised CSTN and suggests that there my be some sort of ligand-mediated control of receptor expression levels.

65.01

INCREASED EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 IN THE ANTERIOR CINGULATE CORTEX IN BIPOLAR DISORDER

Sue Davis, Alan J. Thomas, Nicol Ferrier, Rajesh N. Kalaria, John T. O'Brien
Institute for Ageing and Health, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE.

Structural abnormalities and pathological alterations have been reported in prefrontal regions in affective disorders, whilst a systemic immune-inflammatory response has been reported in unipolar depression. We examined whether expression of the inflammatory markers intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) is increased in the anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC) in the affective disorders. Paraffin sections from schizophrenic, depressed, bipolar, and control brains were obtained from the Stanley Foundation Brain Bank (n=15 in each group). Sections were stained for ICAM-1 and VCAM-1 using standard immunocytochemistry. Sets of images were obtained from grey matter and associated white matter and the areal fraction occupied by CAM immunoreactivity estimated using quantitative image analysis. ICAM-1 immunoreactivity in bipolar ACC was significantly higher than in control ACC (grey:P=0.001; white P=<0.001), schizophrenic ACC (P=0.016;white P=0.025), and depressed ACC (white; P=0.049). No differences between groups were detected for ICAM-1 in the DLPFC, whilst VCAM-1 immunoreactivity was too low for meaningful analysis. These findings represent the first tissue evidence for the presence of an inflammatory response in the ACC in bipolar affective disorder. This may be associated with the previously described neuronal and glial loss in this area in such subjects.

65.02

FOCAL BRAIN INJURY STIMULATES HEPATIC RELEASE OF CHEMOKINES THAT CONTROL MOBILISATION AND RECRUITMENT OF LEUKOCYTES TO THE BRAIN

Sandra J. Campbell*, Angus Butchart*, Fernando Pitossi§, Mariela Chertoff§, Sara Waters#, V. Hugh Perry# & Daniel C. Anthony*
**Molecular Neuropathology and #CNS Inflammation Group, University of Southampton, S016 7PX, UK. §Foundation Institute Leloir, University of Buenos Aires- CONICET Av. Patricias Argentinas 435 (1405) Buenos Aires, Argentina.*

We have previously established that hepatic CXC chemokines, behaving as acute phase proteins, can regulate neutrophil mobilisation and recruitment following focal IL-1b-mediated injury to the rat brain.To determine whether this response was specific to CXC chemokines or a whether it is a more generalised phenomenon in response to acute brain injury, we examined brain and liver production of MCP-1, a CC chemokine, when adult rats were challenged intrastriatally with TNFa. Four hours after the intrastriatal injection of TNFa MCP-1 mRNA and protein were induced in the liver. Serum MCP-1 levels were also elevated at 4 hours, which is consistent with peak mobilisation of leukocytes into the circulation. Both neutrophils and monocytes were mobilised into the circulation, but only monocytes were recruited to both the liver and the brain. MCP-1 expression peaked in the liver at 6 hours whereas recruitment to the brain was delayed until 24 hours. Elevated hepatic and serum MCP-1 is implicated in the control of the leukocytosis and leukocyte recruitment to the brain and liver since neutralisation of peripheral MCP-1 prior to injection of TNFa into the brain was sufficient to inhibit these phenomena. Thus distinct patterns of hepatic chemokine production amplify the CNS inflammatory response.

65.03

REACTIVATION OF IMMUNE RESPONSE BY PERIPHERAL INFECTION CAUSES INCREASED REGIONAL CEREBRAL BLOOD VOLUME

Broom, KA. *Anthony, D. Blamire, A. **Waters, S. **Perry V. Styles, P. Sibson, N.
*MRC Biochemical and Clinical Magnetic Resonance Unit, Department Of Biochemistry, University Of Oxford; *Molecular Neuropathology Laboratory, School of Biological Science, University of Southampton; ** CNS Inflammation Group, School of Biological Science, University of Southampton.*

Peripheral infections may provoke relapses of MS, but limited experimental data exists to define the underlying mechanisms. We have used Magnetic Resonance Imaging (MRI) to study the evolution and reactivation of a focal type-IV hypersensitivity reaction in the rat brain, which displays many of the features of MS pathology. We have discovered that a peripheral challenge with E coli-derived lipopolysaccharide (LPS) causes the reactivation of quiescent (by MRI) cerebral lesions. Reactivation is observed as an initial increase in regional cerebral blood volume (rCBV) six hours after the peripheral LPS challenge, which is followed by re-opening of the blood brain barrier (BBB) at 24-72 hours after the challenge. The increased rCBV observed in this study following challenge may partly reflect increased focal IL-1beta expression in the brain. The discovery that the presence of bacterial cell wall products in the periphery can reactivate a lesion within the CNS may have profound implications for the treatment of MS. This study also suggests that monitoring regional changes in perfusion may be of value in detecting early inflammatory events in the brain that are not associated with BBB breakdown.

65.04

STREPTOCOCCUS PNEUMONIAE DAMAGES THE CILIATED EPENDYMA OF THE BRAIN DURING MENINGITIS.

Robert A. Hirst,*+, Bejal Gosai*+, Andrew Rutman*, Peter W. Andrew+ and Christopher O'Callaghan*.
**Department of Child Health, +Department of Microbiology and Immunology, University of Leicester, POBox 65, Leicester, LE2 7LX, UK.*

Streptococcus pneumoniae meningitis remains a disease with poor outcome for the patient. A region of the brain that has been neglected in the study of meningitis is the ependyma. In this study we have examined whether the ependymal layer is affected by S. pneumoniae in a rat model of meningitis. In meningitis rat brains the mean ependymal Ciliary Beat Frequency (CBF) in the 4th and lateral ventricles (20.2+2Hz) was significantly ($p<0.05$) reduced compared to control (33.6+1.5Hz). In the meningitis group, there was a significant ($p<0.05$) loss of cilia in the 4th and lateral ventricle when compared to control. The ventricle floors were the most denuded of cilia in both ventricles. There was no significant difference in control CBF between the 4th and lateral ventricle. Scanning and transmission electron micrographs will also be presented. In addition to the immediate consequences of the loss of ependymal ciliary function, it is likely that loss of adult neural stem cells in the ependymal lining of the brain, may affect the rate of brain repair after meningitis. We are currently investigating the various virulence factors produced by S. pneumoniae that influence the damage to the ependymal tissue. Such information will allow us to determine areas of virulence that may be targeted by adjunctive therapy.

66.01

INTRINSIC OPTICAL IMAGING REVEALS THE RETINOTOPIC ORGANIZATION OF RAT VISUAL CORTEX

P Cotton, I Schiessl and N McLoughlin
Dept of Optometry and Neuroscience, UMIST, Manchester, M60 1QD.

Optical imaging of intrinsic signals has been used to record the retinotopic organization of the visual cortex in number of different species. In this study, we present the first retinotopic maps obtained from the rat visual system. We used a thinned skull preparation to record the intrinsic optical signals under conditions of monocular stimulation. A computer monitor was used to project either vertical or horizontal drifting bars at a fixed spatial frequency. Stimuli were projected from one of two or three adjacent segments of the screen whilst the other one or two segments were masked. Complementary stimuli were used to generate differential maps. A CCD camera was used to record optical signals from the contra-lateral visual cortex and images were processed using both first frame analysis and extended spatial decorrelation: an algorithm based on the principles of blind source separation. Here we show that activating discrete areas of visual space in both the horizontal and vertical domain produces complementary stripes of activation parallel to the horizontal and vertical meridians respectively. These optical maps confirm the gross retinotopic organization of the rat visual cortex obtained in previous electrophysiological recording studies.

66.02

NEURONAL RESPONSES TO SUBLIMINAL CUES PREDICTING REWARD MEASURED BY fMRI

Clark L, Elliott R, Williams S*, Deakin JFW and Anderson IM.
University of Manchester, Neuroscience and Psychiatry Unit and Imaging Science and Biomedical Engineering, Stopford Building, Oxford Road, Manchester M13 9WL, UK.*

Human studies have indicated that unconscious influences can be used to modulate behaviour. Imaging research suggests that medial and orbital (OFC) prefrontal cortices may respond to stimuli that subjects are not consciously aware of. OFC neurons are also known to be involved in reinforcement processing, coding the relative values of specific reinforcers and expected outcomes of actions. The aim of this study was to investigate BOLD responses to subliminal stimuli predictive of reward, in order to explore the neuronal basis of covert/implicit decision-making.12 healthy volunteers were scanned while performing a computerised decision making task. The correct choice was signalled by a subliminal predictive cue.
None of the subjects reported conscious awareness of the predictive cues. Preliminary analysis of the imaging data identified regions where differential BOLD responses were observed to subliminal predictive cues. These regions included lateral OFC bilaterally, medial OFC, superior frontal gyrus (BA10) and left hippocampus.
If confirmed the preliminary findings imply that lateral and medial OFC are responsive to cues predictive of reward, even when predictive cues are not consciously processed. These findings may have important implications for understanding the pathophysiology of various psychiatric disorders, where the covert, as well as overt, processing of reinforcement cues may be abnormal.

67.01

OPTIMAL TRANSMISSION OF POPULATION CODES THROUGH LAYERED NETWORKS

Mark van Rossum and Alfonso Renart
Neuro-Informatics Program, 5 Forrest Hill, Edinburgh.

We study the transmission of population coded signals through layered networks of Integrate-and-fire neurons.The quality of the transmission is estimated by trying to read out the transmitted signal and measuring its error.We find that a center-surround connectivity profile gives optimal performance. This result suggests that the abundance of center-surround layouts is related to preserving information present in population coded signals.

67.02

ON THE ROLE OF DENDRITIC FEEDBACK IN SYNAPTIC INTEGRATION

M. Griffin; D.M. Halliday
Department of Electronics, University of York, YORK, YO10 5DD.

Synaptic integration in single neurones involves spatial temporal interactions between large numbers of inputs to a dendritic tree. We use a computer model to characterise the relationship between the membrane potential fluctuations in different locations of the dendritic arbor during different patterns of synaptic input to a simulated compartmental model of a motoneurone. We look at a single input applied in isolation, and as part of large scale synaptic input, provided by 996 independent synaptic inputs, to a model consisting of a soma plus 12 tapered dendrites of varying electrotonic length. Correlation analysis of membrane potential fluctuations reveals a complex interaction, suggesting the presence of feedback. Traces of the axial membrane current indicate that a feedback mechanism occurs in the dendrite following individual synaptic inputs. This feedback mechanism takes the form of a reversal of the axial membrane current flowing in the distal direction. This feedback, which occurs on the time scale of 1-2 ms, tends to smear out the effects of individual inputs and is likely to contribute to the correlation structure seen between membrane potential fluctuations in the dendritic tree, and has implications for our understanding of synaptic integration.

67.03

A RAPID SEARCH TECHNIQUE FOR FINDING MAXIMAL CONDUCTANCES IN BIOPHYSICAL MODELS OF NEURAL MEMBRANES

R. Wood and K. Gurney
Psychology Department, University of Sheffield, Sheffield, S10 2TP.

One demanding and time consuming aspect of developing biophysical neural models is the process of obtaining an appropriate set of parameters. In many cases, experimentally determined values are not available whilst, in others, the experimental data show considerable variation from cell to cell. This is particularly evident with the maximal ionic conductances. These are highly variable, and may evolve over time so that taking averages of values determined across several studies is not a fruitful approach (Golowasch et al., 2002, J. Neurophys. 87, 1129-1131). While the modeller may hand-craft the parameters to fit experimental data, it is much more efficient to use a parameter optimisation technique. We present a novel optimisation routine for finding a set of maximal conductances by matching to current injection data. We present two main results. First, the search is able to find exact solutions, as demonstrated by searching against `data` obtained from simulated current injection experiments. Second, close fits to biological data are possible, as shown by modelling using data for the striatal medium spiny neuron (Nisenbaum and Wilson, 1995, J. Neuroscience, 15, 4449-4463). The routine is deterministic and, unlike other popular algorithms, computationally efficient, arriving at a solution after only a few iterations.

68.01

COMPLEX OSCILLATORY ACTIVITY INDUCED IN RAT CEREBELLAR SLICES *IN VITRO* BY METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION

S. Middleton, M.O. Cunningham, P. Chadderton*, F.E.N. LeBeau, E.H. Buhl, M. Hausser* & M.A. Whittington.
*School of Biomedical Sciences, The Worsley Building, University of Leeds, LS2 9JT*Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT.*

Cerebellar recordings in vivo have revealed oscillations across a range of frequencies (5 – 200 Hz), which appear to be associated with mossy fiber input. Here we demonstrate that these rhythms can be generated in vitro in cerebellar slices in response to metabotropic glutamate receptor activation. Coronal cerebellar slices were prepared from CRUS 1 OF adult male Rat cerebellum and maintained in an interface chamber. Field recordings were taken from the Purkinje cell layer following bath application of 60 uM DHPG. 9/13 slices demonstrated a single frequency, persistent field oscillation (61 ± 6 Hz, 19 ± 7 uV2.Hz-1). 4/13 slices demonstrated oscillations with bimodal spectra (frequencies 11 ± 2 Hz, 42 ± 12 uV2.Hz-1, and 40 ± 2 Hz, 15 ± 2 uV2.Hz-1). In slices with a single frequency of oscillation AMPA/kainate receptor blockade (NBQX, 10 uM) increased field power by 14%, GABA(A) receptor blockade (bicuculline, 30 uM) reduced power by 70% and reduction in gap junction conductance (carbenoxolone, 0.2 mM) reduced power by c.60%. Given the dense GABAergic synaptic network, these preliminary data suggest a predominantly inhibition-based local circuit rhythm may be established in response to glutamatergic input to the cerebellum.

68.02

PARVALBUMIN GABAERGIC NEURONS PLAY A ROLE IN PACING KAINATE-INDUCED (THETA) ACTIVITY IN THE RAT MEDIAL SEPTUM/DIAGONAL BAND OF BROCA (MS/DB) *IN VITRO*

H.L. Garner, M.A. Whittington, E.H. Buhl and Z. Henderson.
School of Biomedical Sciences, University of Leeds, Leeds LS2 9NQ.

We have previously demonstrated extracellular field activity at theta frequency (4–15 Hz) can be evoked in the MS/DB slice in vitro in the presence of kainate. To investigate the involvement of neuronal populations of the MS/DB in this activity, we carried out simultaneous intracellular and extracellular field recordings. Longitudinal MS/DB (450 um) slices were prepared from male Wistar rats and placed in an interface recording chamber. Persistent theta oscillations were induced by bath application of 100 nM kainate. Intracellular recordings were made from three types of MS/DB neuron during extracellular field recordings of kainate-induced theta-frequency activity. These neurons were identified as fast-spiking, slow-firing and regular-spiking according to previous criteria. The fast-spiking type (n= 4) displayed spontaneous and persistent rhythmic single spiking activity at theta frequencies. The slow-firing (n= 2) and regular-spiking (n= 1) types displayed non-rhythmic single spiking activity. Previous studies have shown that fast-spiking and regular-spiking neurons in the MS/DB are GABAergic and that parvalbumin is localised selectively in the fast-spiking type neuron; conversely the slow-firing neurons were shown to be cholinergic. We conclude that the parvalbumin GABAergic neurons may pace rhythmic theta activity in the MS/DB.

In memory of E. H. Buhl.

68.03

DOPAMINE MODULATES GAMMA FREQUENCY OSCILLATIONS IN THE RAT HIPPOCAMPUS *IN VITRO*

Paul Cilia La Corte, Nima J. Ghadiri, Anne King, Miles A. Whittington, Eberhard H. Buhl and Fiona E.N. Le Beau
School of Biomedical Sciences, Clarendon Way, University of Leeds, Leeds, LS2 9NQ.

Dopamine is an important neuromodulator of hippocampal function. The hippocampus receives a dopaminergic input from midbrain sources and all five dopamine receptor subtypes (D1-D5) have been identified in the hippocampus. Our aim was to assess whether dopamine was capable of modulating the fast network oscillations that occur in the hippocampus and which are thought to underlie a variety of cognitive processes. Persistent gamma frequency oscillations (20-80 Hz) can be evoked in the rat hippocampal slice using either bath application of kainate (200-300 nM) or the cholinergic receptor agonist carbachol (20 uM). Dopamine (100 uM) caused a marked reduction in the power (33.7 ± 5.5%) of the kainate evoked gamma frequency oscillation (n = 8). This reduction in gamma frequency activity was partly mimicked by application of the D1 receptor agonist SKF 38393 (40 uM) where a reduction in power of 21.9 ± 4.1% (n = 3) was observed. In contrast the D2 receptor agonist quinpirole (10 uM) had no effect on the power (5.18 ± 16.6%) of the gamma frequency activity (n = 3). These results demonstrate that dopamine can modulate the kainate- evoked gamma frequency activity observed in the hippocampus in vitro.

In memory of the late Eberhard Buhl.

Supported by the MRC.

68.04

OSCILLATORY ACTIVITY IN THE SUBSTANTIA GELATINOSA OF THE RAT SPINAL CORD IN VITRO IS DEPENDENT ON BOTH CHEMICAL AND ELECTRICAL NEUROTRANSMISSION

Paul F. Cilia La Corte, Fiona E.N. LeBeau, Eberhard H. Buhl, Anne E. King and Aziz U.R. Asghar
School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK.

Ventral horn rhythmic activity is dependent on synaptic neurotransmission and on electrical coupling across gap junctions (Tresch & Kiehn, 2000, Nature, 3, 593-599). This study determined whether oscillations in the substantia gelationsa (SG) of the dorsal horn are dependent on chemical and/or gap junction-mediated neurotransmission.Wistar rats (12-14 days) were anaesthetised (Urethane, 2g/Kg i.p.). Lumbar spinal slices were cut and field recordings made from SG. Potassium (KCH3SO4, 1.5 M) was pressure ejected nearby the field electrode. Potassium elicited an oscillation for 5-20s with a dominant frequency of 7.7 ± 0.1 Hz (n=10). Disruption of synaptic transmission with TTX (1uM, 30min) or a calcium-free perfusate (45min) reduced the power amplitude and power area of the oscillation. Subsequent application of the gap junction blocker octanol (1mM, 45min) further reduced the power amplitude and area of the remaining potassium-evoked oscillation. There was no change in frequency with TTX or calcium-free perfusate in the absence or presence of octanol.
The results suggest the dependence of neuronal oscillatory activity in SG upon both synaptic mechanisms and on gap junctions. These rhythms could be involved in modulating nociception.

Paul Cilia La Corte supported by a BBSRC Ph.D. studentship.
Dedicated to the memory of Professor Eberhard Buhl.

68.05

THE CONTRIBUTION OF AMPA AND NMDA RECEPTORS TO THE GENERATION OF OSCILLATORY ACTIVITY IN SUBSTANTIA GELATINOSA NEURONES OF THE RAT SPINAL CORD *IN VITRO*

Aziz U.R. Asghar, Siobhan Reilly, Fiona E.N. LeBeau, Eberhard H. Buhl and Anne E. King.
School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK.

Studies in rat spinal cord ventral horn in vitro show that AMPA and NMDA receptors modulate potassium-induced network oscillations (Bracci et al., 1998, J. Neurophysiol., 79: 2643-2652). This investigation determined whether antagonism of AMPA/Kainate or NMDA receptors could influence potassium-induced oscillations in the substantia gelatinosa (SG) of the dorsal horn in vitro.
Lumbar spinal slices were cut from cords of Wistar rats (age 12-14 days) terminally anaesthetised with urethane (2g/Kg i.p.). Field recordings were made from SG and potassium (KCH3SO4, 1.5M) was pressure ejected close to the field electrode. Ejection of potassium evoked an oscillation of 5-15 s duration with a dominant frequency of 7.7 ± 0.1 Hz (n=12). Bath application of the AMPA/Kainate antagonist CNQX (10 uM) reduced the peak power amplitude (66 ± 10%) and the power area (64 ± 8%) of the rhythm. The NMDA receptor antagonist D-AP5 (50 uM) had no effect on the oscillation. Neither antagonist had any effect on the peak frequency.
These data demonstrate that potassium-evoked oscillations in SG are partly dependent upon AMPA/Kainate, but not NMDA, receptor-mediated neurotransmission. These AMPA/Kainate-dependent rhythms may play a role in the processing of nociceptive signals.

Dedicated to the memory of Professor Eberhard Buhl.

68.06

THE SLOW AFTERHYPERPOLARIZATION (SIAHP) MODULATES KAINATE EVOKED GAMMA FREQUENCY OSCILLATIONS IN THE MOUSE HIPPOCAMPUS *IN VITRO*

J.E.Driver(1), R.D.Traub(2), E.H.Buhl(1), M.A.Whittington(1) and F.E.N.LeBeau(1).1.
School of Biomed. Sci. Univ. of Leeds, Leeds, UK; 2. Dept. Physiol. & Pharmacol. SUNY Health Sci. Ctr. Brooklyn, NY, USA.

sIAHP regulates hippocampal neuronal firing properties such as accommodation of action potential firing during bursts of activity. These effects are well characterised but effects on the behaviour of an oscillating network are unclear. The influence of the sIAHP on gamma frequency oscillations (30-80Hz) was investigated using mouse hippocampal slices maintained in an interface chamber. Gamma oscillations were induced by bath application of kainate (50-200nM). The L-type calcium channel antagonist, nifedipine (10uM) was bath applied to decrease the sIAHP. Nifedipine caused a gradual, but non-significant increase in the power of the gamma oscillation up to 101±36.6% after 45 minutes (n=10; p>0.05). The agonist, Bay K 8644 (1uM) caused a significant decrease in the power of the gamma oscillation of 23±5.1% after only 15 minutes (n= 8; p<0.01). A further significant decrease in power of 53±6.5% (n=8; p< 0.01) was seen after 30 minutes application. These results suggest that modulation of the sIAHP can affect kainate induced gamma frequency activity. In particular the L-type calcium channel agonist Bay K 8644 significantly disrupted gamma oscillations. These results suggest that the sIAHP has a direct effect on the behaviour of an oscillating network.

In memory of the late E.H. Buhl.

68.07

A PHARMACOLOGICAL GAMMA TO BETA FREQUENCY SHIFT IN OSCILLATIONS IN THE RAT HIPPOCAMPAL SLICE *IN VITRO*

M.J. Gillies1, M.O. Cunningham1, R.D. Traub2, C. Davies3, E.H. Buhl1 & M.A. Whittington1
1. School of Biomedical Sciences, Worsley building, University of Leeds, Leeds, UK, LS2 9NL2. Department of physiology & Pharmacology, SUNY Health Sciences, Brooklyn, NY11203, USA3. GSK, 3rd Avenue, Harlow, Essex, UK, CM19 5AW.

Beta frequency oscillations (15-30Hz) in the neocortex in vivo follow stimulus-evoked gamma frequency oscillations (30-80Hz). Psychophysiological tests in humans suggest beta oscillations represent a memory consolidation phase of stimulus perception. in vitro, tetanically-induced gamma->beta shifts are associated with an increase in EPSP amplitude, supporting this conjecture. We present here a model of a pharmacological, persistent gamma->beta shift that shares some of the characteristics of tetanically-induced gamma->beta. Animals were humanely sacrificed under anaesthesia, transcardially perfused with sucrose ACSF, and 450um slices obtained. Gamma oscillations were induced in the stratum pyramidale of CA1 by application of mGluR agonist DHPG. Additional application of [10-20uM] 5-HT induced a shift in peak frequency to the beta range, localised to the SP of CA1. Beta oscillations were associated with an increase in EPSP amplitude, and an increase in pyramidal cell action potential firing. In contrast to tetanically-induced gamma->beta, changes in slow AHP did not appear to play an important role in inducing a frequency shift in this model.

In memory of the late E.H. Buhl.

68.08

CHARACTERISATION OF HIGH FREQUENCY ACTIVITY IN THE MEDIAL ENTORHINAL CORTEX *IN VITRO*

Mark O. Cunningham1, Ceri H. Davies2, Roger D. Traub3, Eberhard. H. Buhl1 & Miles A. Whittington1
1. Department of Biomedical Sciences, University of Leeds, Worsley Building, Leeds, UK.2. GlaxoSmithkline, Harlow, Essex, CM19 5AW, UK3. Departments of Physiology and Pharmacology, and Neurology, State University of New York Downstate Medical Centre, 450 Clarkson Avenue, Box 31, Brooklyn, NY 11203.

Fast network oscillations in the hippocampus which range from approximately 150 to 250 Hz are termed ripples. They are thought to be due to simultaneous excitation of pyramidal cells and interneuronal networks and represent IPSPs on the somata of the pyramidal cells due to feedback from interneurones. However, it has been hypothesized that gap junctions located between axons of principal neurons mediate ripples observed *in vitro*.
Extracellular recording techniques in the medial entorhinal cortex (mEC) slices revealed ripples only in layer III of the mEC. They had a mean frequency of 192.5 ± 7.2 Hz (mean ± S.E.M.) and were approximately 100-200 mV in amplitude (n=3). Ripples were observed in the absence of synaptic transmission (ACSF [Ca++]0), had a mean frequency of 215.5 ± 11.8 Hz (n=5) and also displayed similar amplitude range to those observed in physiological ACSF. There was no significant difference in the frequency in either condition ($P > 0.05$). In the presence of gap junction blockers, octanol (500 mM) and carbenoxolone (200 mM), ripples were suppressed.
In agreement with previous work these ripples are located in a layer of the mEC that exhibits principal neuron electrical coupling, supporting the notion that gap junctions are crucial in the generation of these ripples.

This work was supported by the MRC and GSK plc.

69

THE BIOLOGY OF OLIGODENDROCYTE GENERATION AND MIGRATION

Monique Dubois-Dalcq
Department of Neuroscience, Pasteur Institute, Paris, France.

Rapid progress in neural stem cell biology has prompted us to investigate how different signalling pathways control multipotential neural precursor (NP) growth, the generation of oligodendrocyte progenitors (OP) and their migration toward the rodent forming white matter. Endogenous Nrg1 neuregulins enhance NP growth whereas inhibition of Nrg1 signalling by soluble Erb receptor favors the initiation of oligodendrocyte differentiation. The latter also requires the instructive influence of Sonic hedgehog which induces oligodendrocyte specific transcription factors. Many OP originate from Shh expressing regions in ventral diencephalum at E14 and others are generated from the neocortex which starts to express Shh at E17. Shh not only triggers oligodendrocyte fate but also enhances OP mitosis, favoring expansion of this lineage. The chemokine receptor CXCR4 expressed on NP and OP can signal radial migration and/or directed chemotaxis toward CCL12, an alpha chemokine secreted by meninges and neurons in vivo. NP migration is facilitated by the PSA moiety on NCAM as NP engineered to sustain PSA expression can generate migrating OP and oligodendrocytes which down regulate the transgene only when they start to myelinate. Manipulating molecular signals inducing fate and migration of NP may help design strategies to enhance myelin repair in Multiple Sclerosis and Leukodystrophies.

AUTHOR INDEX

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

X

Y

Z