PLENARY LECTURES

L1 Cellular basis of neurometabolic coupling and its relevance for functional brain imaging

Magistretti P.J.

Brain and Mind Institute, Ecole Polytechnique Federale de Lausanne (EPFL), Switzerland; Centre de Neurosciences Psychiatriques, CHUV and Universite de Lausanne, Switzerland

Considerable progress has been made recently in the understanding of the cellular and molecular mechanisms that underlie the coupling between neuronal activity and glucose utilization by the brain. A central role in this coupling is played by astrocytes, which are strategically positioned through (a) processes that largely ensheath synapses and express receptors and reuptake sites for various neurotransmitters including glutamate, and (b) through other processes, the astrocytic end-feet, which surround intraparenchymal capillaries and express, among other molecules, glucose transporters. The essential steps in this coupling involve the sodium-coupled reuptake of glutamate by astrocytes and the ensuing activation of the Na-K-ATPase. This process triggers glucose uptake and its glycolytic processing, resulting in the release of lactate from astrocytes, which fuels the neuronal energy demands associated with synaptic transmission. A large body of *in vitro* and *in vivo* experimental evidence from our group as well as from others, supports this model often referred to as „the astrocyte-neuron lactate shuttle". This body of evidence provides a molecular and cellular basis for interpreting data obtained with functional brain imaging studies.

L2 Extracellular proteolytic signalling in neuronal plasticity

Kaczmarek L.

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Understanding of c-Fos/AP-1 transcription factor has been great challenge in neurobiology. We have documented its expression patterns in context of neuronal plasticity, including learning and memory, and identified TIMP-1 (tissue inhibitor of matrix metalloproteinases) as AP-1 target in the brain. We have shown that TIMP-1-dependent MMP-9 (matrix metalloproteinase-9) is upregulated in the dentate gyrus (DG) neurons in response to kainate-evoked seizures. This upregulation was observed at the level of mRNA abundance and its apparent translocation towards the activated dendrites. Furthermore, enzymatic activity of the MMP-9 was markedly increased throughout the dentate gyrus and dendrites of the granule neurons. Such selective, limited to the dentate gyrus, response to kainate is rare and of great interest, since the DG is the only part of the hippocampus that is spared of neurodegeneration and undergoes plastic changes. We have found that MMP-9 may be directly involved in breaking down beta-dystroglycan at the synapse, and may play a role in a retrograde synaptic signaling. Recently, we have found further support for synaptic localization of the MMP-9 as well as functional evidence for MMP-9 to play a role in neuronal plasticity, including learning and memory. In conclusion the aforementioned results and considerations raise an intriguing possibility that TIMP-1/MMP-9 extracellular proteolytic system may act as an AP-1 target in neuronal plasticity.

L3 Becoming a neuron: Molecular mechanisms underlying neurogenesis

Miller F.

Hospital for Sick Children, Toronto, Canada; University of Toronto, Toronto, Canada

The mechanisms that underlie the genesis of the mammalian nervous system from embryonic multipotent precursors are still largely undefined. This lecture will focus upon two different aspects of this issue. The first part of the lecture will focus upon the way that growth factor cues encountered in the environment of embryonic cortical precursors direct the differentiation of neurons versus glial cells. The second part of the lecture will focus upon characterization of a novel, multipotent neural crest-related precursor cell from skin, termed SKPs, and will describe both our ongoing work addressing the basic biology of these precursors, to our work asking whether such a precursor could be used therapeutically for the damaged or degenerating nervous system.

L4 The ascidian larva: The neurobiology of a model chordate and the evolutionary origins of the vertebrate brain

Meinertzhagen I.A.

Dalhousie University, Halifax, Canada

Many secrets of the vertebrate brain lie in its ancestry from the brains of basal chordate groups, such as ascidians. Among the latter, the CNS of the tadpole larva of the sea squirt *Ciona intestinalis* provides a powerful model. With little more than 330 cells, two-thirds within its brain or sensory vesicle, the larval CNS of Ciona is a chordate nervous system in miniature. Neurulation and its genetic basis, as well as the gene expression territories of this tiny constituency of cells, all follow a chordate plan from a neural plate, giving rise to clear structural homologies with the vertebrate brain. Recent advances in documenting the structure and function of this tiny brain are fueled by the release of the genome and EST expression databases and by the development of methods to transfect embryos by electroporation. Immediate prospects to test the function of neural genes are based on the isolation of mutants, as well as for the disruption of gene expression by morpholino oligo-nucleotides. Coupled to analyses of larval swimming, optophysiological methods offer the additional prospect to analyze the function of a CNS built on a vertebrate plan, adopting the cell-by-cell approach possible in invertebrate ganglia. Examples of advances in the anatomy and neurobiology of the Ciona larval will help broaden appreciation for the opportunities this tiny brain provides to workers in neuroscience.

L5 The multiple effects of mood stabilizers and antidepressants on neuroprotection: Translating basic findings into clinical practice

Young, L.T.

Centre for Addiction and Mental Health, University of Toronto, Ontario, Canada

Bipolar disorder has been well characterized clinically for many years and has some unique responses to medication with an excellent response in a subgroup of patients. Unlike several other psychiatric disorders such as depression and schizophrenia, however, pathophysiological models for bipolar disorder have been lacking. Studies on postmortem brain and on the molecular pharmacology of drugs like lithium and valproate have recently provided some very compelling answers. Techniques including the study of signal transduction, neuroprotection, and the application of genomics have been particularly helpful. In this presentation, work from the author's laboratory and other centres will be reviewed to illustrate some of the advances which have been made with the techniques in understanding the pathophysiology of bipolar disorder and the mechanism of action of mood stabilizing drugs. Several themes have emerged which include: specific and sustained effects at multiple targets in signal transduction pathways, shared targets between agents such as lithium and valproate, identification of several key neuroprotective target genes, prevention of cell loss and damage after treatment with agents such as lithium and valproate. These data strongly support the work to identify molecules that target these pathways and phenomenon as mood stabilizing agents. Furthermore, the work supports the use of established mood stabilizing agents beyond their roles in the treatment of mania and depression.

L6 Pain, learning, and brain plasticity

Flor H.

Dept. of Neuropsychology and Clinical Psychology at the University of Heidelberg, Central Institute, Mannheim, Germany

Recent neuroscientific evidence has revealed that the adult brain is capable of substantial plastic change in areas that were formerly thought to be modifiable only during early experience. These findings have implications for our understanding of chronic pain. Functional reorganization in several brain areas related to the processing of pain was observed in neuropathic and musculoskeletal pain. In chronic low back pain and fibromyalgia patients the amount of reorganizational change increases with chronicity, in phantom limb pain and other neuropathic pain syndromes cortical reorganization is correlated with the amount of pain. These central alterations may be viewed as pain memories that influence the processing of both painful and nonpainful input to the brain. Learning processes that contribute to the development of pain-related memory traces are predominantly implicit and involve processes such as sensitization, operant and classical conditioning or priming. Cortical plasticity related to chronic pain can be modified by behavioral interventions that provide feedback to the brain areas that were altered by pain memories. These behavioral interventions can be enhanced by pharmacological agents that prevent or reverse maladaptive memory formation.

SYMPOSIUM I
METABOTROPIC GLUTAMATE RECEPTORS: ROLE IN NEURODEGENERATION AND NEUROPROTECTION

S1.1 Role of metabotropic glutamate receptor 1 in neuronal apoptosis

Pshenichkin S., Dolinska M., Klauzinska M., Luchenko V., Grajkowska E., Wroblewski J.T.

Dept. of Pharmacology, Georgetown University, Washington, USA

Group-I metabotropic glutamate receptors, mGluR1 and mGluR5, known to regulate intracellular calcium homeostasis through their G protein-mediated coupling to phospholipase C, have been often implicated in various models of neuronal toxicity. While mGluR5 may exert neuroprotective actions, the role of mGluR1 in neuronal death is unclear. Using primary cell cultures, we demonstrate that mGluR1 is endowed with intrinsic toxic properties, and causes neuronal apoptosis, which depends on the level of receptor expression but not on the agonist-stimulated receptor activity. In fact, mGluR1 stimulation by its endogenous agonist glutamate abolishes the toxic receptor action and promotes cell survival. Such properties are characteristic of a heterogeneous family of dependence receptors which control neuronal apoptosis. As a dependence receptor, mGluR1 mediates neuronal death in response to reduced glutamate concentrations, but promotes survival in response to the trophic action of glutamate. Similarly to other dependence receptors, its mechanism of action may involve the proteolytic cleavage of the receptor intracellular C-terminal domain. Our results reveal a new dual role for mGluR1 and a new mechanism of its action that may play a crucial role in the development of the nervous system and may participate in cellular responses to toxic stimuli.

S1.2 Behavioural characterisation of noncompetitive mGluR1 and mGluR5 antagonists

Danysz W., Dekundy A., Gravius A., Pietraszek M., Sevostianova N.

Merz Pharmaceuticals, Frankfurt am Main, Germany

Recently, metabotropic glutamate receptors have gained a great deal of interest as therapeutic target. We performed a verification of the therapeutic potential of mGluR1 and mGluR5 antagonists (both group I) using selective antagonists such as EMQMCM and MTEP/MPEP respectively. EMQMCM produced the most promising effects in animal models of anxiety (e.g., context freezing or fear potentiated startle), depression (e.g., swim test), cocaine abuse (sensitization), and pain (e.g., formalin pain). For antagonists of mGluR5 clear effects were obtained in models of anxiety, depression, alcohol abuse (e.g., sensitization), L-DOPA-induced dyskinesia and pain. No evident activity was seen in models of Parkinson's disease (e.g., haloperidol-induced catalepsy or rotation after SNc system lesion). A separate set of experiments was devoted to study potential side effects related to motor co-ordination, psychotomimetic-like activity and learning impairment. Ataxia was observed following moderate dose of EMQMCM and high dose of MTEP. On the other hand, an enhancement of psychotomimetic-like effect of MK-801 was seen in prepulse inhibition test after MTEP but not EMQMCM. In learning tasks, both agents produced at high doses task dependent impairment. Thus, both mGluR1 and mGluR5 antagonists show a promising profile, however they are not free of side effects as previously suggested.

S1.3 NAAG and mGluR3 receptor in naturopathic pain and schizophrenia animal models

Wroblewska B., Olszewski R.T., Bukhari N., Bzdega T., Neale J.H., Kozikowski A.P., Zhou J.

Georgetown University, Washington DC, USA

NAAG is cleaved by two extracellular peptidases: GCPII and GCP III (yielding glutamate and NAA). Since NAAG is co localized with many neurotransmitters the effects on presynaptic release maybe important in physiology and pathology. We developed urea-based compounds which are potent NAAG peptidase inhibitors. Intrathecal and intravenous administration of these inhibitors suppressed the expression of cFos IMR induced in paw formalin pain model suggesting an action on sensory spinal transmission. Peptidase inhibitors also attenuated level of mechanical allodynia induced by partial sciatic nerve ligation – the effects were blocked by LY341495 suggesting that NAAG (agonist of mGluR3) activates mGluR3 receptor to produce an analgesic effect in naturopathic and inflammatory pain. Stimulation of group II mGluRs decreases the disruptive effects of phencyclidine on working memory, stereotypy, and locomotion in rats. Peptidase inhibitors significantly reduced several of PCP-induced motor activations. Group II antagonist, LY341495, administered with peptidase inhibitors prior to PCP treatment reversed the effects of peptidase inhibitors indicating the involvement of mGluR3 receptors. These data support the view that NAAG peptidase inhibitors may represent a potential therapeutic approach in schizophrenia as modeled by PCP and may be useful tools in pain studies.

S1.4 MTEP – a new, selective antagonist of the metabotropic glutamate receptor subtype 5 (mGluR5) – produces antiparkinsonian-like effects in rats

Pilc A., Ossowska K., Konieczny J., Wolfarth S.

Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

The aim of the present study was to examine a potential antiparkinsonian-like action of 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), a new non-competitive antagonist of mGluR5 in the rat models. This compound has affinity for mGluR5 in a nanomolar concentration range and seems to be superior to the earlier known antagonists in terms of its specificity and bioavailability. Catalepsy and muscle rigidity induced by haloperidol were regarded as models of parkinsonian akinesia and muscle rigidity, respectively. MTEP at doses between 0.5–3 mg/kg i.p. decreased the haloperidol-induced muscle rigidity measured as an increased muscle resistance of the rat's hind leg in response to passive extension and flexion at the ankle joint. The strongest and the longest effect was observed after the dose of 1 mg/kg. MTEP (0.5–3 mg/kg i.p.) decreased also the haloperidol-increased electromyographic (EMG) activity recorded in the gastrocnemius and tibialis anterior muscles. MTEP (3 and 5 mg/kg i.p.) dose-dependently inhibited the catalepsy induced by haloperidol. The present study confirms earlier suggestions that the antagonists of mGluR5 may possess antiparkinsonian properties. However, selective mGluR5 antagonists may be more effective in inhibiting parkinsonian muscle rigidity than parkinsonian akinesia.

S1.5 Role of group I metabotropic glutamate receptors in homocysteine neurotoxicity

Zieminska E., Stafiej A., Kozlowska H., Lazarewicz J.W.

Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Homocysteine (HCY) is a recently recognized risk factor in neurodegeneration. Among the proposed mechanisms of HCY-evoked neurotoxicity the role of the NMDA receptor-mediated excitotoxicity has been suggested. Our data from *in vivo* experiments revealed that HCY induces mobilization of intracellular calcium, which is mediated by the group I metabotropic glutamate receptors (mGluRs GI), while *in vitro* studies utilizing primary cultures of rat cerebellar granule cells (CGC) demonstrated that HCY induces inositol phosphate formation, also partially sensitive to mGluRs GI antagonists. Antagonists of both, mGluR1 and mGluR5 almost completely prevented acute and subchronic HCY-evoked degeneration of CGC only in the presence of the NMDA receptor antagonists, which alone exhibited weak neuroprotection. These data point to obligatory synergism of the mGluRs I and NMDA receptors in mediating HCY neurotoxicity. HCY induced only a slight increase in the intracellular calcium concentration, and strongly activated caspases 3 and 12. These effects were sensitive to mGluR GI and NMDA receptor antagonists. Although the exact mechanisms of participation of mGluRs GI and NMDA receptors in HCY neurotoxicity remain unclear, our data suggest that they include activation of caspases, whereas calcium signaling seems to be less pronounced.

SYMPOSIUM II
PROTEOMICS IN NEUROSCIENCES

S2.1 Proteome of the central nervous system in drug dependence

Silberring J., Noga M., Suder P., Bodzon-Kulakowska A.

Faculty of Chemistry and Regional Laboratory, Jagiellonian University, Krakow, Poland

Drug dependence is a serious health problem in developed countries and its etiology is still unknown. A solid evidence has been reported that drugs of abuse may disturb metabolism of neuropeptides and proteins, thus, affecting protein patterns. The aim of the work was the search for, and identification of the potential markers of drug dependence after morphine administration. The research was focused on the identification of molecular mechanisms involved in these processes and clarification of the role of particular proteins in drug dependence. In particular, we developed the cell model of drug dependence involving rat cortical cells in primary culture. Here, with application of TCA/acetone precipitation, Laemmli 2-D system, and nano-LC-MS/MS, we were able to identify eleven possible morphine dependence markers. Proteins assigned with the accession numbers P20788, P04906, P07895, P39069, Q9WTV5, P35213, P35291, Q8VI04 P35291 were found to be down-regulated after morphine administration, whereas those designated as P11598, P46462, Q06547 were up-regulated in the morphine-treated animals, as compared to controls. To our knowledge, this paper reveals, for the first time, the potential candidates for the dependence markers found in the rat brains using proteomics approach. Further extensive work is necessary to reveal, which changes are physiologically relevant.

S2.2 Application of proteomic approaches in psychiatric disorders
Ekman R.

Institute of Clinical Neuroscience, Unit Neurochemistry, Goteborg University, Sweden

Maintaining brain health and plasticity throughout life is an important public health goal. Accumulating evidence suggest that burnout or exhaustion is not merely a subjective experience, but may lead to changes in the brain-endocrine-immune axes and play a role in progression of autoimmune-neurodegenerative diseases, as well as in depression and post traumatic stress disorders. The progress in proteomics and peptidomics the last years offers us new challenges to study changes in the protein- peptide synthesis and metabolism. These strategies offer new tools to follow post-translational modifications and other disturbed chemical processes that may be indicative of pathophysiological alteration(s). The talk will address different practical aspects of applications of mass spectrometry in clinical neuroscience, as well as protein microarray technology, illustrated by examples from our laboratory.

S2.3 Proteomics approaches in search for aberrant protein in brain
Lubec G.

Department of Pediatrics, Division of Pediatric Neuroscience, Medical University of Vienna, Austria

Abstract not received

S2.4 Neuroproteomics of retinas regenerating ganglion cell axons *in vitro*
Koenig S., Rose K., Thanos S.

University of Muenster, Muenster, Germany

In this work, differential proteomic analyses were performed using rat (*Rattus norvegicus*) and monkey (*Callithrix jacchus*) retinas as a model to examine the posttraumatic ability to regenerate axons. The study intended to investigate if the initial axon growth *in vitro* is associated with the synthesis of new or modulation of pre-existing proteins that can be detected by the proteomic techniques based on 2D-gel electrophoresis and mass spectrometric peptide fingerprinting and sequencing. Organ culture systems of rat and monkey retina were established. Fresh retinas obtained from cadavers of various ages were explanted with the ganglion cell layer facing a growth-supporting matrix (regenerative group). Laminin-1 proved to be best suited for that purpose. Explants with no contact to laminin-1 and non-explanted retinas served as controls (non-regenerative group). Total protein extracts from both groups were separated by 2D-PAGE and landmark and differentially expressed proteins were identified depending on the age of the animal or regeneration status. Particularly, calmodulin, GAP43, alphaA-crystallin, FABP, and CRABP seem to be selectively up-regulated after axonal growth. Ongoing analysis aims at a deeper understanding of the role of these candidates for the regeneration process and growth cone formation. Supported by the Deutsche Forschungsgemeinschaft.

SYMPOSIUM III
NEURONAL MECHANISM OF MAMMALIAN CIRCADIAN TIMING SYSTEM

S3.1 Locus of function in the circadian visual system
Morin L.P.

Dept. of Psychiatry, Stony Brook University, Stony Brook, NY, USA

The suprachiasmatic nucleus (SCN) sits within the context of the visual system. It receives photic information directly from the retina and indirectly from the retinorecipient intergeniculate leaflet (IGL). As research reveals the intricacies of circadian clock mechanics in the SCN, so does elaboration of the increasingly complex anatomical context in which the circadian clock operates. This presentation will provide a glimpse of this context in the hamster, extending from the retinal ganglion cells that project to the SCN and other visual nuclei, including the IGL. The extensiveness, bilaterality and reciprocity of IGL connections with much of the brain imply that this nucleus may have specific functions related to eye movement regulation. The anatomy also demonstrates linkages between the IGL and regions contributing to sleep and vestibular function, suggesting functional involvement of the IGL with these two systems as well. Although specific connections between the sleep or vestibular and circadian systems have, in some instances, been established, the IGL anatomy suggests that it contains at least two cell populations, one concerned with circadian rhythm regulation and another likely to be concerned with visuomotor, sleep and equilibrium-related functions. Supported by NIH grant NS22168 from NIH and National Space Biomedical Research Institute grant HPF0027 *via* NASA agreement NCC 9-58.

S3.2 Electrophysiological properties of the suprachiasmatic nucleus and its responsiveness to light
Meijer J.H.

Dept. of Neurophysiology, Leiden University Medical School, Leiden University, Leiden, Netherlands

Neurons of the suprachiasmatic nucleus have a genetic basis for rhythm generation. The rhythmic production of clock gene products (or proteins) results in rhythmicity at the level of the membrane of SCN neurons, rendering a rhythmic output of electrical impulse activity. As such, the SCN imposes its rhythm on other brain structures and functions as a pacemaker, driving rhythmicity in other parts of the central nervous system. The electrical activity of SCN neurons is elevated by retinal illumination. Recordings in freely moving rats with stationary electrodes show that light responses are sustained, unlike other light responses in the CNS, and are dependent on light intensity. The characteristic light response of SCN neurons ensures that the pacemaker entrains to the environmental light-dark cycle and is able to track changes in day length. In the rat, the ventral SCN is directly driven by retinal fibres and, interestingly, responds with an immediate shift in phase to a change in the light dark cycle. The dorsal SCN, on the other hand, responds indirectly and resets more slowly. The dorsal and ventral SCN communicate by a GABA-ergic pathways which inhibits the ventral but, unexpectedly, excites the dorsal SCN. The asymmetric coupling between dorsal and ventral SCN may explain that the ventral SCN is dominant is setting the final phase of the clock.

S3.3 Reconfiguring cellular ensembles within the suprachiasmatic nucleus

Schwartz W.J.

Dept. of Neurology, University of Massachusetts, Medical School, Worcester, MA 01655, USA

The circadian clock in the suprachiasmatic nucleus (SCN) is composed of multiple single-cell circadian oscillators, and a challenge now is to learn how individual cells are assembled to create an integrated tissue pacemaker that can orchestrate the temporal programs of whole organisms. By measuring SCN gene expression (*in situ* hybridization) as an assay of clock activity, we have found that assembled cellular oscillators can assume different configurations within the SCN, giving rise to unusual bimodal locomotor activity patterns. Thus, in hamsters maintained in constant light, splitting of the single *circa*-24 h activity bout into two *circa*-12 h components appears to be the consequence of a paired SCN that is reorganized into two oppositely-phased, left- and right-sided circadian pacemakers. In rats exposed to an artificially short light-dark cycle, the simultaneous expression of two stable circadian motor activity rhythms with different period lengths corresponds to the desynchronization of circadian pacemakers in the ventrolateral and dorsomedial subdivisions of the SCN (as previously defined by regional differences in their cyto- and chemo-architecture and topography of afferents and efferents). These kinds of reconfigurations (left/right, dorsal/ventral) of regional oscillators should provide a powerful approach for understanding the tissue organization and outputs of the SCN in intact, behaving animals.

S3.4 Electrophysiological properties of the intergeniculate leaflet – the element of nonphotic entrainment pathway

Blasiak T., Lewandowski M.H.

Institute of Zoology, Jagiellonian University, Krakow, Poland

The pacemaker for the most of the circadian rhythms is located in the suprachiasmatic nuclei of the hypothalamus (SCN). Phase of the rhythm generated by SCN undergoes adjustment by the influences from the environment as well as internal signals from the body. There are two main neuronal pathways that participate in this entrainment process. The first one, originating in the retina, conveys to SCN information about the presence of external light – photic input. The second one, originating in the intergeniculate leaflet of the lateral geniculate nucleus (IGL) is involved in the adjustment of the circadian pacemaker by factors like a general arousal, ongoing activity of the animal and some pharmacological manipulations (motor activity, food intake, administration of benzodiazepines, etc.) – nonphotic inputs. At the same time IGL receives direct retinal innervation that can influence its output to the SCN. It has been suggested that both inputs (photic and nonphotic) interact in the IGL, though details of this process are unknown. In the lecture, a review of the electrophysiological experiments on IGL of the rat is given. Basing on this data, and nauroanatomical and behavioral observations, it is hypothesized how light, at the level of IGL neuronal network, can gate flow of nonphotic information to the circadian pacemaker.

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SYMPOSIUM IV
NEURONAL MECHANISMS INVOLVED IN PARKINSON'S DISEASE

S4.1 Is oxidative stress in Parkinson's disease mediated by a change in the ferritin structure?

Friedman A.

Medical University, Warsaw, Poland

Oxidative stress is one of the possible pathways of neurodegeneration leading to Parkinson's disease. In the production of free radicals iron plays a crucial role by inducing Fenton reaction. The concentration of iron in PD compared to control as well as the source of this iron available for Fenton reaction in parkinsonian substantia nigra (SN) remain controversial. Our studies with the use of Mossbauer spectroscopy did not confirm an increase of the concentration of iron in PD SN and demonstrated that most of iron within SN is bound to ferritin. A comparison of the structure of ferritin from SN of patients who died with autopsy proven PD, patients who died without clinical symptoms of PD but who had Lewy bodies in SN (Incidental Lewy Bodies cases ILB pre-clinical stage of PD), and controls (no clinical symptoms of PD, no Lewy bodies at autopsy) revealed a significant decrease of the concentration of L ferritin both in PD and ILB vs. control. As L ferritin is related to a safe storage of iron within this protein, a decrease of L ferritin may be a starting point for an efflux of iron from the ferritin shell. This iron may become available for Fenton reaction leading to death of nervous cells in SN.

S4.2 Slowly progressing degeneration of dopaminergic nigrostriatal neurons induced by a herbicide – paraquat (PQ) administration in rodents

Ossowska K.

Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

An influence of the long-term PQ administration on the nigrostriatal system was examined in rats. PQ was injected at the dose of 10 mg/kg i.p. for 4–24 weeks. After the 4-week treatment PQ reduced the number of tyrosine hydroxylase (TH)-immunoreactive neurons of the rostro-central substantia nigra and then (after 24 weeks) across the whole length of this structure by 26%. Striatal levels of dopamine, its metabolites and turnover were elevated (4–8 weeks), then returned to control values and dropped by 25–30% after 24 weeks. [3H]GBR12,935 binding to dopamine transporter in the striatum was decreased after 4–8 weeks, then returned to control values (12 weeks), and was lowered after 24 weeks. Twenty four-week PQ administration decreased also the striatal TH level. Moreover, PQ activated 5-HT and noradrenaline systems during the first 12 weeks but no decreases in levels of these neurotransmitters were found after 24 weeks. The results seem to suggest that the long-term PQ administration produces a slowly progressing lesion of nigrostriatal neurons and delayed deficits in dopaminergic transmission.

This study was supported by the State Committee for Scientific Research (KBN) as the solicited research project PB2-MIN-001/PO5/18.

S4.3 Dopamine and adenosine receptor interaction as basis for the treatment of Parkinson's disease

Morelli M.[1], Carta A.R.[1], Simola A.[1], Tronci E.[1], Pontis S.[1], Pinna A.[2]

[1]Dept. of Toxicology, University of Cagliari, Cagliari, Italy; [2]CNR Institute for Neuroscience, Cagliari, Italy

The search of alternative therapies for the treatment of PD is very active and adenosine A2A receptors, for their negative interaction with dopamine D2 receptors have became particularly attractive. In this study we report the results obtained with the A2A receptor antagonist SCH 58261 in the unilateral 6-hydroxydopamine (6-OHDA) rat model of PD and in the tacrine model of PD tremor. In 6-OHDA lesioned rats, acute administration of SCH 58261 counteracted the motor deficits induced by the lesion and potentiated the turning behavior induced by L-DOPA whereas in the tacrine model of PD, SCH58261 antagonised tacrine-induced bursts of tremulous jaw movements. In chronic studies, SCH 58261 + L-DOPA in contrast to L-DOPA alone, did not induce long-term increase in GAD67 mRNA in striatum and globus pallidus whereas the increase in GAD67 mRNA produced by the dopaminergic lesion in the substantia nigra was counteracted by SCH 58261 + L-DOPA. The data suggest that A2A receptor antagonists may be beneficial in motor impairment and tremor which characterize PD. Furthermore the neuronal modifications observed in rat basal ganglia after chronic treatment with SCH 58261 + L-DOPA as compared to L-DOPA alone, suggest that such treatment might not produce detrimental long-term responses in basal ganglia areas

S4.4 Caffeine, adenosine A2A receptors, and neuroprotection in PD

Schwarzschild M.A.

Massacusetts General Hospital, Boston, USA

A remarkable convergence of epidemiological and laboratory data has raised the possibility that caffeine reduces the risk of developing Parkinson's disease (PD) by blocking adenosine A2A receptors and preventing the degeneration of nigrostriatal dopaminergic neurons. Several studies of large prospectively followed populations have demonstrated that the consumption of coffee or tea (but not decaffeinated coffee) is associated with a lower the risk of developing PD. In animal models of PD, caffeine and more specific antagonists (or genetic knockout) of the A2A receptor can protect dopaminergic neurons. Other studies demonstrating protection by A2A receptor inactivation in animal models of stroke, Huntington's disease and Alzheimer's disease suggest a more global role of A2A receptors in neuronal injury and degeneration. Although the cellular and molecular mechanisms by which A2A receptors contribute to neuronal death are not yet established, several intriguing possibilities have emerged. Now with initial clinical data substantiating the anti-parkinsonian symptomatic benefit of A2A receptor blockade, the prospects for a complementary neuroprotective benefit have enhanced the therapeutic potential of A2A antagonists in PD.

S4.5 Rodent models of L-DOPA-induced dyskinesia: What do they tell us?

Cenci-Nilsson M.A.

Lund University, Lund, Sweden

Dyskinesia is a major complication of L-DOPA treatment in Parkinson's disease, but its underlying mechanisms are poorly understood. We have characterized models of L-DOPA-induced dyskinesia in rats and mice. Dopamine (DA) denervating lesions are performed by unilateral injection of 6-hydroxydopamine (6-OHDA) in the nigrostriatal pathway. The animals are then treated with daily doses of L-DOPA that are sufficient to ameliorate akinetic features without inducing overt signs of dyskinesia upon their first administration. During a few weeks of treatment, most animals develop abnormal involuntary movements (AIMs), which mainly affect the side of the body contralateral to the lesion. These movements are not seen in animals that receive chronic treatment with long-acting DA agonists. The severity of L-DOPA-induced rodent AIMs is significantly reduced by the acute administration of compounds that have antidyskinetic efficacy in parkinsonian primates. We are using these rodent models in order to identify biochemical and molecular changes that are associated with a dyskinetic motor response to L-DOPA. The lecture will present the main findings that have emerged from these studies. The proposed pathophysiological model will attempt to link alterations of striatal dopamine release with post-synaptic perturbations of intracellular signalling and gene expression, finally leading to an abnormal output from the basal ganglia nuclei.

SYMPOSIUM V
MOLECULAR BASIS OF OPIOID ADDICTION

S5.1 Mu opioid receptor regulation by internalization and transcription

Hollt V.

Deptartment of Pharmacology and Toxicology, Otto von Guericke University, Magdeburg, Germany

In contrast to fentanyl or opioid peptides morphine is not able to cause mu opioid receptor (MOP-r) internalization. We recently demonstrated in MOP-r expressing HEK293 cells that the endocytotic potencies of a wide variety of opioids are negatively correlated with their ability to cause receptor desensitization/tolerance. This indicates that endocytosis counteracts tolerance by inducing fast receptor reactivation by receptor recycling. MOP-r endocytosis (e.g., by the opioid peptide DAMGO) is preceded by a rapid and strong phosphorylation of Ser-375 at the COOH-tail. Mutation of Ser-375 to alanine inhibited the DAMGO-induced receptor internalization. In contrast, morphine which does not induce endocytosis causes a slow and less intense phosphorylation at Ser-375 of long duration. We showed recently that activation of phospholipase D2 (PLD2) is required for opioid-induced internalization. Another regulation of MOP-r occurs at the level of gene transcription. We found that transcription of the MOP-r gene can be enhanced by cytokines, such as IL-4, IL-6 and TNF-alpha in neuronal and/or immune cells. The up-regulation is mediated by transcription factors, such as AP-1, members of the STAT family (STAT-1; STAT-3, STAT-5) and/or NFkappa-B which bind to specific elements within the MOP-r gene promoter. There is strong evidence that the regulation of MOP-r by cytokines plays an important role in inflammatory pain.

S5.2 Involvement of the endogenous opioid system in drug addition
Maldonado R.

University Pompeu Fabra, Barcelona, Spain

Several studies have suggested that the endogenous opioid system could represent a common neurobiological substrate for the addictive properties of different drugs of abuse. The involvement of mu-opioid receptors in the addictive related behavioural responses induced by THC, nicotine and MDMA was explored by using knockout mice deficient in mu-opioid receptors and other components of the endogenous opioid system. The acute behavioural responses induced by THC and MDMA were not modified in these mutant mice, whereas a decrease in nicotine antinociception was observed in mu-knockout mice. Nicotine withdrawal was attenuated in mu-knockout mice. In contrast, cannabinoid withdrawal was not modified in mu-knockout mice, whereas it was attenuated in knockout mice deficient in the pre-proenkephalin gene and in double knockout mice deficient in mu and delta opioid receptors. In addition, the conditioned place preference induced by THC and nicotine was abolished in mu-knockout and pre-proenkephalin knockout mice. However, the rewarding effects of MDMA and its effects on the extracellular levels of dopamine in the nucleus accumbens were not modified in these mutant mice. Taken together, all these results show that mu-opioid receptors activated by endogenous opioid peptides derived from pre-proenkephalin, participate in the in the addictive properties of nicotine and THC. However, mu-opioid receptors are not involved in the rewarding effects of MDMA.

S5.3 Molecular mechanism of morphine tolerance: The role of glycogen synthase kinase 3 and cyclin dependent kinases
Rodriguez Parkitna J.M.

Dept. of Molecular Neuropharmacology, Inst. of Pharmacology, PAS, Krakow, Poland

Repeated administration of morphine is associated with the development of tolerance. We have found that i.t. administration of either a glycogen synthase kinase 3 (GSK3) inhibitor, bromoindirubin-3'oxime or a cyclin dependent kinase (CDK) inhibitor, roscovitine completely abolished tolerance to morphine analgesia. Administration of 10 mg/kg morphine i.p. to Wistar rats twice daily for eight days resulted in complete tolerance to its analgesic effects as measured by the tail flick test. When 1.41 pmol of roscovitine or bromoindirubin-3'-oxime was administered i.t. every day 15 min prior to morphine, the development of tolerance was blocked. Additionally, a single i.t. injection of 14.1 pmol of either kinase inhibitor was able to reverse already developed tolerance. The dose of the inhibitor required for reversing tolerance indicated that its effects on CDKs were crucial for this effect. Administration of either inhibitor has caused an increase in the abundance Ser9 phosphorylated GSK3 beta in morphine treated rats and reversal of morphine tolerance was always associated with an increase of phospho-GSK3 beta. A single i.t. injection of the inhibitor had no effect on phosphor-GSK3 beta abundance in naive rats, therefore chronic morphine treatment caused a “switch” in cellular signaling involving GSK3 beta and CDKs.
Supported by grant PBZ-KBN-033/P05/2000

S5.4 Cocaine – acute and repeated differentially influences the expression of PSA-NCAM-positive neurons in rat hippocampus
Wedzony K., Mackowiak M., Markowicz-Kula K., Fijal K.

Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Alterations in the PSA-NCAM expression are known to accomplish a variety of neuroanatomical rearrangements in the brain structure. Therefore we investigate whether cocaine administered acutely (15 mg/kg, i.p.) or repeatedly (15 mg/kg i.p., once a day for five consecutive days) alters PSA-NCAM expression. The number of PSA-NCAM immunopositive cells was determined at several time points after cocaine treatment: 6 h and 1, 2, 6, 10 days (acute treatment), or 6 h and 1, 2, 4, 6 days (repeated treatment). It was found that single injection of cocaine induced a time-dependent decrease in the number of PSA-NCAM cells in the dentate gyrus. The decrease was observed on 1 day after cocaine treatment and lasted for at least 6 days. In contrast, an increase in the number of PSA-NCAM positive cells in the dentate gyrus was observed 2 and 4 days after the last dose of repeated cocaine. It is concluded that cocaine can evoke long-lasting changes in the PSA-NCAM protein expression in the dentate gyrus and that the direction of cocaine induced PSA-NCAM changes depends on the regimen of cocaine administration. It is postulated that cocaine may have impact on hippocampal plasticity and subsequent processes that are controlled by plastic changes in the hippocampal structure.

SYMPOSIUM VI
ANIMAL MODELS OF BRAIN DISORDERS

S6.1 A Drosophila model to study the role of Presenilins in Alzheimer's disease
Boulianne G.L.

The Hospital for Sick Children, Toronto, Canada

Presenilins were identified as causative factors in familial Alzheimer's disease and also play an essential role in Notch signalling during development. Presenilins function in a multimolecular gamma-secretase complex, which cleaves transmembrane proteins including Notch and amyloid precursor protein. To gain further insight into the function of Presenilins we searched for presenilin interacting genes in Drosophila. Here we show that loss-of-function mutations in Fkbp13 suppress dominant presenilin phenotypes. We also find that Fkbp13 binds directly to Presenilin and that Presenilin protein, but not RNA, is reduced by >80% in Fkbp13 null mutants. Finally, we show that FK506, which binds Fkbp13, also reduces Presenilin and PEN-2 levels and thereby decreases gamma-secretase activity *in vivo*. Together, our data demonstrate that Fkbp13 is an essential component of the Presenilin pathway. We propose that Fkbp13 is required to stabilize Presenilin protein allowing for formation of a functional gamma-secretase complex.

S6.2 An endogenous neuroprotective compound – 1MeTIQ – prevents the cocaine addiction in self-administration model in rat: Neurochemical correlates

Antkiewicz-Michaluk L., Romanska I., Filip M., Michaluk J.

Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Cocaine is the most popular psychostimulant and abuse drug producing locomotor activation and rewarding effects through an increased dopaminergic transmission in mesolimbic structures. The endogenous 1,2,3,4-tetrahydroisoquinolines (TIQs) exist in mammalian brain and play physiological role as natural regulators of different neurotransmitter systems, in particular dopaminergic one. Our earlier papers have shown that the most interesting compound among of them is 1MeTIQ, expresses neuroprotective activity, and prevents the morphine addiction. In the light of above data it seemed of interest to examine the effect of 1MeTIQ on cocaine-induced self-administration and on dopamine (DA) and noradrenaline (NA), and their metabolite concentrations in rat brain structures using HPLC method. An animal model that seems to be the most adequate for studying the craving and relapse phenomenon is the self-administration procedures. The most interesting finding was that 1MeTIQ significantly inhibited self-administration of cocaine, and antagonized the biochemical changes of DA metabolism within VTA, NAc and NA metabolism in BrSt induced by priming dose of cocaine. In conclusion we suggest that partial agonist/antagonist activity of 1MeTIQ on A10 dopaminergic neurons is responsible for its anti-addictive properties.

S6.3 Animal models of schizophrenia as a neurodevelopmental disorder

Van Ree J.M.

Rudolf Magnus Institute of Neuroscience, Utrecht, the Netherlands

According to the neurodevelopmental hypothesis of schizophrenia, this disorder results from damage early in life that interacts with normal maturational events. We investigated a putative rat model for schizophrenia, in which the effects of early neonatal (postnatal day 7 (Pd7)) basolateral amygdala lesion were compared to those of a lesion later in life (Pd21). The reciprocal innovation between the basolateral amygdala and the prefrontal cortex became not mature before Pd13. Pd7 lesioned rats displayed behavioural disturbances later in life, i.e., locomotor stereotypy, diminished habituation, decreased social behaviour, decreased prepulse inhibition of acoustic startle response and altered response to stressful stimuli. These disturbances were not observed in the Pd21 lesioned rats, except for a disruption of social behaviour. In addition, the Pd7 lesioned rats were behaviourally hypersensitive for apomorphie and phencyclidine. D1-like and particularly D2-like, but not D3-receptor levels were reduced following a Pd7, but not a Pd21 lesion. This effect was found in the mesolimbic, but not the nigrostriatal dopamine system. Furthermore, dopamine turnover was increased in the mesolimbic, but not the striatal regions. Cannabinoid (CB1) receptor levels were increased in the striatal, but not in the mesolimbic regions. These and other data contribute to the validation of the neonatal amygdala lesion as an animal model for schizophrenia.

S6.4 Insect models of heavy metal neurotoxicity

Pyza E.

Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow, Poland

Toxic effects of heavy metals on the nervous system are well known in both vertebrates and invertebrates and have been connected to neurodegenerative changes observed in the brain. Since insects show similar mechanisms of transduction, transmission and processing of information in the nervous system they can be used to study effects of various substances, including toxic ones, on these processes. Flies have already been used as organism models in neurobiology to study basic processes in neurons and in neuronal networks, as well as to study synaptic and neuronal plasticity. Heavy metals; zinc, copper, lead and cadmium are present in the environment and accumulate in organisms, however, because of the blood-brain barrier their concentrations in the brain in low. They affect, however, concentrations of light elements in nerve cells including concentrations of Na, K, Cl, P, and S. Moreover, depending on their concentration, they affect cell morphology inducing cell swelling or shrinking. It was also observed, in case of Pb and Cd, that they abolish a circadian rhythm in neuronal plasticity of interneurons in the fly's visual system which are known to show rhythmic changes of axon sizes correlated with their activity.

SYMPOSIUM VII
THE NEURAL CORRELATES OF COGNITIVE FUNCTION IN MAN

S7.1 What kind of attention is impaired in neglect?

Marzi C.A.

Department of Neurological Sciences and Vision, University of Verona, Italy

I will describe our recent experiments carried out both on healthy and on brain-damaged participants with the aim of casting light on the neural mechanisms of two types of spatial attention: endogenous or controlled and exogenous or automatic. In all experiments we used a simple visual reaction time (RT) paradigm and two conditions of stimulus presentation, one requiring endogenous and the other one requiring an exogenous orienting of attention. We recorded event-related potentials (ERP) during performance of the task. RT was on average faster in the endogenous than in the exogenous condition and the amplitude of the P1 component of the ERP was correspondingly larger in the former condition. In contrast, the amplitude of the N1 component was larger in the latter condition. In a separate fMRI experiment we found an occipital activation in the endogenous and a frontal-parietal activation in the exogenous condition. In another ERP experiment we studied patients with spatial hemineglect and we found that there was a selective decrease of the amplitude of the N1 component in response to unilateral visual stimuli presented to the affected left-hemifield/right hemisphere. This suggests that the impairment of neglect patients concerns exogenous rather than endogenous attention.

S7.2 Electrophysiological correlates of memory
Nowicka A.

Nencki Institute of Experimental Biology, Warsaw, Poland

The role of the competent hemisphere in modulation of the cortical activity during memory tests was investigated. The divided-visual field paradigm was employed. It allowed the direct stimulation of one of the hemispheres. In working-memory study with verbal stimuli, alpha band desynchronization at frontal sites was stronger in the directly stimulated hemisphere and this effect was more pronounced in the left hemisphere (competent in verbal processing). Two ERP studies aimed at: (i) determining the sensitivity of the ERP repetition effects to the visual field of stimuli presentation and the type of visual information (verbal vs. non-verbal); (ii) the influence of handedness on ERP repetition effects to words. In both studies, repetition effects were observed in the late components of ERP recorded at frontal sites. Repetition effects, however, were present in the ERP data only in case of the direct stimulation of the competent hemisphere. Stimulation of the incompetent hemisphere resulted in lack of the repetition effect in either hemisphere.

S7.3 Neural correlates of long-term memory encoding in young adults, elderly adults and AD patients
Binder M., Urbanik A.S., Sobiecka B., Kozub J.

Jagiellonian University, Krakow, Poland

The aim of the study was to assess differences in the neural correlates of memory functions in pathological and normal aging using fMRI. Three groups were examined in a MR scanner: young and elderly healthy controls, and probable AD diagnosis subjects. In separate scanning sessions subjects memorized complex geometrical figures and paired-associates. Their memory performance was scored. Both control groups' behavioral results were better than for the AD group. Differences in observed brain activity were seen in frontal regions and occipital lobes, extending to temporal structures. For each group this pattern was dissimilar. For the AD group moderate activation in the occipital lobe was observed, but no activation in frontal lobes. In turn, the elderly group revealed weak activation in occipital lobes and activation in the frontal lobes. Finally, in the young adults group there was prominent activation in occipital lobes, as well as in the frontal lobes. Our study revealed differential patterns of brain activation in the studied groups during memory encoding. Our results suggest that a successful encoding requires an involvement of frontal lobes, that are probably responsible for strategic aspects of memory functions. Activity patterns in the elderly control group suggest that frontal lobes can compensate for the deteriorated visual memory which is known to decline over time.

S7.4 The neural correlates of behavior control
Regard M.

University Hospital Zuerich, Neuropsychology Unit, Zuerich, Switzerland

Clinical observations and studies in patients with focal brain lesions and in patients with non-substance addictions (e.g., eating disorder, gambling) suggest manifestations of impulse dyscontrol to depend upon side of hemispheric dysfunction. To investigate the functional and anatomical characteristics of the cortical regulation of impulse control, behavioral, physiological and anatomical studies were conducted with healthy controls and some patients with impulse control disorders. Testing consisted of neuropsychological tasks sensitive to frontal lobe function and of the registration of saccadic eye movements while solving lateralized tasks. Frontal regulatory circuits in healthy subjects were studied by repeated transcranial stimulations (rTMS) applied over the left and the right dorsolateral frontal area combined with PET. Furthermore, using rTMS over the same areas, we manipulated habitual responses. rTMS effects on regional cerebral blood flow and on behavior are in line with the clinical studies and support the notion of an asymmetrical frontal regulation of complex behavior as impulse control.

SYMPOSIUM VIII
FUNCTIONAL PLASTICITY OF THE CEREBRAL CORTEX

S8.1 The relationship between synaptic plasticity and experience-dependent plasticity studied in the barrel cortex
Fox K., Hardingham N., Wright N.

Cardiff University, Cardiff, UK

Studies into synaptic plasticity mechanisms over the past 20 years have revealed a wealth of detail about the molecular components governing and expressing plasticity. However, it is still unclear which of these mechanisms are employed in the whole organism to control and express naturally induced forms of plasticity. Previous work from our lab has implicated alpha-CaMKII autophosphorylation in experience-dependent potentiation in the barrel cortex. However, experience-dependent depression is not CaMKII dependent in point-mutants lacking the CaMKII autophosphorylation site and neither is repotentiation from the depressed state. In this talk I will describe studies that suggest equivalence between the molecular mechanisms involved in experience-dependent depression and spike pairing induced synaptic depression and that implicate a PKA dependent mechanism in repotentiation from this depressed state *in vitro* and *in vivo*.

S8.2 Intracortical inhibition in learning-dependent plasticity
Kossut M.[1], Siucinska E.[1], Urban-Ciecko J.[1], Tokarski K.[2], Hess G.[2,3]

[1]Nencki Institute of Experimental Biology, Warsaw, Poland; [2]Institute of Pharmacology, Krakow, Poland; [3]Jagiellonian University, Krakow, Poland

Procedural learning can modify receptive fields in primary sensory cortex. We developed a sensory conditioning paradigm that changes the properties of neurons in cortical representation of vibrissae, the barrel cortex. Using the vibrissae-to-cortical barrels pathway in mice we investigated participation of inhibitory neurotransmission in learning-dependent modifications of cortical representations. Plasticity of cortical representation of vibrissae is induced in adult mice by pairing stimulation of whiskers with aversive reinforcement in a classical conditioning paradigm. Post-training mapping of brain activity pattern with [14C]2-deoxyglucose revealed that vibrissae stimulated during the training, activate an enlarged cortical area. Within the plastic representation, in situ hybridization to GAD mRNA showed increased expression GAD 67 but not GAD65 mRNA. This was accompanied by increased density of GAD and GABA immunoreac-

tive neurons. Elevation in the number of GAD67 immunoreactive puncta was found in a localized subregion of cortical layer IV. The neuronal population in which GAD expression was increased was not immunoreactive for parvalbumin. Electrophysiological recordings from cortical slices taken from trained and control mice, revealed that paired pulse depression was selectively enhanced in one of the intracortical pathways from the trained to adjacent barrel, indicating increased intracortical inhibition. Amplitude of the field potential, evoked by stimulation of vertical pathway from layer VI to layer IV within the trained column, was smaller than in the control column. Whole cell path recordings from excitatory neurons from layer IV barrels showed increased frequency of spontaneous IPSPs after the training. The role of enhanced inhibition in the cortical representation modified by associative learning will be discussed.

S8.3 Toward understanding the mechanisms of human motor learning
Classen J.

Wuerzburg University, Wuerzburg, Germany

Motor learning may evolve from an initial short-lasting stage into a subsequent functionally different stage. Immediately after training of a novel motor task, a memory for the trained movements is present in motor cortex (M1). In addition to practice, motor observation is a strong stimulus to generate this memory. Concomitant to the presence of a motor memory, learning of a second motor skill is impaired, in relation to the naive acquisition of the first similar skill (anterograde interference). Long-term recall of the first motor skill is disrupted if a conflicting skill is trained or repetitive transcranial magnetic stimulation is applied during a short time period after the acquisition of the first skill (retrograde interference). It is possible that these distinct phenomena may map onto similar mechanisms. A non-invasive Hebbian stimulation protocol, termed paired associative stimulation (PAS), induces long-term potentiation (LTP)-like and long-term depression-like changes in human M1. Immediately after training a novel dynamic motor task, the capacity of M1 to undergo plasticity in response to PAS was abolished. When retested after 6 hours, PAS-induced plasticity recovered to baseline levels. Application of the PAS protocols after motor training did not prevent the consolidation of motor skills evident as performance gains at later retesting. Properties of LTP-formation may be linked with human short-term motor memory formation and possibly motor learning.

S8.4 Reorganization of human motor cortex by weak direct current stimulation
Nitsche M.A.

Georg-August-University, Dept. of Clinical Neurophysiology, Goettingen, Germany

Stimulation with weak direct currents (tDCS) elicits modulations of motor cortical excitability during as well as after for about one hour after the end of stimulation, if stimulation lasts sufficiently long. It is applicable in animals as well as non-invasivly and painlessly in human studies. Anodal stimulation enhances, while cathodal stimulation diminishes exitability. The effects are localised intracortically. While during stimulation tDCS modulates resting membrane potential, the after-effects involve a modulation of NMDA receptor strength. This technique could evolve as a promising tool in neuroplasticity research, since it has been shown to result in modifications of cortical functions like implicit motor learning, visuo-motor coordination and improved fine motor skills in chronic stroke patients with paresis of the upper limb. Here, an overview is given on the basic and functional effects of weak direct current stimulation as well as technical preconditions and currently available safety criteria.

SYMPOSIUM IX
ANIMAL BEHAVIOUR AND ITS NEURAL MECHANISMS

S9.1 Neurobiological basis of insect social behaviour
Godzinska E.J.

Nencki Institute of Experimental Biology, Warsaw, Poland

Extensive research devoted to causal factors underlying insect social behaviour was so far focused mainly on its ontogeny, function and evolution. Neurobiological basis of insect social behaviour was so far relatively little known. I will present a review of recent advances in the research devoted to that topic, including the results of current research carried out by my team. I will discuss recent experimental data concerning behavioural, anatomical and neurochemical correlates of the transition nurse-forager, a developmental phenomenon contributing in the crucial way to the phenomenon of division of labour encountered in insect societies. I will also discuss neurochemical mechanisms of aggressive/dominance behaviour of ants, honeybees and bumblebees, of foraging behaviour of these insects, of responses of honeybees and ants to aliens and to nestmates, and of interactive behaviour shown by ant workers when reunited with a nestmate after a period of social deprivation. Lastly, I will discuss the results of a recent experiment of my team in which we tried to throw more light on the possible involvement of the hypothetical phenomenon of social reward in the mediation of social behaviour displayed by the ants reunited after a period of social deprivation by comparing neurochemical mechanisms underlying two different reward-related phenomena: isolation-induced trophallaxis in carpenter ants, and sensitization to cocaine in Drosophila fruit flies.

S9.2 Exploratory behavior as a function of environmental novelty and complexity in male and female rats
Pisula W.

Warsaw School of Social Psychology, Warsaw, Poland

Laboratory rats show a positive response to low- or non-stressful novel events. The novel event may involve a number of aspects of the stimulus field. It is usually associated with a change in the level of environmental complexity. Most studies concerning novelty-related behavior involve the introduction of novel objects or the rearrangement of familiar objects. The purpose of the present study was to determine the degree of exploratory behavior in response to environments of increased and decreased complexity. Both directions of environmental change are conditions of novelty. A two-way manipulation was used in this study: increasing and decreasing the complexity of the environment. Rats of both sexes showed increased exploration (locomotor activity) to exposure to novelty, no matter which manipulation was applied. However, female and male rats behaved differently to the two types of novelty. Males responded more to novelty that resulted from the introduction of an

unfamiliar object. The results obtained demonstrate that novel stimulation, whether it be of increasing or decreasing complexity, has reward properties. We speculate that male-specific behavior directed toward unfamiliar objects may serve an adaptive function.

S9.3 Development of food preferences in mammals: A behavioural view
Stasiak M., Walasek G.

Nencki Institute of Experimental Biology, Warsaw, Poland

The processes of food acquisition are necessary for the preservation of the animal's integrity. Studies on the development of food preferences show that the food selection may be dependent on innate, social and/or experiential factors. The emphasis of the lecture is on an experiential factor, that is, the role of feeding experience with food during early period of life upon the food selection or food acceptance habits of the adult mammal. The data are presented in a broader investigative context, which includes behavioural data obtained on carnivores and rodents, especially on domestic cats and laboratory rats. The importance of the two contradictory tendencies established by prior dietary experiences with nutritionally complete foods on later food preferences: the primacy effect (a preference of adult animals for their rearing diet) and the novelty effect (a preference for a novel diet), is stressed. Moreover, the role of palatability of the foods is underlined; the concept of palatability corresponds to taste pleasure, liking or happiness, in contrast to appetite or craving, indicating a want or need. Finally, the utility of instrumental conditioning for food reward is presented as an important means to gain a better understanding of the behavioural aspect of the development of food preferences in mammals.

SYMPOSIUM X
DEVELOPMENT AND EVOLUTION OF THE NEOCORTEX

S10.1 Avian pallial primordia compared to mammalian ones in the light of molecular markers
Puelles L., Ferran J.L., Sandoval J., Garcia-Calero E., Martinez-de-la-Torre M.

University of Murcia, Faculty of Medicine, Dept. of Human Anatomy, Murcia, Spain

The status of evidence on pallial subdivisions in the avian and mammalian telencephalon will be discussed in the light of recent and novel data. These results bear on the issue of defining the cortex homolog in birds, as well as on the notion of the claustroamygdaloid complex and its inner subdivision.

S10.2 Maintenance of the radial glial morphology in corticogenesis
Gierdalski M., Juliano S.L.

Uniformed Services University, Bethesda, USA

A model of cortical dysplasia resulting from disruption of the earliest generated neocortical cells by means of injections of an antimitotic methylazoxymethanol (MAM) into pregnant ferrets has been established. Short-term arresting of cell division during corticogenesis leads to a set of effects including a very thin and poorly laminated neocortex, disturbed radial glia, with early differentiation into astrocytes, disorganization of reelin-containing Cajal-Retzius cells and impaired migration of neurons into the cortical plate. We hypothesized that early interference in the normal cortical development removes a factor instrumental in maintaining radial glia in their normal elongated shape. We found that co-culture of cortical slices from MAM-treated newborns with explants of the normal cortical plate reorganizes the radial glia toward their normal morphology and improves migration of neurons into the cortical plate. Series of studies involving various treatments of the impaired cortical slices *in vitro* allowed us to narrow the search of the factor promoting radial morphology of glia down to neuregulin, one of the ligands of erbB receptors.

S10.3 Patterning of the cerebral cortex area map
Grove E.A.

University of Chicago, Chicago, USA

Thalamic innervation of each neocortical area is vital to cortical function, but the developmental strategies that guide axons to specific areas remain unclear. We took a new approach to determine the contribution of intracortical cues. The cortical patterning molecule FGF8 was misexpressed in the cortical primordium to rearrange the area map. Thalamic axons faithfully tracked changes in area position, and innervated duplicated somatosensory barrel fields induced by an ectopic source of FGF8, indicating that thalamic axons indeed utilize intracortical positional information. Because cortical layers are generated in temporal order, FGF8 misexpression at different ages could be used to shift regional identity in the subplate and cortical plate either in or out of register. Thalamic axons showed strikingly different responses in the two different conditions, disclosing sources of positional guidance in both subplate and cortical plate. Unexpectedly, axon trajectories indicated that an individual neocortical layer could provide not only laminar but also area-specific guidance. Our findings demonstrate that thalamocortical axons are directed by sequential, positional cues within the cortex, and implicate FGF8 as an indirect regulator of thalamocortical projections.

S10.4 Pattern of c-Fos expression in the neocortical parts of the limbic system during development and maturation
Morys J., Ludkiewicz B., Badowska-Szalewska E., Klejbor I., Domaradzka-Pytel B.

Dept. of Anatomy and Neurobiology, Medical University, Gdansk, Poland

The limbic system plays a crucial role in emotional and learning processes. On the basis of c-Fos protein activation we studied how the activity of neuronal populations is spread out in the limbic part of the cerebral cortex and amygdala after open field test in the rat during the maturation process. The material consisted of rat brains of various postnatal ages (from P0 to P120). Open field test (OF) was applied throughout 10 min. After fixation brains were stained with use of immunohistochemical method for c-Fos and examined with a confocal Bio-Rad system. At birthday in rats exposed to OF we noted c-Fos activity mainly in the layers II and III of piriform cortex as well as in the deep layers of the neocortical parts of the limbic cortex. Then it increased and stabilized about the 5th week of life. The medial nucleus, anterior cortical nucleus and bed nucleus of accessory olfactory tract play crucial role in the amygdalar OF response. During the first

postnatal week the density of c-Fos-ir cells in amygdala was low, and then (up to P90) it significantly increased; after this period systematic decline of the density of c-Fos-ir cells was observed. Our results suggested that during maturation of various parts of limbic system c-Fos-positive cells are strongly activated in response to stress stimuli.

S10.5 Reduction of size in mammalian evolution: Influence on brain size and neocortical division
Turlejski K., Djavadian R.

Nencki Institute of Experimental Biology, Warsaw, Poland

In many mammalian lineages body and brain size gradually increased in evolution. Concurrent expansion of neocortex resulted in emergence of an increasing number of functional fields. However, in many mammalian lineages there were periods of body and brain size decrease resulting in proportional or selective reduction of cortical areas. We were investigating proportions of cortical areas in mice and shrews that differ in size. In mice and Sorex shrews differences in brain size does not correlate with the number of cortical areas that are precisely scaled down within their lineage pattern. In shrews the number of cortical fields (about 10) is close to that postulated for a prototypic mammalian brain. Differences in proportions of the cortical fields were visible between families and genera of shrews. *Crocidura russula* has proportionally smaller areas S1 and V1 than *Sorex araneus* that is of similar size. Area V1 was reduced independently in various mammalian lineages, depending on the size of visual input. Therefore, two independent developmental processes may act during scaling down: first leads to a proportional reduction and the second to reduction of the number of specific receptors resulting in selective reduction of areas. It is postulated that in the early development cortical areas are genetically labeled, but later some are not supported by the reduced thalamic input.

SYMPOSIUM XI
OF THE BRITISH NEUROSCIENCE ASSOCIATION
MECHANISMS OF HIPPOCAMPAL PLASTICITY

S11.1 Activity-dependent control of rapid presynaptic Ca^{2+} signalling at individual central synapses
Rusakov D.A.

Institute of Neurology, University College London, London, UK

Rapid, activity-driven modulation of Ca^{2+}-dependent synaptic release by presynaptic receptors contributes critically to the fundamental mechanisms of information processing in the brain. To probe these mechanisms at a single-synapse level, we combined confocal/two-photon microscopy with single-cell electrophysiology in acute brain slices. We monitored and analysed fast, action potential evoked Ca^{2+} transients in several types of individual presynaptic terminals that represent major synaptic circuitries in the hippocampus and cerebellum. We identified sub-cellular mechanisms by which synaptic release is regulated through activation of (a) presynaptic GABAA receptors at glutamatergic synapses formed by hippocampal mossy fibres, and (b) presynaptic glutamate receptors (AMPA and group III metabotropic types) in GABAergic terminals of hippocampal and cerebellar interneurons. The ability to probe individual synapses reveals important organisation principles that contribute to short-term plasticity and the differentiation of synaptic release control within the apparently homogenous synaptic populations.

S11.2 Low expression levels of NR1 N598R NMDA receptors alter functional and structural properties of the dentate gyrus impair spatial learning
Schoepfer R.

University College London, London, UK

The NMDA-receptor (NMDAR) system has a special role in spatial learning and mechanisms underlying synaptic plasticity, such as hippocampal long-term potentiation (LTP). Its coincidence detection property and signaling pathways are crucial in this context. The introduction of the N598R point mutation into the NR1 subunit (NR1R) results in agonist-dependent and APV-sensitive NMDARs that are Mg^{2+} insensitive and Ca^{2+} impermeable and, therefore, cannot act as coincidence detectors. We have obtained animals of three genotypes expressing different relative amounts of mutant (NR1R) and wild-type NR1, namely NR1R/-: 100/0%; NR1R/+: 50/50% and NR1Rneo/+: 5/95%. NR1Rneo/+ mice express a hypomorphic variant of the NR1 N598R gene. This results in the expression of a mixed population of NMDA receptors, with the vast majority being wild-type, together with a minority of mutant receptors. *In vivo* recordings from these animals revealed specific deficits in synaptic plasticity in the hippocampal formation. Furthermore these animals showed impairments in spatial learning, reversal learning and retention. Our data suggest that minor changes in NMDA-receptor physiology can cause dramatic consequences in synaptic signaling, and provide genetic evidence for a critical involvement of the DG in spatial learning.

S11.3 Glutamate receptors and synaptic plasticity
Bashir Z.I.

Bristol University, UK

The mechanisms of synaptic plasticity (e.g., long-term potentiation and depression – LTP and LTD) in the hippocampus have been the subject of intense investigation. In particular the role of glutamate receptors in synaptic transmission and synaptic plasticity has been the focus of many studies. Over the years the pivotal roles of NMDA receptors and mGlu receptors in different forms of synaptic plasticity have been elucidated. However, some surprising results are beginning to emerge concerning the roles of different NMDAR subtypes in different forms of plasticity. In addition, the mechanisms by which mGlu receptor activation results in LTD in hippocampus is beginning to become clearer. The above studies will form the basis of this presentation.

S11.4 Structural basis of hippocampal plasticity following stress and learning: Electron microscopical studies
Stewart M.G.

The Open University, Milton Keynes, United Kingdom

Memory formation following learning is generally believed to result from alterations in synaptic efficacy and the hippocampus plays a crucial role in this process. There is, however, no consensus as to the nature of morphological changes in synapses and neurons, in part because of the differing nature and time scales involved in the various models studied, coupled with diverse methodological approaches to measuring morphometric parameters. Most previous work has been based upon mathematical manipulations of 2-dimensional images. Alternatively, 2-photon imaging offers the opportunity to examine changes in neurons and spines in 3 dimensions but is: (a) best applied to slices; and (b) is of limited use in the study of synaptic membranes, because its resolution is in the micron range. Here we have examined morphological plasticity in thorny excrescences and dendrites of CA3 of rat hippocampus, and in CA1, following water maze learning and a restraint stress paradigm. Unbiased stereology and 3-dimensional reconstruction techniques were applied to ultrathin serial sections of CA1 and CA3 hippocampal tissue. Our data show that: (i) in both CA3 and CA1 there is an increase in the surface area and size of postsynaptic densities 24 h following spatial learning; (ii) the effects of stress on synaptic morphology can be rapidly reversed by spatial training.
Supported by BBSRC.

SYMPOSIUM XII
NEUROPLASTICITY AND NEUROREHABILITATION

S12.1 Brain plasticity in stroke rehabilitation
Johansson B.B.

Wallenberg Neuroscience Center, Lund University, Sweden

The adult brain retains a capacity for plasticity and functional reorganization throughout the life span. Despite permanent tissue loss most surviving stroke patients improve with time. The mechanisms involved may vary with post-ischemic time and the type and location of the lesion. Experimental data indicate that a stimulating environment improves functional outcome, increases dendritic branching and number of dendritic spines, and can influence endogenous stem cell proliferation and differentiation after focal brain ischemia. That training of specific functions is important and can alter cortical representation areas, cortical maps, and significantly improve motor function has been verified in many clinical studies. Several new rehabilitation methods based on basic neurobiological principles have been introduced and will be discussed. However, more studies comparing different new models and long term follow up studies are needed. Intense training under shorter periods seems to be more efficient than the same amount of training spread over longer periods. This has been indicated in studies on training of motor functions as well as aphasia. The attitude, coping capacity, motivation and social interaction and support of the individual patient are nonspecific but important factors for successful rehabilitation.

S12.2 Cortical plasticity contributing to child development, adult learning, and neurorehabilitation
Merzenich M.

Keck Center for Integrative Neuroscience, UCSF, San Francisco, USA

Cortical recording and imaging studies in developing and adult animals and humans have provided us with an increasingly clear understanding of the phenomenology of cortical plasticity, as it accounts for the development of the specific skills and abilities of the developing child and for the acquisition of new skills and abilities in the adult brain. They also provide us with an increasingly clear understanding of the contributions of experience and learning to variations in child and adult achievement, to the complex interplay between genetic and environmental contributions to human performance variation, and to the origin of the expressions of specific developmental and adult impairments. We have used this growing understanding of neurogenetics and brain plasticity phenomenology to develop a new class of models of human disabilities (e.g., in development, impairments in language and reading acquisition, autism, cerebral palsy, schizophrenia; in adults, acquired focal dystonias, Parkinsonism, memory/cognitive losses in aging), and to design brain plasticity-based therapeutic strategies designed to ameliorate or reverse them. Several of these treatment models have now demonstrated that key behavioral and neurological expressions of developmental and adult impairments can be substantially re-normalized by this integrative neuroscience-based therapeutic approach.

S12.3 Functional integration of grafted embryonic neurones into the circuitry of the host spinal cord
Slawinska U.

Nencki Institute of Experimental Biology, Warsaw, Poland

Transplantation of embryonic CNS tissue provides an important method in investigations related to neural development, plasticity and regeneration. The aim of our studies was to investigate whether the grafted neurones are able to establish appropriate connections and integrate into the neural circuitry of the host spinal cord. In the first model, an intraspinal transplantation of serotonergic neurones below the level of total transection induced an improvement in hindlimb motor function in adult spinal rats. The nature of this improvement was examined using pharmacological agents that interfere with 5HT2 serotonergic transmission. Our results revealed that the graft-induced restitution of hindlimb locomotor functions was brought about by the new serotonergic innervation. In the second model, an integration of the grafted motoneurones into the circuitry of the host spinal cord was investigated. In addition to previous findings that the grafted motoneurones are able to survive, develop and extend their axons into the re-implanted ventral root, we demonstrated (using chronic EMG recordings) that they became successfully integrated and were activated appropriately during locomotor movements. Thus, our experiments revealed that grafted neurones are able to establish appropriate connections with the circuitry of the host spinal cord that control movements of hindlimbs.

S12.4 Neurite outgrowth inhibitors at nodes of Ranvier
Xiao Z.C.

Dept. of Clinical Research, Singapore General Hospital, Singapore, Singapore; Institute of Molecular and Cell Biology, Singapore, Singapore

The last two decades have witnessed a tremendous effort of utilizing the neurite outgrowth inhibitors to combat axonal injury and promote functional regeneration both *in vitro* and *in vivo*. Although these molecules show potent inhibition on neurite outgrowth in tissue culture, most null mutation animal models have so far revealed no significant improvement in the CNS regeneration. Are these so-called inhibitors, Tenascins, chondroitin sulphate proteoglycans, MAG, Nogo, OMgp, really the culprits of regeneration failure? This disparity between the *in vitro* and *in vivo* behaviours of these inhibitors has led us to reassess the physiological roles and functions that they take on. And it appears that previous reviews on classical neurite inhibitors have overlooked Notch, which does inhibit neurite outgrowth in postmitotic neurons. So in this presentation, we attempt to sit these molecules in the context of active axoglial interactions that establish the polarized organization of myelinated axons. That is, not only do the inhibitory molecules signal to their neuronal receptors to influence the axonal polarization and channel function, but also some axonal molecules signal back to oligodendrocytes *via* certain receptors, such as Notch, to mediate oligodendroglial generation.

SYMPOSIUM XIII
BEHAVIORAL GENETICS: GENETIC BASIS OF NEUROPHYSIOLOGY AND BEHAVIOR

S13.1 Introduction to behavioral genetics: Nature or nurture
Swiergiel A.H.

Dept. of Animal Behavior, Inst. of Genetics and Animal Breeding, PAS, Jastrzebiec, Poland

There is no more doubt that genotype plays a role not only in determining simple behavioral patterns, e.g. motor activity, but also complex behaviors such as social interactions, learning, eating and also those observed in mental health disorders. The evidence comes from the twin studies, analyses of chromosomal abnormalities, gene polymorphism, gene expression and models involving selected, mutated or transgenic animals. In the coming years the main tasks are to identify genes that are involved in complex behavioral disorders as well as genes implicated in mediating undesirable (stressful) environment x genotype interactions resulting in behavioral disturbances. Eventually, this may lead to new treatments targeting specific genes that mediate behavioral disorders.
Supported by KBN Grant 3PO4C0582 and the EU Framework 6 NEWMOOD Integrated Project to AHS

S13.2 A genomic approach in human mood disorders
Deakin B.

Neuroscience Psychiatry Unit, University of Manchester, Manchester, England

Depression is a common, very complex behavioral disorder. Gender, social and familial (genetic) factors increase risk for depression, but little is known about how these influences work in brain, least at all at the molecular level. NEWMOOD project: "New molecules in mood disorders: A genomic, neurobiological and systems approach in animal models and human disorder" aims to identify changes in gene expression which are common to animal models of depression and to the human condition. The new genes then become new candidates for causation and targets for drug development. NEWMOOD focusses on three underlying psychological processes which mediate vulnerability to depression: the inability to experience pleasure, excessive fear, excessive sensitivity to stress. This approach enables to cross-validate findings in humans and animal models more reliably than relying on depression as the link: measures of the component process of depression are likely to be close to the underlying molecular mechanisms. Research involves: isolating molecular, behavioral, neurotransmitter and stress hormone mechanisms shared by animal models of genetic, developmental and acquired vulnerability to depression, identifying which of the mechanisms identified in animal models of vulnerability also occur in humans with genetic, developmental and acquired vulnerability to depression, identifying which mechanisms are reversible by known and novel antidepressants.
Supported by 6FP IP NEWMOOD.

S13.3 Selective breeding of mice for swim analgesia: Coinheritance of unselected behavioral traits
Sadowski B.

Inst. of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland

Swiss-Webster mice have been selectively bred over 20 years toward divergent magnitudes of swim stress-induced analgesia (SSIA) produced by 3 min swimming. Nociception was assessed on a hot plate and mice displaying long post-swim latencies were selected to build up a high analgesia (HA) line, whereas those manifesting short latencies constituted a low analgesia (LA) line. These lines appeared differentiated also with respect to other pain-related traits. The opioid form of SSIA prevails in the HA line and non-opioid SSIA is seen in the LA line, correlating with, respectively, high and low sensitivity to opioid analgesics. Interestingly, the lines have also inherited behavioral traits that were not intended in the selection protocol, and have no direct relationship to SSIA. Thus, the HA line manifests high magnitude of acoustic startle response, contrasting with the low startle response of the LA line. Secondly, the HA line appears less active than the LA line in the open-field test. Thirdly, the HA line displays depressive-like patterns of behavior in the forced swim or tail suspension tests, which are antagonized by antidepressant treatments. Finally, HA mice are relatively poor learners of two-way avoidance, contrasting with its good acquisition by the LA line. These differences in coinherited behaviors are thought to depend on higher emotionality of HA than of LA mice.

S13.4 Studies on genetic etiology of depression using selected mouse lines
Sacharczuk M.

Inst. of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland

Depression is an etiologically heterogeneous disease characterised by numerous changes in cognitive, psychomotor and emotional processes. It is conditioned by many factors including those of a genetic character. For the studies of a role of genotype x environment interaction in depression, a new animal depression model of mice selected for low and high stress-induced analgesia is used. The lines differ in depression-like behavior, responses to antidepressants and susceptibility to develop drug and alcohol dependence. They are examined for quantitative traits loci (QTL) connected with an intensity of response to factors causing depression and to determine the specific profile of gene expression during depression and its modification by antidepressants. "Depression microarray" is used to confirm the significance and expression of genes identified in a linkage analysis. Also, the research aims at determining the primary cause of hippocampal neurogenesis disturbances; it is not known whether the neurogenesis reduction is of a primary character or a secondary to depression incident. Confirmation of relation between specific loci, expression of genes and behavior will suggest that depression is determined not only by psycho-physical processes but also the genetic constitution. Analysis of relations between phenotype and genes identified as result of QTL analysis may assist in developing of new antidepressants.

S13.5 Allelic variation of serotonin receptor 1A function and complex traits
Lesch K.P.

Department of Psychiatry and Psychotherapy, Wuerzburg, Germany

Individual differences in anxiety-related personality traits have been associated with variation of genes related the serotonin (5-HT) pathway, specifically with a functional C-1019G single nucleotide polymorphism (SNP) in the transcriptional control region of the 5-HT1A receptor gene (HTR1A). The human and animal literature on the topic will be reviewed and converging data from behavioral and neuroimaging studies that suggest a non-linear association between HTR1A-1019 genotype and its influence on cognitive and neural systems engaged in attention to negative emotional stimuli will be presented.

S13.6 Temperamental traits postulated by the Regulative Theory of Temperament and the dopamine D4 receptor (DRD4), serotonin transporter (5-HTT) and dopamine transporter (DAT1) gene polymorphisms
Dragan W.L., Oniszczenko W.O.

Faculty of Psychology and Interdisciplinary Center for Genetic Behavior Research, Warsaw University, Poland

Cumulative evidence from family and twin studies suggests that genetic mechanisms underlie at least a portion of individual differences in personality traits. The genes coding for the serotonin transporter (5-HTT) and dopamine D4 receptor (DRD4) and transporter (DAT1) have been investigated in a number of studies, but the findings have been inconclusive. In the present study we investigated possible associations between temperamental traits postulated in Strelau's Regulative Theory of Temperament and the most widely studied polymorphisms in aforementioned genes: a VNTR in intron 2 (5-HTT VNTR) and a functional 44 bp deletion/insertion in the promoter region of 5-HTT (5-HTTLPR), a 48 bp repeat in exon 3 of DRD4, and a 40 bp repeat in 3'UTR of DAT1. Two hundred healthy, mutually unrelated females of Polish origin were assessed by the FCB-TI and were typed using PCR. We found a significant associations between the 5-HTTLPR polymorphism and two temperamental traits: Activity (F=4.5, P=0.012) and Endurance (F=5.68, P=0.004). We also noted an association between DRD4 gene and Endurance (F=5.2, P=0.024). Our results may provide an evidence of a possible small contribution of 5-HTT and DRD4 genes to individual differences in temperamental traits.

SYMPOSIUM XIV
OF THE BRITISH NEUROSCIENCE ASSOCIATION
NEURAL DIFFERENTIATION OF NON-EMBRYONIC STEM CELLS

S14.1 Using stem cells to repair the Parkinsonian brain: Will it work?
Barker R.A.

Cambridge Centre for Brain Repair and Department of Neurology, Cambridge, UK

Parkinson's disease is a chronic neurodegenerative disorder of the central nervous system, which is characterised by the loss of dopaminergic neurons and their projection from the substantia nigra in the brainstem to the striatum. This loss of dopaminergic cells leads classically to a movement disorder characterised by tremor, rigidity and bradykinesia. As a result of this localised pathological loss of cells within the brain, therapeutic treatments are available which target this network. Thus, effective drug treatments exist in the early stages of Parkinson's disease but with time these become less effective and produce their own side effects. As a result alternative therapies have been explored including the use of stem cells, and in this talk i will discuss how such cells may be of benefit in treating this condition.

S14.2 Phenotypic and differentiation properties of normal and tumor human neural stem cells
Vescovi A.L.

Stem Cell Research Institute, DIBIT, Hospital San Raffaele, Milan, Italy

Transformed neural precursors that display all of the critical features of adult neural stem cells have recently been implicated in the establishment, growth and recurrence of pediatric and adult brain tumors. Similar, but not identical to their normal counterpart, tumor neural stem cells (TNSCs) from human glioblastomas (GBMs) emerge as unipotent (astroglial) *in vivo* and multipotent (neuronal-astroglial-oligodendroglial) in culture. TNSCs act as tumor-founding cells down to the clonal level, establish tumors which resemble the main histological, cytological and architectural features of the

human disease to an extent never observed before, even through serial transplantation. Notably, while TNSCs from different GBMs exhibit common general characteristics, patient-specific properties emerge from a more detailed functional and molecular analysis. Here, we report on the results of a combined investigation on the functional and phenotypic properties of TNSCs isolated from various GBMs. Expression of well over forty surface antigenic markers – amongst which AC 133, various members of the integrin family and many receptors for various cytokines and growth factors – was assessed on purified TNSCs by cytofluorimetric analysis and compared to their growth and differentiation capacity and to their tumorigenic potential.

S14.3 Adult solutions for adult problems?
Joannides D., Hunt D., Chandran S.

Dept. of Clinical Neurosciences, University of Cambridge, Cambridge, UK

Somatic stem cells until recently have been regarded as niche resident lineage restricted cells capable of tissue renewal. In contrast embryonic stem cells are distinguished by the ability to generate and contribute to all germ layer derivative cell populations. However, considerable interest has been generated in the last few years by a series of reports that suggest adult somatic stem cells may possess a differentiation potential greater than previously ascribed. The idea of phenotypic potential beyond that of the tissue of origin has obvious biological interest and therapeutic implication for a range of diseases including neurodegenerative processes. Against this background the neural potential of adult human skin and bone marrow populations will be discussed.

S14.4 Validating the success and failure of neural development by stem cells *in vitro*
Przyborski S., Horrocks G., Christie V., Croft A.

University Of Durham, UK

Cultures of stem cells can provide amenable systems to investigate the molecular mechanisms that regulate cell growth and differentiation. This is particularly applicable to the study of human development and the formation specific human tissue types that may be used for drug discovery, toxicological testing and potentially cell replacement therapy. It is essential that any such culture model is validated. In our laboratory we have examined the ability of various stem cell systems to form neural derivatives *in vitro*. First, we have demonstrated that cultured embryonal carcinoma stem cells form functional neural tissues in a predicted and orderly fashion closely following the development of neurons *in utero*. Second, we have investigated the ability of bone marrow-derived mesenchymal stem cells (MSCs) to form neural tissues under defined culture conditions. Detailed analysis suggests that MSCs which form cells with neural-like morphologies may have been previously misinterpreted as neuronal differentiation. We suggest that such changes in cell shape and expression of neural genes may be accounted for by the aberrant behaviour of cells in response to their growth environment. These examples will be employed to show that validation of neural differentiation is essential in the use of cell-based assays to explore the pathways that control nervous system development.

S14.5 Human cord blood-derived neural stem/progenitors: The state of play
Domanska-Janik K.

NeuroRepair Dept., Medical Research Institute, Warsaw, Poland

Somatic stem cells (SC) are notorious for the difficulty encountered when attempts are made to expand them *in vitro*. Studies conducted by our group documented that neural progenitor cells (HUCB-NPs) can be derived from human umbilical cord blood (Buzanska et al. 2002). Due to repeated expansion and selection of these cells we have established the first clonogenic human umbilical cord blood neural stem cell like line (HUCB-NSC). In the presence of neuromorphogens, cultured rat astrocytes or hippocampal slices, primary NPs cultures and NSCs-like line can attain advanced neuronal phenotypes as assessed by various marker proteins and gene expression. Moreover, these cells can form neurospheres or neurosphere-like entities – the commonly approved hallmark of NSC. Recently our attention has been focused on the characteristic of supposed ancestor cells present in freshly isolated cord-blood mononuclear fraction (MN). Using Oct4 and Sox2 expression analysis (Oct4 as a marker of pluripotency and Sox2 – typical for both, pluripotent and neural stem stages) as well as Hoechst-low labeled "side population" measurements, we have found a small subpopulation of MN cells with putative pluripotent "ESs-like" characteristic from which neuronal-committed cells could originate. Besides strictly controlled culture conditions, the decisive factors on MN cells fate seem to be culture density/intercellular communication.
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KONORSKI'S AWARD LECTURE

K1 Modulation of GABAergic currents: A close look at the time scale of synaptic transmission
Mozrzymas J.W.

Lab. of Neuroscience, Dept. of Biophysics, Wroclaw Medical University, Poland

Synaptic transmission is a major mechanism of rapid signaling between neurons and in the mature brain inhibition is mediated by GABAergic currents. The time course of synaptic currents depends on the kinetics of postsynaptic receptors as well as on the amount and time exposure of synaptic agonist. The time course of synaptic currents can be modulated by several physiological factors (e.g., changes in pH) or by exogenous compounds (e.g., clinically relevant drugs). While synaptic current recording is easy, it is usually insufficient to describe the mechanisms determining the kinetics and modulation of synaptic transmission. Ultrafast perfusion system allows to apply drugs within tens of microseconds, enabling to reasonably reproduce highly dynamic conditions of synaptic receptor activation, while controlling the concentrations of both neurotransmitter and modulators. Using this approach, the mechanism of GABAergic IPSCs modulation by protons was investigated. It was shown that alterations in IPSCs induced by changes in pH resulted from modulation of affinity and desensitization of GABAA receptors. It is proposed that combination of classical IPSC recordings with measurement of current responses to rapid GABA applications is an excellent tool to explore the mechanisms of synaptic current modulation at the time scale of synaptic transmission.
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