POSTER SESSION I

Development [P1]

P1.01

NON-INVASIVE ASSESSMENT OF IMMUNOSUPPRESSION AND PREVENTION OF ALLOGRAFT REJECTION USING BIOLUMINESCENCE IMAGING

Janowski M.[1], Engels C.[1], Gorelik M.[1], Lukomska B.[2], Bulte J.[1], Walczak P.[1]

[1] Department of Radiology, Johns Hopkins University, Baltimore, MD, USA; [2] NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Neurotransplantation of allogeneic cells is currently being practiced in double-blinded clinical trials. Overall, the general outcome of these studies has been disappointing and may depend in part upon suboptimal control of host immune response. Immune rejection of allografts has been studied in animal models, but most investigations have been limited to histopathological studies, which can not report on the time course of rejection. One technique that can be used to study the biodynamics of graft rejection over time is bioluminescent imaging (BLI) based on the constitutive expression of the reporter gene luciferase. Using BLI, we report here on the fate of intracerebral grafts of firefly luciferase-positive glial restricted precursors, that were followed for 3 weeks in immunocompetent Balb/C mice under administration of different immunosuppresive regimens. Controls included immunodeficient rag2 -/- mice and non-immune suppressed immunocompetent Balb/C mice. Immunodeficient mice revealed continuous growth of BLI signal. Non-immune suppressed mice showed graft rejection in approximately 75% of 23 animals; the other 25% was characterized by absence of apparent rejection and also continued growth of BLI signal. The administration of cyclosporine (10 mg/kg bw, i.p.) was less effective for prevention of graft rejection (55% of 7 animals), than rapamycin/FK506 (both 1 mg/kg bw, i.p.) two drug combination (20% of 10 animals). Graft rejection was observed exclusively during the second week postgrafting, apparently as a sudden disappearance of BLI signal. Histological evaluation of transplanted cells corroborated well with the BLI data. Inflammatory processes were not observed in immunodeficient mice but, surprisingly, a significant infiltration of inflammatory cells still occurred in grafts with excellent survival. Immunosuppressive treatment did not seem to affect that and it was similar in both graft rejecting and graft accepting mice.

P1.02

THE ROLE OF ADHESION MOLECULE CD44 IN DENDRITIC TREE ARBORIZATION

Joanna Dzwonek J.[1], Gorlewicz A.[1], Konopka A.[1], Swiech L.[2], Jaworski J.[2], Wilczyński G.[1]

[1] Laboratory of Molecular and Structural Neuromorphology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland.

Dendritic arborization patterns define neuronal subtypes, and have important functional implications, determining how signals coming from individual synapses are integrated. Developing dendrites of neurons are responsive to extrinsic signals. Although several secreted proteins, cell surface receptors and adhesion molecules have been recently shown to be involved in dendrite morphogenesis, the role of extracellular matrix (ECM) components and molecular mechanisms of signal transduction from ECM to the neuronal cells involved in these processes are still poorly understood. The main component of the ECM in the brain is hyaluronan (HA). The major receptor for hyaluronan is CD44 adhesion molecule which mediates the response of cells to their extracellular microenvironment. The aim of this study was to investigate the role of CD44 in regulation of dendritic tree arborization. First, we examined the expression pattern of CD44 at the protein and RNA level in the rat brain by immunohistochemical, in situ hybridization and in situ PCR assays. All our experimental approaches clearly point to the neuronal localization of CD44, in addition to widely accepted presence in glia. Next, we investigated the role of CD44 in the hippocampal neurons cultured *in vitro* using the shRNA technology and anti-CD44 function-blocking antibody. The morphometric analysis show that cells with diminished expression of CD44 have more complex dendritic tree then control cells. Moreover, we have shown that treatment of neuronal cells with CD44 blocking antibody caused activation of small Rho GTPases (Cdc42, Rac1 and RhoA), which were previously shown to regulate dendritic tree arborization. These observations indicate that the members of small Rho GTPase family can be downstream effectors of CD44 in neuronal cells. The results of our experiments point to the importance of CD44 protein for the development of dendritic tree.

P1.03

THE POTENTIAL ROLE OF FAK- AND PYK2 COUPLED PATHWAY IN THE NEUROGENESIS IN GERBIL HIPPOCAMPUS AFTER GLOBAL ISCHEMIA

Ziemka-Nałęcz M., Wójcik-Stanaszek L., Zając H., Zalewska T.

NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Recently published data indicate that in physiological conditions proteolytic remodeling of extracellular matrix (ECM) by matrix metalloproteinases (MMPs) participates in the stem cells development. Signal derived from ECM may activate specific intracellular signaling pathways which involve non-receptor tyrosine kinases such focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2), key components responsible for the flow of information to the cell. FAK and Pyk2 might act through a diverse array of downstream molecules such a phosphatidylinositol 3-kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK). Activation (PI3K)/Akt and ERK pathways in neural precursors plays a central role in induction of adult neurogenesis. These prompted us to evaluate the possible involvement of FAK/PYK2-coupled pathway in the regulation of neurogenesis-associated processes stimulated by transient global ischemia in gerbil hippocampus. For this purpose we checked if there is temporal relationship between activation/phosphorylation of these kinases and proliferation and/or determination of neural progenitor cells. We found that short-term (5 min) ischemia increased Pyk-2 phosphorylation level in dentate gyrus (neurogenic part of hippocampus) after 2 and 4 weeks of recovery, the time when we observed the intensive proliferation rate and differentiation of progenitors toward neuronal phenotypes. In contrast, in the CA1 region of the hippocampus the level of phosphorylated Pyk-2 was slightly reduced after 2, 4 and 6 weeks of reperfusion. At the same time the level of phosphorylated FAK was significantly increased in both investigated hippocampal regions. In contrast activation of ERK and Akt kinases was significantly reduced in all investigated time points with more pronounced effect in CA1. The elevation of PYK-2 activity in dentate gyrus might suggest the involvement of this kinase in the post-ischemic stimulation of neurogenesis after global ischemia.

P1.04

MILD LABORATORY STRESS ELEVATES FOS EXPRESSION IN THE AMYGDALA AND HYPOTHALAMUS IN RATS HIGH-RESPONSIVE TO NOVELTY

Irena Majkutewicz I., Myslinska D., Jerzemowska G., Plucinska K., Wrona D.
Department of Animal Physiology, University of Gdansk, Gdansk, Poland.

Responsiveness to novelty is often used as a measure of inter-individual vulnerability to stress loads and drug abuse. The aim of this study was to determine the relationship between individual behavioral profile and brain structures activation. Possible influence of stressful laboratory routines on manifestation of these individual differences was investigated. Male Wistar rats (n=21) were subjected to the novelty test and divided into high (HR) and low (LR) responders to a new environment according to median. Randomly chosen 6 LRs and 5 HRs rats were handled and carried out from the vivarium to the laboratory for nine days (carried group), remaining rats stayed in their home cages (control group, 5 HRs and 5 LRs). One week after the last carrying, an immunohistochemical detection of Fos protein in selected brain areas was performed. Carried HRs showed significantly higher Fos expression in all studied nuclei of the amygdala and most of the hypothalamic areas as compared to LRs and also to control rats. Carried LRs showed elevated density of Fos+ cells only in the stress-related paraventricular and supraoptic hypothalamic nuclei. Surprisingly, inter-individual (HR vs LR) differences in brain activation was found in carried rats only. We conclude that mild stress evoked by some laboratory routines reveals constitutive differences between the individuals reflected by an increased activity of the amygdala and hypothalamus.

P1.05

NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY ACTIVATES HIPPOCAMPAL STEM/PROGENITOR CELLS

Gregor A., Kozlowska H., Zalewska T.
NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Birth asphyxia remains a frequent cause of perinatal morbidity and mortality. During perinatal hypoxic-ischemic (HI) brain injury neuronal cells are damaged and lose their function or die. Recently, it has become clear that ischemic brain injury stimulates neural stem cell proliferation and differentiation in cerebral neurogenic areas – subventricular zone (SVZ) and dentate gyrus (DG) of the hippocampus frequently injured after the perinatal HI. There is a tremendous speculation, that the induction of these progenitors after injury may represent an endogenous mechanism for brain regeneration. To study the response of hippocampal progenitors to neonatal HI brain damage, we utilized an established model of HI induced in rats of postnatal day 7 (PND7). The left common carotid artery was ligated and then after 60 min of recovery, the animals were exposed to hypoxia (7.4% oxygen for 75 min). The hypoxic undamaged hemisphere served as control for developmental modification. In addition, age-matched sham-operated rats were also used as controls. At 4, 10, 14, and 21 days following hypoxia, pups were perfused transcardially with PBS followed by 4% PFA. To determine the proliferation profile animals were injected with BrdU (50 mg/kg) at various days after HI and immunopositive cells were analyzed the next day. At 4 - 14 days after HI the presence of BrdU-positive cells was seen in both, ipsi- and contralateral hemispheres, with the greatest number of dividing cells in the ischemic side. Thereafter, cell proliferation appeared to be reduced. The labeling pattern revealed structure-dependent differences. At 4 days after

the insult the highest density of cells incorporating BrdU was seen in hilus, whereas at longer survival time the majority of labeled cells were located in the DG. To confirm that the BrdU-positive cells represent dividing progenitors we used double staining: BrdU/Ki67, BrdU/PSA-NCAM and immature neuronal marker - DCX.

P1.06

NEUROGENIC POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS TRANSPLANTED INSIDE 3D GELATIN/LAMININ SCAFFOLDS OR WHARTON JELLY IMPLANTS INTO ADULT RAT BRAIN

Anna Jablonska A.[1], Drela K.[1], Sarnowska A.[1], Wanacka E.[1], Jurga M.[2], Dainiak M.[3], Jungvid H.[3], Forraz N.[2], McGuckin C.[2], Domanska-Janik K.[1], Lukomska B.[1]

[1] NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland; [2] Parc Technologique de Lyon-Saint Priest, Cell Therapy Research Institute, Saint Priest, France; [3] Protista Biotechnology AB, IDEON, Lund, Sweden.

Mesenchymal stem cell (MSC) transplantation offers new therapeutic avenue for neurological diseases, however limitted survival of exogenous cells in the host brain is a major setback. The aim of this study was to evaluate the efficacy of using biodegradable scaffolds or Wharton jelly implants contained MSC after their transplantation into rat brain.Materials and methods: Adult Wistar rats were transplanted with MSC derived from human umbilical cord (hUC-MSC) (i), hUC-MSC localized inside biodegradable gelatin/laminin scaffolds (hUC-MSC/GL) (ii) or Wharton jelly implants (hUC-MSC/WJ) (iii). Results: hUC-MSC cultured *in vitro* expressed CD73, CD90, CD166 and Oct3/4, Nanog1, Nestin markers. 7 days after hUC-MSC transplantation only few viable donor cells were observed in the rat brain surrounded by heavy infiltration of macrophages/microglia (ED1+) and activating astrocytes (GFAP+). Hence transplantation of hUC-MSC/GL resulted with better cell survival compared with hUC-MSC grafted in suspension. There was no donor cell migration out of the scaffolds however hUC-MSC lodged inside G/L scaffolds attained early neural markers. The inflammatory cell influx observed around the scaffolds was less intense with few ED1+ cells present in the core of scaffold. Similarly, all transplanted hUC-MSC/WJ remained in 3D tissue implants. Some of these cells adopted NF200, A2B5 or GFAP phenotypes during 7 days of observation. Concomitantly only scarce infiltration of immunoreactive cells was seen. Conclusions: Transplantation of hUC-MSC in 3D G/L scaffolds or hUC-MSC/WJ implants into adult rat brain improves survival of donor cells and induces their spontaneous transition into cell of neural lineage. It seems that 3D structures protect cells localized inside them from the host immune cells and may allow the diffusion of nutrients and other factors to propagate cell survival and differentiation into neuronal lineage. *Supported by MSHE grant no N401 014235 and Fondation Jerome Lejeune grant.*

P1.07

EFFECT OF MATRIX METALLOPROTEINASES INHIBITION ON THE PROLIFERATION AND DIFFERENTIATION OF HUCB-NSCS CULTURED IN THE PRESENCE OF ADHESIVE SUBSTRATES

Szymczak P., Sypecka J., Wójcik-Stanaszek L., Zalewska T.

NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Cell adhesion to extracellular matrix (ECM) generates intracellular signals that modulate cell survival, proliferation, migration of neural precursor cells and differentiation *in vitro.* The function of ECM in developmental processes may be associated with particular patterns of proteolysis of ECM. Among the proteases the matrix metalloproteinases (MMPs) represent family of enzymes responsible for the modification of ECM components and by this may influence cell development. The aim of our study was to determine the potential of native ECM proteins: fibronectin, laminin and collagen, on the proliferation and differentiation of HUCB-NSCs cultured in serum free condition. In an effort to elucidate the engagement of MMPs we have checked the effect of inhibitors - SB-3CT, GM6001 and doxycycline on the above development-associated processes. Among the tested substrates the highest proliferation rate (evaluated by using anti-Ki67) was noted in the presence of fibronectin. Fibronectin also occurred most permissive substrate in HUCB-NSCs differentiation toward neuronal fate. The endogenous activity of MMPs (assayed by in situ zymography) corresponds to the rate of cell proliferation. Addition of MMPs inhibitors resulted in the significant suppression of proliferation potential and inhibition of neuronal cell generation (TUJ1- and MAP2-positive) with simultaneous promotion of oligo- and astroglial cells (expressing GALC and S100β, respectively). In conclusion, our results suggest that MMPs might be an important component in neurogenesis-associated processes. *Supported by MSRHE grants: 0154/B/P01/2010/38 and 0345/B/P01/2010/38.*

P1.08

BINOCULAR DEPRIVATION REVEALS EARLY PLASTICITY-DEPENDENT REGION- AND LAYER-SPECIFIC *zif268* EXPRESSION PROFILES IN CAT PRIMARY VISUAL CORTEX (AREA 17)

Laskowska-Macios K.[1], Zapaśnik M.[1], Hu T.T.[2], Kossut M.[1], Arckens L.[2], Burnat K.[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Neuroplasticity and Neuroproteomics Unit, K.U.Leuven, Leuven, Belgium.

Binocular pattern deprivation (BD) alters visual cortex circuitry development, here for the first time studied via analysis of region- and layer-specific expression of the activity reporter gene *zif268*. We describe its profile in cat area 17 after BD in comparison to age-matched controls. Three BD conditions were applied; the first four (4BD) or six (6BD) months from eye-opening, and a late onset BD in the 3rd and 4th month of age, preceded by 2 months of normal vision (2N2BD). Cats were exposed to overnight darkness prior to 1-hour light stimulation and then sacrificed; kittens at the end of the BD period, adults after 2 years of visual training upon BD. Radioactive in situ hybridization was applied to analyze *zif268* mRNA levels. Juvenile and adult BD cats showed elevated *zif268* mRNA levels in peripheral area 17 compared to central area 17, while in controls the signal was similar throughout area 17. 2N2BD kittens had such a BD pattern only in layer IV. All juvenile control and BD kittens, except 4BD, showed a similar inter-laminar *zif268* expression profile for central area 17. The least active was layer IV and the most active layer V/VI. Adult control and BD cats displayed such a pattern throughout area 17. Furthermore in BD kittens, layers IV and II/III had a higher level of *zif268* mRNA than in control area 17. 4BD resulted in a distinctive elevation of the *zif268* mRNA level: in adults in all peripheral area 17 layers as compared to controls; in juveniles in layer IV throughout area 17 and in central layer II/III compared to controls and other BD kittens. We conclude that the quality of visual input during the initial 4 months of life plays a crucial role in establishing the inter-laminar circuitry within primary visual cortex in cat. We suggest that early BD arrests the developmental processes in central and peripheral representations leading to a continued differential *zif268* gene expression ratio in central versus peripheral area 17 into adulthood. *Project co-financed by the European Union from the European Regional Development Fund within the frame of International PhD Projects Programme (MPD4-504).*

P1.09

DISSECTING OF MICROENVIROMENTAL NISHES FOR OPC DIFFERENTIATION

Ulanska J.[1], Zhao C.[2], Pohl H.[3], Suter U.[3], Kaminska B.[1], Franklin R.J.M.[2], Zawadzka M.[1]

[1] Deparment of Cell Biology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] MRC Cambridge Centre for Stem Cell Biology and Regenerative Medicine and Department of Veterinary Medicine, University of Cambridge,Cambridge, UK; [3] Institute of Cell Biology, ETH-Hönggerberg, Zurich, Switzerland.

New myelinating oligodendrocytes during CNS remyelination are derived from proliferating oligodendrocyte precursor cells (OPC). Using cre/lox technology for OPC fate mapping we have previously demonstrated that these cells give rise not only to oligodendrocytes but also astrocytes and Schwann cells in response to demyelination. We have demonstrated that Schwann cells, derived form OPC, occupied almost exclusively the tissue around blood vessels in astrocyte-deficient areas. It suggests that the peri-vascular microenvironmental niche is the critical determinant of this alternative fate choice phenomenon. The aim of present study was to identify and characterise the microenviromental factors and their downstream cellular effectors determining the fate of adult CNS precursor cells which could be modulated in order to control the remyelination process. We used a well-defined demyelination model that involves bilateral stereotaxic injection of ethidium bromide into the brain white matter of adult rats. At 6 and 10 days after lesion animals were sacrificed and the fresh frozen sections were subjected to immunohistochemical detection of endothelial cell markers, to establish the area of vascular and non-vascular niches. Tissue was then microdissected from pre-defined niches by using infrared and ultraviolet laser capture microdissection system. The microdissected cells represent the *in vivo* state of the cells at the time of cells recruitment and early differentiation. The tissue was collected as a separate niche pools and RNA was extracted for global gene expression analysis. The major outcome of present work is identification of microenviromental factors and quantitative spatial and temporal profile of their expression in the specific niche. Our data serves as a starting point to explore in details the molecular pathways involved in multipotentiality of precursor cells induced by mixed extrinsic microeviromental signals.

P1.10

EPIGENETIC STIMULATION OF PLURIPOTENCY GENES IN CORD BLOOD DERIVED NEURAL STEM CELLS

Szablowska-Gadomska I., Winiarska H., Buzanska L.

NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Epigenetic cues are potent tools for *in vitro* control of the stem cell fate decisions. Since human iPSc, can be derived from any tissue of the body and are characterized by unlimited self-renewal and potential to differentiation into all cell types of the body, they are considered as a good source for autologous transplantation. In this report we were studying the process of induction of pluripotency in neural stem cells derived from human cord blood using only epigenetic stimulation by small molecules and changing oxygen tension. “Pluripotency” is regulated by the set of genes including the expression of Oct4, Nanog and Sox2. Methylation status of the promoters of “pluripotency” genes as well as the chromatin histon

acetylation determine self-renewal and differentiation of stem cells. However the low oxygen environmental condition and small molecules have been used only as additional factors for enhancing induction of pluripotency state. We were testing the influence of lowered (5%) oxygen conditions as well as TSA and RG-108 (histon deacetylase and DNA methyltransferase inhibitors respectively) on the expression of Oct4, Sox 2, Rex1 and Nanog genes in HUCB-NSC (human umbilical cord blood neural stem cells). Cells were cultured from 5 days until one month in serum-free medium supplemented with TSA and/or RG-108 in 5% oxygen. Our results show that low oxygen tensions can activate Oct4 and Nanog genes in HUCB-NSC. Small molecules: TSA and RG-108 enhance this process and additionally induce expression of Sox2 and Rex1. The time of cultivation of the cells in low oxygen conditions and the developmental stage of the cells are the important factors for the induction of the expression of "pluripotency" genes. Our observations confirm that the low oxygen tensions promote maintenance of undifferentiated state of the cells. *Sponsored by grant from Polish Ministry of Scientific Research and Higher Education No. NN302 597838.*

P1.11

ANALYSIS OF NEURAL POTENTIAL OF MESENCHYMAL STEM CELLS DERIVED FROM HUMAN UMBILICAL CORD

Drela K.[1], Jablonska A.[1], Jurga M.[2], Domanska-Janik K.[1], Forraz N.[2], McGuckin C.[2], Lukomska B.[1]

[1] NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland; [2] Cell Therapy Research Institute, CTI-LYON, Lyon, France.

Mesenchymal stem cells (MSC) emerged as promising candidates for therapeutic applications in regenerative medicine and tissue engineering. The ability of MSC to differentiate into multiple different cells of mesodermal origin has offered therapeutic tool for the treatment leukaemias and other malignant diseases. Recently, MSC have been shown to ameliorate a variety of neurological dysfunction. The critical question that remains unanswered is whether MSC can trans-differentiate into neural cells. In an attempt to clarify this issue we explored the expression profile of different markers by mesenchymal cells isolated from human umbilical cord Wharton jelly (HUC-MSC) and compare their expression with neural stem cell line derived from human umbilical cord blood (HUCB-NSC) established in our laboratory. Materials and methods: Gene expression pattern in HUC-MSC (hMSC Lonza medium) and HUCB-NSC (DMEM/F12 medium+2% FBS) was performed by RT-PCR and quantitative RT-PCR reactions. Total RNA was isolated from cultured cells and RT-PCR was performed by using gene-specific primers. The target gene value of each sample was normalized by the GAPDH value. Concomitantly immunocytochemical analysis of gene-related proteins was employed. Results: Direct comparison of the expression profiles demonstrated that HUC-MSC, in addition to pluripotent (Oct-4, Nanog) genes, spontaneously express neural genes: Nestin, NF-200, βIIITubulin, and GFAP. Concomitantly non-induced expression of neural proteins was found. The subsets of HUC-MSC were positive for several markers including: SSEA-4, Nestin, NF-200, βIIITubulin, GFAP and A2B5. Summary and conclusions: We have demonstrated that MSC derived from human umbilical cord Wharton jelly cultured *in vitro* acquire neural progenitor-like properties by expressing neuronal and astrocytic specific markers. *Supported by MSHE grant No. N401 014235 and Foundation Jerome Lejeune grant as part of the Novus Sanguis research consortium.*

P1.12

PROTECTION OF NEURAL STEM CELLS FROM MeHgCl EFFECT AT DIFFERENT BIOFUNCTIONAL DOMAINS

Kozlowska H.[1], Zychowicz M.[1], Mehn D.[2], Dziedzicka D.[3], Nowak J.[2], Stepien P.[3], Rossi F.[2], Buzanska L.[1]

[1] NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland; [2] Nanobiosciences Unit, Institute of Health and Consumer Protection, European Commissions Joint Research Centre, Brussels, Belgium; [3] Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland.

The methylmercury chloride (MeHgCl) is known to cause developmental neurotoxicity in humans. This neurotoxic reagent can induce cell death due to several cellular mechanisms including phosphorylation dependent pathways and disruption of microtubule assembly. The non transformed neural stem cell line obtained from human cord blood (HUCB-NSC) has been previously shown to be susceptible to MeHgCl in developmentally dependent manner. In this report we are trying to find out whether developmental sensitivity of HUCB-NSC to MeHgCl depends upon the type of adhesive biomolecules in functional domains. Cell growth platforms microspotted with fibronectin, vitronectin and poly-L-lysine have been used to compare differentiation potential of HUCB-NSC into neuronal or astrocytic cells at various MeHgCl concentrations ranging from 0.05 to 1 μM. Developmental decisions of HUCB-NSC whether to differentiate into neuronal or glial lineage were observed at non cytotoxic concentrations of MeHgCl and were dependent on the type of bioactive domain. Generally, adhesive domains protected HUCB-NSC from cytotoxic effect of MeHgCl, since on plastic surface even the low-

est concentration of toxicant (0.05 μM) significantly diminish the cell number after 48 h of incubation time as shown by Alamar Blue assay. The same tendency was observed in the proliferation response as shown by Ki67 presence or BrdU incorporation. *Supported by MSHE grant No. 2211/B/P01/2010/38 and No. 5978/B/P01/2010/38, and European Commission Joint Research Centre NanoBioscience Action.*

P1.13

EFFECTS OF PATTERN DEPRIVATION ON THE DEVELOPMENT OF THE MOTION-SENSITIVE POSTERIOR MEDIAL LATERAL SUPRASYLVIAN (PMLS) VISUAL AREA OF THE CAT AS REVEALED BY *zif268* GENE EXPRESSION

Zapasnik M.[1], Laskowska-Macios K.[1], Hu T.T.[2], Kossut M.[1], Arckens L.[2], Burnat K.[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Neuroplasticity and Neuroproteomics Unit, K.U.Leuven, Leuven, Belgium.

Long-term binocular pattern deprivation (BD) from eye opening results in severe global motion perception impairment in children and cats. We recently showed in cat that late onset deprivation (month 3 and 4 following 2 months of normal vision; 2N2BD), also leads to global motion perception impairments. Interestingly, 2 months of BD from eye opening facilitates motion perception, while a continuous 4 month BD period from birth (4BD) does not result in significant impairment. To test how these BD outcomes are reflected at the level of neuronal activity we compared the expression profile of the activity reporter gene *zif268* in PMLS in three separate BD conditions. Adult and juvenile 4BD, 6BD and 2N2BD cats and age-matched controls were used. Cats were exposed to overnight darkness prior to 1-hour light stimulation and then sacrificed; kittens at the end of the BD period, adults after 2 years of visual training upon BD. In situ hybridization was applied using an oligonucleotide probe complementary to the nucleotides encoding amino acids 2-16 of the rat *zif268* gene. For 4BD and 2N2BD kittens the *zif268* mRNA level in PMLS was significantly elevated by 50%, as compared to age-matched controls. 6BD kittens also had elevated *zif268* signals, but by 21%. Interestingly, *zif268* expression in PMLS increases with age as in control kittens higher levels were observed after 6 than 4 months of age. In adult cats *zif268* signals were highest in the 4BD group, while 2N2BD cats did not differ from controls. We infer that the developmental pattern of the motion-sensitive area PMLS is affected by early BD. BD most likely prolongs the processes of cortical development, as *zif268* mRNA levels in 4BD and 2N2BD kittens are equally high. Surprisingly, restoration of normal visual input in adult 2N2BD cats leads to normalization of activity levels in PMLS, while in the 4BD group it remains high. These results link high *zif268* gene expression to behavioral performance.

P1.14

THE IMPACT OF LOCAL TISSUE MICROENVIRONMENT ON THE OLIGODENDROCYTE PROGENITOR DIFFERENTIATION

Sypecka J., Sarnowska A., Winiarska H., Domanska-Janik K., Lukomska B.

NeuroRepair Department, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

The oligodendrocyte precursors exhibit many features of neural stem cells and constitute the abundant population of dividing progenitors in the young and adult brain. A question arises if their commitment and development could be modulated by either local tissue-specific or neuropathological signals. The aim of our study was to evaluate the effect of distinct microenvironments (provided by either the spinal cord or the hippocampal slices) on the differentiation of rat neonatal NG2 cells. The hippocampal slice cultures subjected to an ischemic injury (OGD) were used to mimic the traumatized tissue microenvironment. Both the hippocampal and spinal cord slice cultures were established from the same 7-day old rats. The model of an indirect contact (i.e. exclusively by the culture media) in co-culture system was chosen to eliminate the influence of cell-cell contact. The NG2 cells were obtained from 10-day old mixed primary culture of neonatal rat hemispheres. After 7 days in co-culture, the cells were either stained with neural markers or collected for the RNA isolation and real-time PCR. The medium conditioned by hippocampal slices effectively promoted neurogenesis: ~30% of NG2 cells differentiated into TUJ 1-positive neurons. The remaining fraction mostly formed premyelinating and mature oligodendrocytes. The exposition of hippocampal slices to the OGD injury abolished the effect of pro-neuronal induction in co-cultures. In media conditioned by spinal cord slices, neurogenesis was less pronounced (20% neurons) and the oligodendrocyte differentiation was significantly slowed-down. The NG2 precursors have the intrinsic potency for neurogenesis. Heterogeneity of local microenviroment might modify the fate of endogenous or transplanted NG2 cells what should be taken into consideration in potential neurorepair strategies. *Supported by grant 0345/B/P01/2010/38.*

P1.15

EFFECTS OF BLASTOMERE BIOPSY OF PREIMPLANTATION EMBRYOS ON EXPRESSION OF IMPRINTED GENES AND ANXIETY- AND DEPRESSION-LIKE BEHAVIOURS IN MICE

Sampino S.[1], Zacchini F.[1], Swiergiel A.[2], Modlinski J.[3], Loi P.[1], Ptak G.[1]

[1] Comparative Biomedical Sciences, University of Teramo, Teramo, Italy; [2] Department of Animal Behaviour, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland; [3] Department of Embriology, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland.

Prenatal developmental period is a critical window, where any perturbation of embryonic/fetal environment can lead to behavioural abnormalities and diseases in adult life, such as depression and stress disorders. The mechanisms underlying long term effects, induced by early perturbation, are not well described, but an epigenetic origin has been suggested. In this work we wanted to investigate whether and how blastomere biopsy on 8-cells stage embryos can have long-term effects on behaviour. One-month-old mice derived from the biopsed embryos were subjected to a battery of behavioural tests. The animals displayed an increased locomotor and exploration activity ($p<0.05$) and increased anxiety-like behaviour. Interestingly, the depression-like behaviour in the tail suspension test was observed only in female offspring ($p<0.001$). In addition, to investigate the epigenetic mechanism underlying these behavioural alterations, we analyzed expression of imprinted genes Snrpn, Peg1 and Ube3a in blastocysts obtained after biopsy. These imprinted genes are highly expressed in preimplantation embryos, where their epigenetic programming is defined, and in brain, shaping the behavioural phenotype of offspring. Real-Time PCR analysis revealed significant down-regulation of Peg1 ($p<0.05$), Snrpn and Ube3a ($p<0.01$) in blastocysts derived from the biopsed embryos, compared to controls. The results suggest that blastomere biopsy causes an altered expression of imprinted genes in preimplantation embryo. The reduction of expression of these transcripts can cause anxiety- or depression-like behaviours and alteration of locomotory activity in offspring obtained following biopsy of early embryos.

Neural Excitability, Synapses, and Glia: Cellular Mechanisms [P2]

P2.01

CHOLINERGIC MODULATION OF FREQUENCY POTENTIATION AT CORTICAL LAYER 6 INPUT TO POSTEROMEDIAL THALAMIC NUCLEUS

Nersisyan S.[12], Bekisz M.[1], Kublik E.[1], Smyda J.[1], Granseth B.[2], Wróbel A.[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.

Besides sensory input from whiskers, higher order posteromedial nucleus (PoM) of the thalamus, receives rich and complex cortical feedback projections of modulatory and driving type (from layer 6 and 5, respectively). The function of these recurrent pathways is under control of the brain stem neuromodulatory systems. In this study we investigated cholinergic influence on facilitatory properties of cortical layer 6 input to PoM cells. For this purpose we prepared "classical" thalamocortical slices from brains of young (21-28 days) rats. Such slices are suitable for studying the physiology of synapses made on PoM cells by axons from cortical layer 6, because fibers descending from layer 5 are mostly cut off. Membrane potential of PoM neurons was recorded by whole-cell patch-clamp method while cholinergic agonist carbachol (5-8 μM) was added to the bath to activate cholinergic receptors. To evoke excitatory postsynaptic potentials (EPSPs), repetitive series of 5 electrical stimuli at 20 Hz frequency (15 s inter-trial interval, baseline membrane voltage at -56 mV) were applied by stimulation electrode placed at the cortico-thalamic fiber tract in the internal capsule. Bicuculline was present in the bath to block GABAA receptors. We found that carbachol led to almost threefold decrease of the first EPSP's amplitude in the train. At the same time, however, the ratio between the second and the first postsynaptic potential (paired pulse facilitation) became nearly two times bigger. Moreover, with activated cholinergic receptors, the following EPSPs in the train also grew faster in amplitude. Our results indicate that modulatory cholinergic system may increase the frequency potentiation of cortical layer 6 input to PoM, most likely due to decrease of transmitter release through activation of presynaptic cholinergic receptors. *This research and NS was supported by the Foundation for Polish Science through International PhD Program in Neurobiology.*

P2.02

THETA OSCILLATIONS IN A NOVEL *IN VITRO* PREPARATION OF POSTERIOR HYPOTHALAMUS

Kowalczyk T., Gołębiewski H., Caban B., Bocian R., Kaźmierska P., Konopacki J.

Department of Neurobiology, University of Lodz, Lodz, Poland.

Theta rhythm is the best synchronized EEG activity recorded from the mammalian brain. In rodents, this EEG pattern consists of high-voltage, regular, almost sinusoidal oscillations in the frequency range of 3-12 Hz. Hippocampal formation (HPC) is considered to be the main structure involved in the generation of this activity. Extensive anatomical and electrophysiological studies performed in rodents have revealed that the ascending brainstem-hippocampal synchronizing pathway originates in the nucleus reticularis pontis oralis (RPO), next RPO fibres ascend to posterior hypothalamic (PH) region and then PH neurons projects to medial septal area which is well known as a hippocampal theta rhythm "pacemaker". Earlier results suggest that the posterior hypothalamic region is only a modulator of hippocampal formation type II theta. Our preliminary studies suggest that this area is also capable of generation of local type II theta, which can be produced independently of the HPC theta rhythm. The purpose of the present study was to evoke the theta activity by tonic cholinergic (carbachol) or cholinergic/GABAergic (carbachol/bicuculine) bath perfusion in completely deaferentated posterior hypothalamus i.e. in novel PH slice preparations maintained *in vitro.* Experiments were performed on 25 posterior hypothalamic slices delivered from 25 Wistar rats. Slices were perfused with artificial cerebrospinal fluid containing carbachol (50 μM; 10 slices) or carbachol and bicuculine (50 μM and 10 μM respectively; 15 slices). Well synchronized hypothalamic theta activity was recorded in 7 carbachol-treated PH slices and in 13 PH slices perfused with carbachol/bicuculine.

P2.03

NMDA-INDUCED ELECTROPHYSIOLOGICAL ACTIVITY IN HIPPOCAMPAL FORMATION SLICES - ATTEMPTS TO INDUCE RHYTHMIC OSCILLATIONS

Kaźmierska P., Bocian R., Kowalczyk T., Gołębiewski H., Posłuszny A., Konopacki J.

Department of Neurobiology, University of Lodz, Lodz, Poland.

Theta rhythm is a model example of oscillation and synchrony in neural networks of the central nervous system. Cholinergic and GABAergic nature of the *in vitro* and *in vivo* induced theta rhythm has been earlier established. However, according to the recent report by Bland and colleagues (2007) it is suggested that glutamatergic septohippocampal projection represents a third, independent pathway capable of generating hippocampal field and cellular synchrony. With regard to above data, the aim of the study was to investigate whether the stimulation of NMDA receptors is able to induce rhythmic activity in the hippocampal formation maintained in complete de-afferentation. In the present work field potential and intracellular recordings were made from the CA1 and CA3 fields of hippocampal formation during the bath perfusion of the following concentration of NMDA: 1 μM, 3 μM, 10 μM, 30 μM, 50 μM and 80 μM. Application of all but 1 μM NMDA resulted in epileptic activity in aproximate range 0,1 - 1 Hz. The attempts of synchronization were observed in 23% and 31% of all active slices treated with 20 μM and 50 μM NMDA. Since an "effective dose" of NMDA which administration resulted in the highest probability to induce the attempts of synchronization was 50 μM NMDA, the influence of following mixtures on hippocampal EEG activity has been examined: 50 μM NMDA was tested with 10 μM 2-hydroxy-saclofen and 50 μM NMDA was tested with 10 μM bicuculine. Short attempts of synchronization of EEG activity were noticeable in 15% of active slices treated with the first mixture and in 21% of active slices treated with the other. The effect of NMDA was completely blocked after preincubation with 50 μM selective NMDA receptor antagonist D-AP5. I the light of above results it seems that stimulation of NMDA receptor itself is not sufficient to induce theta oscillations in completely deafferented hippocampal formation. *Project supported by the European Union under European Social Fund (HUMAN - BEST INVESTMENT).*

P2.04

mGluR2 AGONIST, DCG-IV, DECREASES SPIKE RATE OF MITRAL CELLS IN THE ACCESSORY OLFACTORY BULB IN ANESTHETIZED MICE

Emilia Leszkowicz E.[1,2], Brennan P.A.[2]

[1] Department of Animal Physiology, University of Gdansk, Gdansk, Poland; [2] School of Physiology and Pharmacology, University of Bristol, Bristol, UK.

The mGluR2 receptor agonist, DCG-IV, has been hypothesised to promote memory formation by disinhibiting mitral cell activity in the accessory olfactory bulb (AOB), leading to increased feedback inhibition from granule cells. We tested a key aspect of this hypothesis by recording DCG-IV effects on mitral cell activity in the AOB of urethane anaesthetised mice. Animals received 1 μl infusion of artificial cerebro-spinal fluid or 10, or 100 pmol of DCG-IV into the AOB. A recording electrode was located in the mitral cell layer and a reference electrode in the granule cell layer. There was no single unit activity in the latter. Single unit activity in the mitral cell layer was recorded before, during and after drug infusion. Local infusions of DCG-IV not only did not disinhibit

mitral cells but actually reduced their firing frequency. The effect appeared during the infusion and was dose-dependent. The 10 pmol DCG-IV-induced decrease in spike rate was deeper and lasted longer than 100 pmol effect. Trends to return to the pre-infusion levels were observed in both groups by the end of 60-min post-infusion period. Thus, this study failed to find a disinhibitory effect of DCG-IV on mitral cells that had been predicted on the basis of *in vitro* data. These findings challenge the established hypothesis that the memory inducing effects of DCG-IV are mediated by mitral cell disinhibition.

P2.05

ABSINTHE INGREDIENT MONOTERPENOID α-THUJONE IS A MODULATOR OF NEURONAL $GABA_A$ RECEPTORS

Czyżewska M.M.[1], Szczot M.[1], Pollastro F.[2], Appendino G.[2], Mozrzymas J.W.[1]

[1] Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland; [2] Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche University of Novara, Novara, Italy.

Monoterpenoid α-thujone is a compound found in absinthe, alcoholic beverage commonly abused (often by famous artists) in late XIX and early XX century. It has been long speculated that α-thujone is responsible for some adverse effects of this liquor including seizures. It has been investigated that the effect of α-thujone is related to its action on $GABA_A$ receptors but a precise pharmacological analysis is lacking. In the present work we investigated mechanism of α-thujone action on GABAergic currents (current responses to exogenous GABA and miniature synaptic currents) in cultured hippocampal neurons. We found that high concentrations (100 - 300 μM) of α-thujone have only modest effect on amplitude of responses elicited by low (3 μM) [GABA], but it prolonged the current rise-time by nearly fivefold and significantly decreased the current fading during prolonged GABA application. At saturating [GABA] (10 mM), the amplitude of current response, elicited by rapid agonist applications, was significantly reduced by 300 μM α-thujone and current onset was also slowed down almost three times but this effect was markedly smaller than for currents evoked by low [GABA]. To assess the impact of α-thujone on desensitization, the time course of currents elicited by prolonged applications of saturating [GABA] were analyzed. In the presence of α-thujone the steady-state to peak value was markedly increased indicating a decrease in the extent of macroscopic desensitization. To check for the effect of α-thujone on synaptic transmission, we measured miniature inhibitory currents (mIPSC) and found that at 300 μM of α-thujone, amplitude and frequency of mIPSC were significantly reduced. In conclusion, our results suggest that α-thujone may act as a low potency allosteric modulator of $GABA_A$Rs. Preliminary kinetic analysis suggests that this compound affects both binding and gating of neuronal $GABA_A$Rs. *This study was funded by the FNP award Mistrz (contract No 7/2008).*

P2.06

BRAIN GABA LEVELS IN WORKERS FROM TWO ANT SUBFAMILIES: INTER-SPECIFIC DIFFERENCES AND EFFECTS OF QUEEN PRESENCE/ABSENCE

Wnuk A.[1], Kostowski W.[2], Korczyńska J.[1], Szczuka A.[1], Symonowicz B.[1], Bieńkowski P.[2], Mierzejewski P.[2], Godzińska E.J.[1]

[1] Laboratory of Ethology, Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Pharmacology and Physiology of the Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland.

Gamma-butyric acid (GABA), a classical amino acid neurotransmitter, is implicated in the mediation of aggressive behaviour in both vertebrates and invertebrates including social insects. In social Hymenoptera queen absence usually has a suppressing effect on worker aggressiveness and may induce modifications of biogenic amine levels in worker brains. Effects of queen presence/absence on worker brain levels of classical amino acid neurotransmitters were so far unexplored. To investigate that question and to elucidate the possible role of GABA in the mediation of ant aggressive behaviour we carried out HPLC measurements of GABA contents in individual brains of workers of two ant species, *Formica polyctena* (subfamily Formicinae) and *Myrmica ruginodis* (subfamily Myrmicinae) reared in queenright or queenless colony fragments. Immediately before killing the ants (only foragers) were tested in dyadic aggression tests consisting of an encounter with a nestmate, an alien conspecific, or a small larva of the house cricket (*Acheta domesticus*). In spite of significantly smaller brain weight of workers of *M. ruginodis*, worker brain GABA levels were significantly higher in *M. ruginodis* than in *F. polyctena*. Queen absence was associated with significantly increased brain GABA levels in *F. polyctena*, but not in *M. ruginodis*. Brain GABA levels of the tested ants did not depend on the type of the aggression test, and no significant interaction was discovered between the aggression test type and queen presence/absence. We demonstrate for the first time that queen presence/absence may influence not only biogenic amine levels, but also levels of classical amino acid neurotransmitters in ant worker brains. Our data also imply that not only brain levels of classical neurotransmitters, but also modifications of these levels induced by changes of social context (queen removal) may significantly differ between various ant phyla.

P2.07

SOMATOSTATIN EXPRESSING CELLS IN EXPERIENCE-DEPENDENT PLASTICITY IN THE SOMATOSENSORY CORTEX OF ADULT MICE

Nowak K.[1], Liguz-Lęcznar M.[2], Cybulska-Kłosowicz A.[2], Zakrzewska R.[2], Kossut M.[2]

[1] Department of Psychology, Warsaw School of Psychology and Humanities, Warsaw, Poland; [2] Laboratory of Neuroplasticity, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Synaptic plasticity in the nervous system is associated with rapid state-dependent changes that require constant adaptation of the balance between excitation and inhibition. Increasing neuronal activity has been shown to stimulate the inhibitory system to preserve the excitation/inhibition homeostasis. Experimental data suggest an increased inhibitory GABA-ergic neurotransmission in brain structures involved in the learning process. Previously we have shown that classical conditioning involving stimulation of a row of facial vibrissae in adult mice results in an increased density of GABAergic interneurones and increased cortical expression of glutamic acid decarboxylase in granular layer of trained row representation. Also, we have found that parvalbumin containing subpopulation was not involved in the observed changes. From numerous subpopulations of GABAergic neurons, somatostatin (SST) containing cells seem to be likely involved in regulation of activity and plasticity of neuronal networks. To test this hypothesis we have used the sensory training protocol that was based on the classical conditioning where tactile stimulation of one row of sensory whiskers (CS) was paired with a tail shock (UCS). The training was continued for 3 consecutive days and lasted 10 min each day (40 trials). Cells expressing SST were assessed using stereology-based counting in both hemispheres in the barrel cortex. We have shown the substantial (22%) increase of SST-containing cells in the trained row representation. The result suggests that somatostatin is involved in learning induced changes of inhibitory cortical network. *Supported by grant No. 2486/B/P01/2010/39 (M.K.) N N301 248639.*

P2.08

FINE-TUNED MMP-9 LEVEL IS CRITICAL FOR LTP MAINTENANCE IN MOSSY FIBER - CA3 HIPPOCAMPAL PATHWAY

Wiera G.[1], Wójtowicz T.[2], Chmielewska M.[3], Wawrzyniak M.[4], Kaczmarek L.[4], Dzięgiel P.[3], Mozrzymas J.[2]

[1] Department of Molecular Animal Physiology, Wroclaw University, Wroclaw, Poland; [2] Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland; [3] Department of Histology and Embryology, Wroclaw Medical University, Wroclaw, Poland; [4] Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Matrix metalloproteinases (MMPs) are a family of endopeptidases involved in degradation and turnover of extracellular matrix proteins. Among them, MMP-9 is implicated in learning and synaptic plasticity. We have shown using inhibitor FN-439 that MMPs play a critical role in LTP maintenance in the mossy fiber - CA3 hippocampal projection (Wójtowicz and Mozrzymas 2010). In the present study we address the specific role of MMP-9 in the plasticity of this pathway. Gel zymography revealed an up regulation of pro- and active form of MMP-9 in homogenates from slice fragments containing mf-CA3 projection, fixed 2 h after LTP induction. Interestingly, this effect was abolished when LTP was induced in the presence of protein synthesis inhibitor (cycloheximide). Moreover, the effects of cycloheximide and FN-439 on fEPSPs were indistinguishable for up to 2 h after LTP induction. Additionally, we observed on western blot a significant decrease in 30 kDa β-dystroglycan digestion product 2 hours after LTP induction. To further explore the mechanism of MMP-9 action, LTP was induced in slices from MMP-9-/- and MMP-9 overexpressing rodents. In slices from KO mice, the late phase of LTP was severely impaired, although not abolished (136% vs. 189% in WT). Surprisingly, in slices from MMP-9 overexpressing rats, LTP was impaired in a similar manner as in KO mice (113% vs. 171%). Moreover, in MMP-9 overexpressing rats, LTP induction was accompanied by increase in MMP-9 level (shown by gel zymography) similar to what was observed in WT rats. These results indicate that fine-tuned MMP-9 balance is critical for LTP consolidation in mf-CA3 pathway, as in the absence of MMP-9 or in conditions of its excess, LTP is impaired. These results may raise a possibility that impairment of optimal MMP-9 activity (implicated in schizophrenia or bipolar mood disorder) may be detrimental for cognitive processes. *Supported by Ministry for Science and Higher Education grants PN/030/2006 and N N401 541540.*

P2.09

PROXIMITY LIGATION ASSAY VISUALIZES IN CULTURED NEURONS THE INTERACTION BETWEEN ENDOGENOUS ORAI1 AND STIM PROTEINS

Gruszczynska-Biegala J., Kuznicki J.

Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, Warsaw, Poland.

Calcium sensors STIM1 and STIM2, located in ER, and calcium channel forming protein ORAI1 are involved in the store-operated calcium entry (SOCE). The process relies on extracellular calcium influx through the plasma membrane channels. In non-excitable cells STIM interaction with ORAI1 is a crucial element of SOCE, but in neurons its mechanism remains unclear. We showed earlier that STIM1 is likely involved in thapsigargin induced SOCE, while STIM2 is mostly active after EGTA-driven depletion of extracellular

Ca^{2+} (Gruszczynska-Biegala et al. 2011). Depletion of calcium from ER increased number of puncta-like colocalization of YFP-STIM1 and ORAI1, but not of YFP-STIM2 and ORAI1. In contrast, reduction of extracellular calcium level triggered puncta formation for both YFP-STIM1/ORAI1 and YFP-STIM2/ORAI1. In this work we aimed to determine whether after SOCE induction it is possible to detect complexes containing endogenous STIMs and ORAI1. We showed that in cultured rat cortical neurons STIM1 and STIM2 can interact with ORAI1 what can be observed by proximity ligation assay (PLA). Using PLA we were able to visualize fluorescent dots, which represent the site where two antibodies are bound: one against ORAI1 and another one against either STIM1 or STIM2. These dots identify likely the complexes between STIMs and ORAI1. The interaction between them was quantified and found to correlate well with the number of exogenous complexes formed under the same conditions. To confirm that observed PLA dots represent authentic STIM-ORAI1 complexes we use different pairs of anti-STIM and anti-ORAI1 antibodies. The positive findings will allow us to confirm that the interaction between endogenous STIMs and ORAI1 occurs in neurons during SOCE *in vivo* and to demonstrate the feasibility of the PLA technique with antibodies of low specificity.

P2.10

SYNAPTIC PLASTICITY IN BARREL CORTEX IS OCCLUDED BY CLASSICAL CONDITIONING TRAINING AND DEPENDS ON THE ACTIVITY OF METALLOPROTEINASES

Lebida K., Urban-Ciećko J., Mozrzymas J.
Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland.

It is well established that classical conditioning paradigms induce plastic changes in the mouse barrel cortex. In particular, tactile whisker stimulation paired with a tail shock affects GABAergic currents in the layer IV in the cell-specific manner. It is thus expected that sensory learning might affect the neuronal networks in the "trained" barrel, possibly altering its ability to express the synaptic plasticity. To test this possibility, we have compared the long-term potentiation (LTP) induction in "trained" barrels in slices from animals which underwent classical conditioning to that in corresponding barrels in control (yoked, pseudoconditioned) mice. To induce LTP, classical pairing protocol was used (stimulation - layer IV, current-clamp whole-cell recordings - layer II/III). Interestingly, while in control mice, pairing resulted in a clear LTP (161% EPSP increase, 30 min after pairing), in trained animals the LTP induction was nearly absent. This result suggests that behavioral learning occludes the synaptic plasticity in the considered model. It has been demonstrated in other brain region (hippocampus) that synaptic plasticity as well as behavioural learning may critically depend on the activity of metalloproteases (MMPs). We were thus interested whether LTP in the barrel cortex depends on these enzymes. To address this issue, pairing protocol was used to induce LTP in the barrel cortex of control animals and MMPs were blocked by a broad spectrum MMP inhibitor (FN-439). We found that pre-treatment of slices with MMPs inhibitor practically abolished LTP indicating that these enzymes play a critical role in the LTP maintenance in this model. In conclusion, these data indicate that behavioural learning occludes the synaptic plasticity in the barrel cortex and that LTP maintenance in this preparation relies on the activity of MMPs. *Supported by the Ministry of Science and Higher Education grants N401 028 32/0664 and NN401541540.*

P2.11

ACTIVITY-DEPENDENT CLEAVAGE OF NECTIN-3 IS MEDIATED BY MMP-9 METALLOPROTEINASE

Rejmak E., Kalita-Bykowska K., Kaczmarek L.
Department of Molecular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Matrix metalloproteinase-9 regulates pericellular environment through cleavage of protein components of the extracellular matrix as well as cell adhesion molecules. Recently, it has been revealed that MMP-9 plays an important role in the synaptic plasticity. However, only one synaptic target for its enzymatic activity, beta-dystroglycan was identified to date. In this report we show that Nectin-3 the Ca^{2+}-independent immunoglobulin-like cell adhesion molecule, is a potential substrate for MMP-9. We found that NMDA receptor activation resulted in robust ectodomain shedding of Nectin-3 in the hippocampal cultures. The effect was abolished in the presence of NMDA receptor antagonists, APV and MK801. In contrast, pretreatment with either nifedipine or CNQX only partially decreased NMDA-induced Nectin-3 shedding. Using EGTA, the calcium chelator, we showed that NMDA-mediated cleavage of Nectin-3 was calcium dependent. In addition, we observed Nectin-3 cleavage in the presence of calcium ionophore ionomycin. To test if MMP-9 is mediating Nectin-3 shedding we pretreated hippocampal neurons with inhibitor of MMP-9 and found that this tretment completely abolishes Nectin-3 cleavage evoked by wither NMDA or ionomycin. Our results suggest that ecodomain shedding of Nectin-3 is Ca^{2+}-regulated event and MMP-9 can potentially be responsible for these cleavages.

P2.12

THE INTERFERENCE OF THE INFRA SLOW OSCILLATIONS CONTROLS THE SIZE OF THE PUPIL OF THE URETHANE ANAESTHETISED RAT

Blasiak T.[1], Kalita P.[2]
[1] Institute of Zoology, Jagiellonian University, Krakow, Poland; [2] Institute of Computer Science, Jagiellonian University, Krakow, Poland.

Infra slow oscillations (ISO) are low frequency (<0.1Hz) fluctuations detected at the various levels of the brain organisation. In the urethane model of sleep at least a few ISOs can be detected in the rat brain: (1) urethane sleep structure – cyclic alternation of brain state between activated and deactivated EEG patterns; (2) rhythmic, neuronal bursting in the the subcortical visual system – e.g., olivary pretectal nuclei (OPN). Our preliminary observation has revealed that, under constant illumination, the pupil size of the anaesthetised rat oscillates with the period in the range of ISO. The present study was aimed to: (1) determine the relationship between the changes in the pupil size and the ISOs observed in the brain; (2) elucidate the neuronal mechanism of observed pupil size changes. The following signals were simultaneously recorded from urethane anaesthetised rats: multisite ECoG, neuronal firing in left and right OPN, video of the left and right eye. Results revealed that changes of the size of the pupils are synchronised with each other and characterized by two dominant ISO frequencies (~0.01Hz and <0.001Hz). A simple mathematical model of iris smooth muscle constriction and relaxation was proposed to verify the hypothesis that observed changes of the size of the pupils are result of the interference of the three ISOs. The model is governed by the linear first order Ordinary Differential Equation that expresses the muscle relaxation and integration of three ISOs. The parameters of the model were fit to the measured area changes using nonlinear least squares algorithm. It is shown that the area changes predicted from the model correlate well with the observed values. Moreover the analysis revealed that taking all three ISOs into account produces significantly better fit of the model to observed data than any one or two signals, which means that observed pupillary oscillations are result of the interference of all three ISOs recorded in the brain.

P2.13

CHANGES IN α1-ADRENERGIC RECEPTOR SUBTYPES IN THE HIPPOCAMPUS OF RATS NON-RESPONDING TO IMIPRAMINE TREATMENT IN THE CHRONIC MILD STRESS MODEL OF DEPRESSION

Rafa-Zabłocka K.[1], Zelek-Molik A.[1], Bielawski A.[1], Piotr Gruca P.[2], Łasoń-Tyburkiewicz M.[2], Papp M.[2], Nalepa I.[1]

[1] Department of Brain Biochemistry, Institute of Pharmacology PAS, Krakow, Poland; [2] Department of Pharmacology, Institute of Pharmacology PAS, Krakow, Poland.

Despite many years of research on depression the mechanism of the disorder remains elusive. Many studies are focused on dysfunction of central monoaminergic systems and some evidence exist for the role of α1-adrenoceptor (α1-AR). There are three subtypes of this receptor - α1A, α1B and α1D, which are widely distributed in brain. The aim of this study was to assess the expression of all three α1-AR, both at the mRNA and at the protein level in the hippocampi of male Wistar rats, subjected to the chronic mild stress (CMS) procedure followed by treatment with antidepressant drug, imipramine (IMI). Five groups of animals were studied: sham-saline; stress-saline; sham-IMI; stress-IMI-responders and stress-IMI-non-responders. The latter included the stressed animals resistant to IMI treatment as indicated by anhedonia test. The mRNA level was measured using qRT-PCR and SybrGreen dye, and the protein level was assessed by Western blotting. We found that mRNA expression of all α1-AR subtypes was significantly elevated only in the IMI-non-responders group (α1A-AR by 76%; α1B-AR by 96%; α1D-AR by 50%, vs. sham-saline). Moreover, stress alone caused an increase in α1A-AR mRNA (by 41%) though the effect was statistically insignificant. Changes found in the protein level were less pronounced. The only difference between IMI-responders and non-responders was found in α1A receptor protein that was decreased by 73% vs IMI-responders. The level of α1D protein was elevated in all IMI treated groups (by about 79%, vs. sham-saline) and the change occurred independently on stress procedure. No change in the α1B protein was found. Our results indicate that although α1A-AR and α1D-AR are involved in mechanism of IMI action, only the α1A receptor seems to be engaged in the phenomenon of resistance to IMI treatment. *Supported by a grant POIG.01.01.02-12-004/09-00 financed by European Regional Development Fund.*

P2.14

EXPRESSION AND FUNCTION OF EXTRACELLULAR MATRIX METALLOPROTEINASE-9 IN HUMAN EPILEPSY

Konopka A.[1], Grajkowska W.[2], Ziemiańska K.[3], Roszkowski M.[2], Rysz A.[3], Koperski Ł.[3], Kaczmarek L.[4], Wilczyński G.[1], Dzwonek J.[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] The Childrens' Memorial Hospital, Warsaw, Poland; [3] Medical University of Warsaw, Warsaw, Poland; [4] Department of Molecular and Cellural Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Focal cortical dysplasia (FCD) is a developmental brain disorder characterized by abnormalities of cytoarchitecture and neuronal morphology. FCD is associated with pharmacologically intractable forms of epilepsy in both children and adults. The mechanisms that underlie FCD-associated seizures are unclear. It is believed that a pathological plasticity, including abnormality of synaptic connections, plays the crucial role in this disease. Recent studies indicate the role of interactions between nerve cells and the extracellular matrix in the processes of plasticity. Matrix metalloproteinases are

enzymes, which are able to degrade the extracellular matrix components, so they can play an important role in these interactions. Results of experiments using rodent models showed that extracellular matrix metalloproteinase-9 (MMP-9) can play an important role in epileptogenesis. There is no data demonstrating that MMP-9 is involved in the development of epilepsy in human. The aim of this study was to determine whether MMP-9 might play a role in FCD - related epilepsy. Expression of MMP-9 was investigated in human brain tissue derived from people suffering from epilepsy. The immunohistochemistry and antibody microarray methods were used. The control group consisted of the autopsy brain samples. The results indicate that the expression of MMP-9 in the human brain tissue with FCD is increased. The highest immunoreactivity occurs in cytoplasm of abnormal neurons. Moreover, among the 7 tested matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-10, MMP-13), MMP-9 is present in greatest concentration in the FCD tissue homogenates. The results support the hypothesis of the possible role of MMP-9 in the development of human epilepsy and give an opportunity to develop new treatments.

P2.15

MODIFICATION OF ELECTROPHYSIOLOGICAL PROPERTIES OF BARREL CORTEX NEURONS INDUCED BY ENRICHED ENVIRONMENT - *EX VIVO* STUDY

Garkun Y.[1], Bekisz M.[2], Zakrzewska R.[1], Kossut M.[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

In laboratory animals, exposure to the enriched environment (EE) induces broad range of modifications in nerve cells at both molecular and anatomical levels. EE also improves animal's cognitive performance in learning and memory tasks. Despite some progress in revealing the effects of EE on synaptic transmission in the hippocampus, scant and inconsistent data are available on the impact of EE on synaptic properties in the neocortex. The aim of the present study was to examine the influence of the EE exposure on neuronal properties in layer IV of the barrel cortex. Twenty five days old mice (bred under standard laboratory conditions) were put for two weeks to the enriched environment (i.e. to bigger cage with playing tools: tunnels, ladders, a running wheel, spatial maze box and with a set of objects of different shape, made from various fabrics). Control mice were housed in standard laboratory cages during the same period of time. Next, we prepared brain slices containing the barrel cortex and performed visually guided whole-cell recordings from excitatory layer IV neurons within barrels B-D. The results were compared between control and EE-exposed animals. We found that EE experience increased the spontaneous firing rate of excitatory layer IV cells. This phenomenon seems to be due to stronger excitatory synaptic input to these neurons, because both frequency and amplitude of spontaneous excitatory postsynaptic currents were bigger after EE exposure, while kinetic properties of spontaneous inhibitory postsynaptic currents as well as intrinsic excitability remained unchanged. Our results indicate that EE selectively enhances excitatory transmission within the cortical representation of whiskers. *The research was supported by the Ministry of Science and Higher Education "PolPostDoc" grant PBZ/MNiSW/07/2006/09 to GY.*

P2.16

IPSILATERAL RETINAL INNERVATION OF OSCILLATORY OLIVARY PRETECTAL NUCLEUS NEURONS – IS IT IMPORTANT?

Orłowska P., Szkudlarek H.J., Sikorska A., Lewandowski M.H.

Department of Neurophysiology and Chronobiology, Jagiellonian University, Krakow, Poland.

The olivary pretectal nucleus (OPN), suprachiasmatic nucleus and the intergeniculate leaflet of the thalamus, play a crucial role in the entrainment of circadian rhythms. All mentioned nuclei receive input from the retina - the only source of information about environmental light in mammals. It is known that rod/cone- and melanopsin-mediated photoreception are needed to display proper responses of circadian pacemaker to light stimuli. Our previous studies described population of OPN cells which fire in an oscillatory mode with a period of about 2 min. This rhythmic firing pattern requires intact excitatory input from the retina. We have shown that blockade of contralateral rod/cone photoreception caused a decrease in firing rate of OPN cells without influencing their rhythmic activity. However, inhibition of contralateral melanopsin photoreception showed 3 types of neuronal responses: complete disappearance (n=5), temporary disturbances (n=5) and no changes (n=2) in oscillatory pattern of OPN cells. To clarify the likelihood that persistence of oscillatory pattern in 2 of the cases is caused by ipsilateral retinal innervation, we performed *in vivo* experiments on Wistar rats combining electrophysiology with intraocular injections. Contralateral eye was injected with glutamatergic receptor antagonists or 2-aminoethoxydiphenylborane to inhibit rod-cone or melanopsin phototransduction respectively. Tetrodotoxin was used to suppress ipsilateral retinal activity. The results have shown that simultaneous blockade of melanopsin phototransduction and ipsilateral retinal activity strongly decreased firing rate of oscillatory cells (10.63 ± 2.81 to 2.60 ± 2.19 Hz) and caused disappearance of their rhythmic spiking. Interestingly, vanishing of the rhythm was temporary in 3 out of 7 cases and recovered oscillations were longer (95.93 ± 30.11 to 128.33 ± 48.31 s). We suggest that ipsilateral retinal innervation may play a role in the oscillatory activity of some OPN cells.

P2.17

KINETIC PROPERTIES OF LEAK CHANNEL CURRENTS IN MEDIAL PREFRONTAL CORTEX (mPFC) PYRAMIDAL NEURONS IN RATS

Książek A., Witkowski G., Szulczyk P.
Department of Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland.

The prefrontal cortex is involved in cognitive function. It has been suggested that dysfunction of mPFC occurs in widespread neuropsychiatric disorders. The purpose of this study was to classify K^+ channel currents along their kinetic properties in mPFC pyramidal neurons. Channel current recordings were performed from dispersed pyramidal neurons in cell-attached configuration in symmetrical K^+ solutions. The kinetic properties of 100 channel currents were analysed. A total of 27% resembled leak, TREK-like channel currents. Their mean amplitude was 6.9 pA, dwell time was 1.31 ms, and NPo was 0.009 at -50 mV. Their outward and inward conductances were 160 and 150 pS, respectively. The channel currents were strongly mechanosensitive. A total of 27% resembled TASK-like channel currents. Their mean amplitude was 2.0 pA, dwell time was 0.58 ms and NPo 0.11 at -50 mV. Inward and outward conductances were 48 and 71 pS, respectively. A total of 34% were BK-like channel currents. Their amplitude was 5.8 pA, dwell time was 1.1 ms, and NPo was 0.03 at +25 mV. Their outward conductance was 177 pS. In cell-attached configuration, the BK channel currents were only outward. The application of Ca^{2+} ions from the intracellular side in inside-out configuration strongly activated these channels. A total of 5% channel currents were not classified. Surprisingly, we did not find voltage dependent K^+ channel currents. We concluded that the leak-type channel currents could be important players in setting the level of membrane potential in mPFC pyramidal neurons.

P2.18

SERUM RESPONSE FACTOR (SRF) REGULATES MATRIX METALLOPROTEINASE-9 (MMP-9) TRANSCRIPTION IN NEURONS

Kuzniewska B.[1], Blazejczyk M.[2], Malik A.[2], Jaworski J.[2], Kaczmarek L.[1], Kalita-Bykowska K.[1]
[1] Laboratory of Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Laboratory of Molecular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland.

MMP-9 is an endopeptidase playing important role in neuronal plasticity. Although multiple factors regulating MMP-9 expression have been described in different cell types, the molecular mechanism directly controlling its transcription in neurons remains poorly understood. The aim of the current study was to determine, whether SRF/c-Fos pathway is involved in the transcriptional regulation of MMP-9 in neurons. Real-Time PCR analysis revealed strong upregulation of MMP-9 mRNA levels after stimulation of rat primary cortical neurons with BDNF. Additionally, elevated MMP-9 gelatinolytic activity was observed. To investigate mechanism of MMP-9 promoter regulation, we used luciferase gene reporter assay system in which luciferase gene is controlled by MMP-9 promoter fragment (-1369/+35). Treatment of neurons with BDNF led to MMP-9 promoter activation, that was dependent on ERK1/2 actvity, as demonstrated using selective inhibitor or overexpressing constitutively active MKK1. As in MMP-9 promoter there are two AP-1 binding sites, we investigated whether AP-1 contributes to the BDNF-mediated MMP-9 transcription in neurons. MMP-9 reporter construct was induced upon overexpression of different AP-1 dimers in neurons, the most potent being those containing c-Fos. Moreover, BDNF-induced activation of the MMP-9 reporter construct was reduced if proximal, but not distal, AP-1 binding site was mutated. Furthermore knocking-down c-Fos expression in neurons by shRNA decreased MMP-9 gene activation in response to BDNF. As c-fos gene is a known target of SRF, we tested whether SRF can contribute to MMP-9 transcription. Inhibition of SRF by the overexpression of dominant-negative mutant of SRF or using shRNA targeting SRF, abolished BDNF-induced activation of MMP-9 promoter. Our data indicate that MMP-9 expression in neurons can be induced by BDNF. The signal propagation could involve ERK1/2 pathway and SRF-mediated transcription of c-fos gene resulting in activation of MMP-9 promoter.

P2.19

THE OREXIN-INDUCED RELEASE OF CYTOKINES IN PRIMARY MICROGLIAL CELL CULTURES

Namiecinska M.[1], Sokołowska P.[2], Biegańska K.[3], Urbanska A.[3], Andrzejczak D.[4], Zawilska J.B.[3]
[1] Laboratory of chronobiology, Institute of Medical Biology PAS, Lodz, Poland; [2] Department of Pharmacodynamics, Medical University of Lodz, Lodz, Poland.

Orexin A and B (hypocretin 1 and 2) are hypothalamic neuropeptides that have been implicated in a variety of physiological functions, including sleep and arousal, reaction to stress, regulation of energy homeostasis and hypothalamo-pituitary-adrenal axis. The neuropeptides exert their numerous actions by interacting with two specific, membrane-bound, G-protein-coupled receptors, OX_1R and OX_2R. We have recently demonstrated that orexin receptors are expressed in rat astrocytes and modify their function. Thus, searching for new orexins roles, in this work we investigated whether these peptides can affect the production of cytok-

ines by microglial cells. Studies were performed on primary microglial cultures from rat cerebral cortex. Orexin A and orexin B stimulated the release of IL-6 as well as TNF-α from microglial cells, as measured by rat IL-6 and TNF-α immunoassay ELISA kits. Interestingly, orexins did not affect the release of IL-1. The obtained results might be important in the aspect of interaction between microglia and neurons/astrocytes in the central nervous system. *Supported by MNiSW (grant No 4254/B/PO1/2010/38) and InterMolMed (grant No POIG.01.01.02-10-107/09).*

P2.20

D1 RECEPTOR AGONIST INCREASES THE AVAILABILITY OF VOLTAGE-GATED SODIUM CURRENTS IN mPFC PYRAMIDAL NEURONS FROM ADULT RATS

Szulczyk B.

Department of Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland.

This abstract presents the effects of D1 receptor stimulation on action potentials and voltage-gated sodium currents in medial prefrontal cortex pyramidal neurons. Perforated-patch and cell-attached recordings (macropatches) from neurons in slices were obtained from adult rats (9 weeks old). Fas synaptic transmission was blocked. Surprisingly, a D1 agonist (SKF 38393, 10 μM) did not significantly change the membrane potential of mPFC pyramidal neurons in perforated-patches. Also, the D1 receptor agonist influenced neither excitability nor single action potential properties of mPFC pyramidal neurons in perforated patches. The maximal amplitude of sodium currents was not influenced by the D1 receptor agonist (17.5 ± 1.9 pA in control and 17.9 ± 1.6 pA in the presence of SKF 38393, n=7, p>0.05). The potential of half-maximal activation (V0.5) was more negative after D1 receptor stimulation (-8.2 ± 2.5 mV in control and -17.8 ± 1.5 mV in the presence of SKF 38393, n=7, p<0.05). Moreover, given depolarization step activated bigger fraction of available current after D1 receptor stimulation (0.98 ± 0.03) than in control (0.62 ± 0.07, -5 mV depolarization step, n=7, p<0.05). The effects were absent with D1 receptor antagonist (SCH 23390) in the bath. In the presence of the antagonist the potential of half maximal activation was -11.6 ± 1.7 mV in control and -9.6 ± 2.6 mV in the presence of SKF 38393 (n=3, p>0.05). Moreover the D1 receptor agonist did not exert its effects on sodium channels activation curve in the presence of kinase A and kinase C antagonists. This suggests that these two kinases are involved in the signal transduction pathway from the D1 receptor. The up-regulation of sodium channels may enhance persistent activity in mPFC pyramidal neurons from adult rats. *Supported by MniSzW, grant No N N401 03 0037.*

P2.21

REPOSITIONING OF SELECTED GENES IN NEURONAL CELL NUCLEUS UPON SEIZURES

Walczak A.[1], Szczepankiewicz A.[1], Szczepińska T.[2], Ruszczycki B.[2], Dąbrowski M.[2], Broszkiewicz M.[1], Siudek M.[1], Pyskaty M.[1], Wilczek E.[3], Szczerbal I.[4], Świtoński M.[4], Pawłowski K.[2], Wilczyński G.[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Molecular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [3] Department of Histopathology, Medical University of Warsaw, Warsaw, Poland; [4] Department of Animal Breeding, Poznan University of Life Sciences, Poznan, Poland.

Even though the molecular mechanisms of gene expression in neurons are quite broadly described in the literature, little is known about the relationship between these processes and the architecture of the neuronal cell nucleus. For example, it is firmly established that waves of gene expression occurring after neuronal stimulation comprise plenty of genes playing important roles in cognitive and epileptic phenomena. However, it has never been examined whether these bursts of transcriptional activity involve any regulation at the level of higher-order nuclear structure. Accordingly, we have performed studies on the structure of neuronal nucleus in epileptic animals. Based on the literature we chose the genes for BDNF and TRKB that play key roles in synaptic plasticity in the brain. We have investigated clustering of these genes in the nucleus by means of fluorescent *in situ* hybridization. We found that in hippocampal neurons the *bdnf-trkb* distance decreases 2 hours after seizure induction compared to control. Moreover, using bioinformatical approach we selected 2 linear clusters of the genes that are upregulated after seizures. One of them was selected on the chromosome 1 and the other one on chromosome 20. Those linear gene clusters appeared to associate 4 weeks after seizure compared to control. Our results suggest that upon epileptogenesis there is a reorganization of neuronal nucleus involving clustering of the genes in three-dimensional space. The phenomenon could occur also in other forms of synaptic plasticity, and should be a topic to follow up in the future.

P2.22

PRESYNAPTIC VOLTAGE FACILITATION OF SYNAPTIC TRANSMISSION BETWEEN CA3-CA3 PYRAMIDAL NEURONS

Bialowas A.[1], Rama S.[1], Zbili M.[1], Fronzaroli-Molinieres L.[2], Carlier E.[1], Giraud P.[1], Debanne D.[1]

[1] UMR 641 Laboratoire de Neurobiologie des Canaux Ioniques, INSERM, Université de la Méditerranée, Marseille, France; [2] Laboratoire de Neurobiologie des Canaux Ioniques, INSERM U641, Université de la Méditerranée, Marseille, France.

The spread of subthreshold somatic voltage fluctuations into the axon modulates spike-evoked synaptic transmission. In neocortical L5 pyramidal neurons, depolarization-induced facilitation of synaptic efficacy is thought to occur through the enlargement of presynaptic action potential (AP) driven by inactivation of axonal Kv1 potassium channels. We report here in hippocampal slice cultures that synaptic transmission at excitatory CA3-CA3 synapses also depends on the membrane potential of the presynaptic neuron (Vm-pre). In synaptically connected cell-pairs, presynaptic APs produced postsynaptic response recorded in voltage (EPSC) or current-clamp (EPSP) configuration. Synaptic transmission was tested when Vm-pre was held continuously at rest (-61 mV), hyperpolarized (-77 mV) or depolarized potential (-48 mV). The presynaptic voltage facilitation (PVF) of synaptic transmission was quantified by normalizing the postsynaptic responses obtained at -48 mV to those measured at -77 mV. In these conditions, PVF amounted to 135 ± 14% and was associated with a decrease in the paired-pulse ratio. We found that PVF was totally occluded by bath application of the Kv1 channel blocker DTX. Time constant of PVF was determined by evoking single presynaptic APs at increasing delays after the onset of a presynaptic depolarization. The measured time constant (2 s) was compatible with the time constant of the inactivation of D-type current carried by Kv1 channels. Using confocal laser scanning microscopy and Fluo-4 fluorescent calcium indicator, we measured calcium transients in axons of CA3 pyramidal neurons. Depolarization of the cell body from -65 to -50 mV enhanced spike-evoked axonal calcium transients by ~30%. Notably, this facilitation followed the time course of the PVF. We conclude that PVF is a short-term plasticity present at excitatory CA3-CA3 synapses resulting from the increase in spike-evoked calcium transients in the axon caused by voltage-inactivation of Kv1 channels.

P2.23

OREXIN (HYPOCRETIN)-INDUCED HIPPOCAMPAL THETA RHYTHM IN URETHANIZED RATS

Bocian R., Kowalczyk T., Kaźmierska P., Gołębiewski H., Konopacki J.

Department of Neurobiology, University of Lodz, Lodz, Poland.

Orexins (OX), also called hypocretins, are bioactive peptides secreted only from neurons located in the lateral hypothalamus. Immunohistochemical studies indicated that OX neurons project diffusely to almost all brain regions. Widespread projection can determinate multifunctional role these neuropeptides. Preliminary investigations indicated that OX neurons are primarily engaged in food intake and body weight regulation but now they are recognized as major regulators of arousal and sleep-architecture. However, to understand what other physiological function are regulated by OX it is necessary to investigate each site of orexins projection. An existence of well defined orexin-immunoreactive fibers and orexin-B receptor-containing cells in the hippocampal formation (HPC) encouraged us to study the role of orexin B (OXB) in production of hippocampal theta rhythm in urethanized rats. Microinjection of OXB (0.2 ug/0.5 ul) into HPC resulted in theta episodes with increased amplitude and power. In contrast to the amplitude and power, theta frequency was not affected. This effect was reversible and lasted about 1.5 hour. In separate experiments the effect of intrahppocampal injection of OXB, after systemic injection of atropine sulphate (the muscarinic receptor blocker, 8 mg/kg), was also examined. Intracerebral administration of OXB in atropine pretreated animals induced again theta oscillations. Results obtained in the present study indicated that the hippocampal orexin-B receptors are actively involved in mechanism responsible for generating theta-band oscillations in anesthetized rats.

P2.24

INTERACTIONS BETWEEN TWO MODIFIERS OF SODIUM CHANNEL FUNCTION OBSERVED ON CELLULAR LEVEL AND IN WHOLE INSECT ORGANISM

Stankiewicz M.[1], Ciołek J.[1], Wyszkowska J.[1], Gilles N.[2]

[1] Department of Biophysics, Institute of General and Molecular Biology, Nickolaus Copernicus University, Torun, Poland; [2] Department of Proteins, Commissariat à l`Energie Atomique, Saclay, France.

Sodium channels play a crucial role in the nervous and the muscle excitability. They are targeted by a variety of natural and synthetic toxins. Some of them show high selectivity to insects. They are considered as potential bioinsecticides. Toxins bind to different sites in sodium channel; moreover allosteric interactions have been observed between them. Interactions between scorpion insect selective sodium channel toxins (alpha and excitatory toxins) binding to sites 3 and 4, and classical insecticides - pyrethroids (deltamethrin and permethrin – binding to site 7) have been tested at the cellular and whole insect organism level. In cockroach (*Periplaneta am*.) isolated giant axon preparation, the application of scorpion toxins, in “threshold” concentration, accelerated the development of pyrethroid effects. Afterwards, action of pyrethroids in control conditions and after pretreatement with scorpion toxins was tested on DUM (dorsal unpaired median) cockroach neurons. Recordings were realized in “in situ” conditions. Surprisingly, the presence of toxins did not increase the pyrethroid effects – rather slowed them down. Tests performed on whole insects confirmed this strange effect. Scorpion toxins have been applied in the vicinity of cockroach nerve cord, at dose inducing soft insect excitation. Effect of post-application of

pyrethroids has been observed. Pretreatment with scorpion toxins did not increase much or not at all the effects of pyrethroids. Studies are continued with different doses of toxins as well as of pyrethroids on neuronal and whole insect level. The aim is to clarify why the synergistic activity and even the summation of the two component effects was not observed. Results may be crucial for pest insect control, whereas co-application of different agents is proposed as a present-time practice. *Work was sponsored by the Ministry of Science and Higher Education, project no. N N303 320637.*

P2.25

INDUCTION OF LONG TERM POTENTIATION IN DG - CA3 HIPPOCAMPAL PATHWAY RESULTS IN INCREASED MMP-2/9 GELATINOLYSIS IN TARGET HILAR AND CA3 NEURONS

Wójtowicz T.[1], Gendosz D.[1], Lebida K.[1], Drulis-Fajdasz D.[2], Wiera G.[2], Capogna M.[3], Wilczyński G.[4], Dzięgiel P.[5], Mozrzymas J.W.[1]

[1] Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland; [2] Department of Molecular Animal Physiology, Wroclaw University, Wroclaw, Poland; [3] Anatomical Neuropharmacology Unit, Medical Research Council, Oxford, UK; [4] Laboratory of Molecular and Systemic Neuromorphology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [5] Department of Histology and Embryology, Wroclaw Medical University, Wroclaw, Poland.

Matrix metalloproteinases (MMPs) are capable of remodeling extracellular matrix and have been implicated in synaptic plasticity, learning and memory. In particular, upregulation of gelatinases (MMP-2 and 9) accompanies long-term potentiation (LTP) in hippocampal Schaeffer collateral-CA1 pathway. However, the role of gelatinases in synaptic plasticity in other hippocampal pathways remains unknown. Recently, we have found that MMPs blockade by FN-439 abolishes LTP consolidation in the dentate gyrus-CA3 (DG-CA3) projection (where LTP expression is presynaptic, Wójtowicz and Mozrzymas 2010). To address the involvement of gelatinases in the plasticity of this pathway, we have combined high resolution in situ zymography with DQ-gelatin (DQ-G) and immunofluorescence in hippocampal sections from slices used in electrophysiological experiments. LTP was evoked by high frequency stimulation (HFS, 4×100 Hz) while baseline (control) stimulation was applied at 0.1 Hz. Following fixation, slices were cut into thin sections, treated with DQ-G and stained against a neuronal marker MAP-2. The intensity of DQ-G fluorescence was quantified for MAP-2 positive neurons using confocal microscopy. Computer- and visually-guided analysis of cytoplasm and proximal dendrites of target hilar and CA3 neurons revealed that LTP induction was associated with a significant increase in DQ-G fluorescence (30% and 35% increase, respectively, n=6 animals, p<0.05). Importantly, cytoplasmic DQ-G fluorescence profile corroborated with immunoreactivity for MMP-9. The overall DQ-G fluorescence signal in MAP-2 and GFAP-negative extracellular space did not differ between control and HFS-stimulated preparations (n=6 animals, p=0.89). In conclusion, we provide evidence that stimulation pattern that evokes LTP in the DG-CA3 pathway, induces a significant up regulation of gelatinases in the cytoplasm of postsynaptic hilar and CA3 neurons. *Support: Grants NN401541540 and UDA-POKL.04.01.01-00-010/08-01.*

P2.26

NEURONAL PML BODIES ARE MODULATED BY ACTIVITY IN ADULT MURINE BRAIN

Broszkiewicz M.[1], Szczepankiewicz A.[1], Yeghiazaryan M.[1], Patel S.[2], Ruszczycki B.[1], Pawlak R.[2], Wilczyński G.[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Cell Physiology and Pharmacology, University of Leicester, Leicester, UK.

PML is a tumor-suppresor protein involved in the pathogenesis of promyelocytic leukemia. In proliferating mammalian cells PML is a principal component of characteristic nuclear bodies, which contain other proteins but do not contain nucleic acids. There are several PML bodies per nucleus. The molecular function of PML protein is unclear, yet the majority of data points to its involvement in regulation of gene-expression and/or intranuclear protein storage and degradation. In the brain PML has been implicated in the pathogenesis of neurodegenerative disorders, glioma and in the control of embryonic neurogenesis. It is not clear whether the protein is expressed, and has a function, in the normal adult brain. Therefore we have investigated the expression and localization of PML at the cellular and subcellular levels, in the adult mouse brain. By immunofluorescence, typical PML bodies were found in a subset of neurons in the cerebral cortex, hippocampus and amygdala. In the cortex, the protein was present predominantly in layer II/III. Stimulation of neuronal activity by a) pentylenetetrazole seizures or b) immobilization stress, greatly increased the size and signal-intensity of the neuronal PML bodies. Our results suggest that function of PML protein in the brain can be associated with neuronal activity and plasticity.

P2.27

5-HT7 RECEPTORS MODULATE GABAergic TRANSMISSION IN THE CA1 AREA OF RAT HIPPOCAMPUS

Tokarski K., Kusek M., Hess G.

Department of Physiology, Institute of Pharmacology PAS, Krakow, Poland.

Hippocampal GABAergic interneurons modulate the activity of principal glutamatergic cells. Hippocampus receives 5-HT innervation originating in raphe nuclei. In this study we aimed at establishing whether the 5-HT7 receptor-dependent modulation of hippocampal functions also involves local inhibitory circuits. We investigated the effects of 5-HT7 receptor activation on the glutamatergic input to stratum lacunosum moleculare GABAergic interneurons and on the GABAergic input to pyramidal cells of the CA1 area. The experiments were performed on hippocampal slices using whole-cell patch-clamp technique. Neurons were visualized and identified by the shape of the soma as well as by the spiking pattern. For the recording of sIPSCs neurons were voltage clamped at 0 mV and sEPSCs were recorded at -76 mV. The amplitude and the frequency of sIPSCs recorded from pyramidal neurons as well as the amplitude and the frequency of sEPSCs recorded from GABA interneurons were measured. To activate the 5-HT7 receptor, 5-CT (a nonselective 5-HT7 receptor agonist) was applied in the presence of WAY 100635 (the 5-HT1A receptor antagonist). The application of 5-CT increased the mean frequency of sIPSCs and sEPSCs while the mean amplitudes of sIPSCS and sEPSCs were not altered. In the presence of a nonselective glutamate receptor antagonist, kynurenic acid, 5-CT-mediated increase in the sIPSCs frequency was still present. These data suggest that the activation of the 5-HT7 receptor results in an enhancement of the GABAergic transmission via two mechanisms. The first one is an enhancement of excitatory glutamatergic input to GABAergic interneurons and the second - an increase of the excitability of GABAergic cells and /or an increase of GABA release due the activation of 5-HT7 receptors located in the perisomatic region of GABAergic cells and/or on GABAergic terminals. *Support: MNiSW grant 0259/B/P01/2010/38.*

Novel Methods and Technology Development [P3]

P3.01
SOUND FREQUENCY DISCRIMINATION: FUNCTIONAL ROLE OF c-FOS IN AUDITORY CORTEX

Gierej D.[1], Lioudyno V.[1], Mikosz M.[2], Nowak A.[2], Blazejczyk M.[3], Filipkowski R.K.[1], Jaworski J.[3], Kaczmarek L.[1], Knapska E.[2]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [3] Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland.

Immediate-early genes, c-fos in particular, are expressed in the brain upon neuronal activation. c-Fos expression is thought to reflect novelty detection and propensity for a plastic change as its levels decrease when the animal fully acquire a new task. However, its functional role in the brain remains largely unknown. In the present study, we used c-Fos immmunolabeling to identify the cortical network components within auditory and motor cortices that subserve sound frequency discrimination in a behavioral context. We designed a protocol in a fully automated cage for mice (IntelliCage), in which animals were supposed to discriminate between two sounds of different frequencies, one signaling safe access to water and another signaling punishment (air puff). c-Fos expression was analyzed at different stages of the training. Within the anterior parts of ventral and dorsal auditory cortices significant changes in c-Fos levels were observed that correlated with the discrimination learning. On the other hand, c-Fos expression in the motor cortex correlated only with motor activity. In order to address questions concerning the role of c-Fos in learning discrimination of sounds, associated with a particular behavioral meaning, RNA interference was used. c-Fos expression in the auditory cortex was blocked using short hairpin RNA (shRNA) delivered by lentiviral vectors. Mice were bilaterally injected with control vectors and harboring shRNA for *c-fos* (experimental). Then, the animals were subjected to sound discrimination training. Both the control and the experimental animals were responsive to the aversive cues, but the experimental group learnt much more slowly and showed faster extinction of the learned behavior than the control group. These results show the functional involvement of c-Fos within the auditory cortex in sound discrimination learning. More generally, they also support the idea that c-Fos marks neuronal plasticity rather than simple activation.

P3.02
ELECTROPHYSIOLOGICAL CORRELATES OF AUDITORY PERCEPTION OF TEMPORAL ORDER

Alicja Moczulska A.[1], Anna Oron A.[1], Skolimowska J.[1], Lewandowska M.[1], Szelag E.[12]

[1] Laboratory of Neuropsychology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Neurorehabilitation, Institute of Clinical Psychology, School of Social Sciences and Humanities, Warsaw, Poland.

The study concerns the effect of task difficulty on perception of temporal order (TO) for auditory stimuli presented in rapid succession. The measurement comprises auditory mismatch negativity (MMN), i.e., a negative potential with latency from 100 to 250 ms, induced by preattentive changes during auditory stimulation (Näätänen et al. 1978). Fifteen healthy volunteers, aged 21-29 years, participated in this study. The stimuli were two white noises of 10-ms (short) and 50-ms (long) durations. Within each pair noises were separated by a silent gap of 160, 60 or 10 ms, corresponding to three different levels of TO task difficulty, spe-

cifically: “easy”, “moderate” or “difficult”. An auditory oddball paradigm was used, thus a standard and deviant stimuli were presented. In half of the subjects deviants were short-long noises and in the other half long-short noises. Standards were long-short or long-short sequences, respectively. The subject’s task was to watch a silent movie on a computer screen without paying any attention to these auditory stimuli. The stimuli were presented in 9 blocks (3 blocks per each difficulty level). Each of them contained 200 stimuli (20% deviants and 80% standards). Both standard and deviant stimuli were presented in random order. The EEG signal was registered from 64 electrodes, using Brain Products EEG with reference to all averaged electrodes. We expect in ‘easy’ task the MMN with higher amplitudes and/or shorter latencies, than in more difficult tasks, corresponding to gaps of 60 ms, or 10 ms.

P3.03

SEPARATING DYNAMICS OF DIFFERENT CELL POPULATIONS FROM MULTIELECTRODE SIGNALS

Wójcik D., Potworowski J., Głąbska H., Majka P., Łęski S.
Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Local field potentials (LFP), low-frequency part of extracellular electric potentials, seem to reflect dendritic processing of incoming activity to neural populations. Long-range nature of electric field leads to correlations even between remote recordings showing sources from millimeters away which complicates analysis of LFP. To get more insight it is convenient whenever possible to look for current sources of the potentials or to decompose the signals into meaningful components using statistical techniques. In Łęski and coauthors (2010) we have combined inverse current source density method with independent component analysis (ICA) to decompose 140 recordings in rat forebrain obtaining physiologically meaningful components across a group of seven animals. To find out what can be really observed with such an approach experimentally we simulated local field potentials generated in a single cortical column in a model of 3560 cells with non-trivial morphologies. Having both the current source density (CSD) and LFP generated by twelve cortical populations included we compared it with independent components obtained in the decomposition of data generated by the whole network. We assumed a set of potential measurements on a regular grid, low-pass filtered it temporally under 500 Hz, reconstructed the sources using kernel current source density and performed the ICA. We found that the recorded evoked activity was dominated by two populations of pyramidal neurons, which were well separated by ICA. Other populations could not be clearly distinguished in the simulated potentials nor in the ICA. *Supported by grants POIG.02.03.00-00-003/09, POIG.02.03.00-00-018/08.*

History, Teaching, Public Awareness, and Societal Impacts in Neuroscience [P4]

P4.01

THE NEED OF TEACHING CLINICAL NEUROPHYSIOLOGY AT FACULTIES OF PHYSIOTHERAPY

Kinalski R.
Department of Clinical Neurophysiology, Jędrzej Śniadecki District Hospital in Bialystok, Bialystok, Poland; Institute of Cosmetology and Health Care in Bialystok ,Bialystok, Poland.

In the EBM era, the clinical neurophysiology (CNF) diagnostic tools, are important because of quantifying in SI units the effects of physiotherapy of the patients with central nervous system damage. In relation to those patients physiotherapy constitutes a key link in which the physiotherapist play a key role. The students of physiotherapy, as well as of the medicine faculties, are not trained by professional clinical neurophysiologists. To show that, in conformity with the standards of physiotherapy education, there is a need to teach CNF as a separate study subject. There are possibilities to reliably utilize the existing in the country professional human capital, that can introduce the lectures and practical training of CNF into the physiotherapy and medicine faculties curricula. To perform an initial assessment to estimate: 1) which issues of CNF are implemented in core standards of education of the physiotherapy and medicine faculty students; 2) in which and how many of the country discricts tertiary education have faculties of physiotherapy; 3) in which districts, bordered territorially by these higher education schools, there sre establischments licensed to conduct training in CNF; 4) what type of scientific status have persons certified in CNF, who are listed in the districts with physiotherapy faculties. 5) how many physicians and teachers of physical culture have received scientific degrees for CNF research associated with rehabilitation and physiotherapy. The need to include CNF into the undergraduate education curricula of the physiotherapists can be executed by utilizing the existing human resources and infrastructure.

Homeostatic and Neuroendocrine Systems [P5]

P5.01

DIFFERENTIAL mRNA EXPRESSION LEVEL OF THE Bcl2 PROTEIN FAMILY IN THE THALAMUS OF RATS REACTIVE AND NON-REACTIVE TO CHRONIC MILD STRESS - THE ANIMAL MODEL OF DEPRESSION

Bielawski A.[1], Rafa-Zabłocka K.[1], Papp M.[2], Nalepa I.[1]
[1] Department of Brain Biochemistry, Institute of Pharmacology PAS, Krakow, Poland; [2] Laboratory of Behavioral Pharmacology, Department of Pharmacology, Institute of Pharmacology PAS, Krakow, Poland.

Apoptosis is controlled by the balance between pro- (Bax) and anti-(Bcl-2, Bcl-xl) apoptotic proteins within the cell. Bcl-2 and Bcl-xl interact with Bax in the outer mitochondrial membrane and regulate the release of cytochrome c, which activates caspases, the main executors of apoptosis. The increased ratio of pro- vs. anti-apoptotic proteins is associated with an enhanced vulnerability to apoptotic activation. The chronic mild stress (CMS) procedure induces depression-like symptoms in animals. The rats subjected for a prolonged period of time to a variety of mild stressors gradually decrease their responsiveness to rewarding stimuli (e.g., consumption of sweet pellets). We aimed to investigate the expression of Bcl-2, Bcl-xl and Bax mRNAs in the thalamus of rats subjected to the 3-weeks CMS. Three groups of male Wistar rats, selected basing on behavioral test of sucrose (1% solution) consumption – sham, stress reactive and stress non-reactive, were considered. The mRNA expression was measured by quantitative RT-PCR applying TaqMan probes. We found that in the thalamus of rats developing anhedonia to sucrose consumption after the CMS, the mRNA expression of both anti-apoptotic (Bcl-2 and Bcl-xl) and pro-apoptotic (Bax) genes was significantly attenuated, though to a various extent. In the stress reactive animals, the Bcl-2 mRNA was decreased by 57% ($p<0.01$), Bcl-xl – by 51% ($p<0.05$) and Bax – by 24% ($p<0.05$), while no change was noticed in the stress non-reactive animals. Further analysis revealed a significant decrease in the Bcl-2/Bax and Bcl-xl/Bax ratios (respectively, by 48% and 25%; $p<0.01$) in the stress reactive animals, and no change in case of the stress non-reactive animals. Our results suggest that the behavioral reactiveness of rats to the CMS is associated with the enhanced susceptibility to apoptotic activation and development of apoptotic processes in the thalamus. *Supported by statutory funds of the Institute of Pharmacology, PAS.*

P5.02

TEMPORAL INACTIVATION OF THE PEDUNCULOPONTINE NUCLEUS SUPPRESSES HIPPOCAMPAL THETA RHYTHM INDUCED BY ELECTRICAL STIMULATION OF THE NUCLEUS PONTIS ORALIS IN RATS

Matulewicz P., Kusmierczak M., Orzel-Gryglewska J., Jurkowlaniec E.
Department of Animal Physiology, University of Gdansk, Gdansk, Poland.

Synchronous neuronal activity in the hippocampus (theta rhythm) can be elicited in urethanized rats with sensory stimulation as well as with electrical or pharmacological stimulation of different nuclei of the brainstem. It is known that two of these nuclei, the nucleus pontis oralis (RPO) and the pedunculopontine nucleus (PPN), play an important role in theta regulation, however, it is still unclear which of them is essential for expression of theta in the hippocampus. In the present study we investigated the effect of temporal inactivation of PPN on the hippocampal theta rhythm induced by electrical stimulation applied to RPO. The experiments were performed on 5 male Wistar rats in deep urethane anesthesia with its level monitored on the basis of breathing rate. Animals were implanted with bilateral recording electrodes into the dorsal hippocampus and stimulation electrode into the RPO. Hippocampal EEG was recorded during repeated electrical stimulation of RPO in control conditions and also following intra-PPN administration of procaine. In all animals electrical stimulation of the RPO (200 - 300 mA, 30 s) induced episodes of robust hippocampal theta rhythm in both hippocampi which lasted for the whole period of the electrical stimulation (30 s) with no latency. After temporal inactivation of the PPN by direct procaine microinjection (20% solution/0.5 µl), electrical stimulations of the RPO were not able to induce synchronous activity in the hippocampus. Neuronal activity within the RPO and PPN nuclei changes during sleep/wake cycle including paradoxical sleep, of which hippocampal theta rhythm is an important indicator. Regular theta rhythm in the hippocampus is also present during urethane anesthesia which was applied in our experiments. Our results indicate that undisturbed neuronal activity within the PPN is crucial for evoking hippocampal theta rhythm with electrical stimulation of RPO, which suggests superior role of the PPN.

P5.03

PRIOR REPEATED STRESS AFFECTS HPA AXIS AND IL-1ß RESPONSES TO ACUTE RESTRAINT

Rachwalska R., Gądek-Michalska A., Szymańska M., Tadeusz J., Spyrka J., Bugajski J.
Department of Physiology, Institute of Pharmacology PAS, Krakow, Poland.

Interleukin-1ß (IL-1ß) level is modulated during multiple stress reactions in both brain structures involved in hypothalamic-pituitary-adrenal (HPA) axis regulation and peripheral systems. Multiple distinct stressors induce different IL-1ß and HPA axis responses. The purpose of the present study was to determine if the effect of prior repeated restraint stress on IL-1ß levels in prefrontal cortex, hippocampus, hypothalamus and plasma may have an impact on alterations induced in HPA-axis responses. Experiments were performed on male Wistar rats which were exposed to 10 min restraint stress twice a day for 3 days. Twenty-four hour after the last stress period rats were injected i.p. with a single dose of IL-1ß, IL-1ß receptor antagonist or saline. After rapid decapitation, trunk blood was collected and prefrontal cortex, hippocampus and hypothalamus were excised and frozen at

-70°C. Total IL-1ß, ACTH and corticosterone (CORT) levels were determined in plasma using commercially available kits. Western blot analyses were performed on brain structures samples. Repeated restraint for 3 days alone substantially augmented the resting plasma levels of both IL-1ß, ACTH and CORT 24 h after the last restraint. Pretreatment with IL-1ß antagonist abolished the increase in ACTH and CORT responses to repeated stress. IL-1ß receptor antagonist also reduced the enhancement of plasma CORT level induced by 10 min stress. This suggests the selectivity of IL-1ß receptors in central and peripheral mechanisms modulating the stress-induced HPA axis responses. These results suggest that repeated stress increases IL-1ß production which activates ACTH and CORT secretion. Repeated stress also markedly enhanced IL-1ß level in brain structures involved in HPA axis regulation. The present results support the role of brain and peripheral IL-1ß in adaptation of HPA response during prolonged stress. *Grant: POIG 01.01.02-12-004/09-00 financed by European Regional Development Fund.*

P5.04

INTERLEUKIN-1ß IN ACTH AND CORTICOSTERONE SECRETION

Tadeusz J., Gądek-Michalska A., Szymańska M., Spyrka J., Rachwalska P., Bugajski J.
Department of Physiology, Institute of Pharmacology PAS, Krakow, Poland.

Interleukin-1ß (IL-1ß) is crucial mediator of adaptative stress response of the hypothalamic-pituitary-adrenal (HPA) axis. The potential relationship between stress and brain IL-1ß has not been elucidated. Central and systemic administration of IL-1ß enhanced HPA axis activity. We examined the role of IL-1ß in ACTH and corticosterone (CORT) secretion and parallel alterations of IL-1ß in plasma and brain structures involved in HPA axis regulation. Experiments were performed on male Wistar rats. Non-stressed groups of rats were injected i.p. IL-1ß, IL-1ß receptor antagonist and saline. Stressed rats were exposed to 10 min restraint twice a day for 3 days. Twenty-four hour after the last stress period rats were treated like non-stressed. After decapitation trunk blood was collected and the brain prefrontal cortex, hippocampus and hypothalamus were excised and frozen at -70°C. Total CORT, ACTH and IL-1ß levels were determined using commercially available kits. Western blot analyses were performed on brain structures. Under basal conditions IL-1ß considerably increased plasma IL-1ß level, in a time dependent manner, more potently 1 h than 2 h following administration. By contrast plasma ACTH and CORT levels were more strongly enhanced at 2 h than 1 h after IL-1ß injection. This indicates that the most potent increase in plasma IL-1ß levels preceded a similar enhancement of ACTH and CORT secretion. IL-1ß receptor antagonist abolished the increase in ACTH and CORT responses to exogenous IL-1ß. Prior repeated stress increased IL-1ß production and sensitized ACTH and CORT responses to exogenous IL-1ß. Repeated stress also enhanced IL-1ß level in brain structures involved in HPA axis regulation and intensified this increase induced by exogenous IL-1ß. These results indicate the selective modulatory role of IL-1ß in HPA axis activation under basal and stress conditions. *Financed by European Regional Development Fund, grant POIG 01.01.02-12-004/09-00.*

P5.05

NEUROPHYSIOLOGICAL RELATIONSHIP BETWEEN ANXIETY AND SOCIAL RANK

Krotewicz M., Wieczorek M., Koprowska M., Strzelczuk M.
Department of Neurophysiology, University of Lodz, Lodz, Poland.

Social rank means that the individuals take various positions in a social group. The individuals high in the social rank have more social opportunities compared to those low in the hierarchy. Social rank may have important consequences for the hypothalamo-pituitary-adrenocortical (HPA) system activity. It is known that the noradrenergic brain neuronal activity is closely related to the control of the HPA system. In our previous studies we observed that the brain NA system is implicated in the control of social position. The obtained data showed that the low social status was correlated with enhance of noradrenaline release. These results suggested to us that the low ranking individuals were in an anxiety state induced by the presence of a high ranking animal, winner of competitive situation. The present study was designed to examine correlations between anxiety indices measured in the popular anxiety models and positions in the social rank. Rats were exposed to open field (OF), transitions (TT) and social competition (SCT) tests. In the SCT rats were paired accordingly to their body weight and time of sugar pellets eating. The comparison of behavioural indices revealed negative correlation between number of consumed pellets and frequency of locomotion registered in OF. Additionally, the analysis of behavioural indices showed positive correlation between number of consumed pellets and behavioural indices measured in TT. Obtained data revealed that braver individuals which willingly explored new arena were successful in the SCT while the animals attribute with anxiety were losers in the social competitive situation. It suggests that anxiety is rather a reason of low ranking position of individuals then an effect of social competition. The animals got better position for competition and receive higher social rank in pairs when previously had presented better exploratory activity in the “stress”-inducing situation.

P5.06

THE EFFECTS OF 8-OH-DPAT ON FOOD INTAKE IN NON- AND FOOD-DEPRIVED MICE

Brosda J., Müller N., Bert B., Fink H.
School of Veterinary Medicine, Institute for Pharmacology and Toxicology, Berlin, Germany.

Brain serotonin (5-HT) plays an important role in the regulation of food intake. The ingestive effects of 5-HT are mediated by a number of different receptor subtypes. The 5-HT_{1A} receptor agonist 8-OH-DPAT produces hyperphagia or a decrease of food intake depending on animal feeding regime. These hyper- and hypophagic effects of the 5-HT_{1A} receptor agonist have been the subject of many studies mostly carried out in rats. However, there is still little known if (1) the effects are mediated by presynaptic 5-HT_{1A} autoreceptors in the raphe nuclei or by postsynaptic 5-HT_{1A} receptors in serotonergic terminal structures and if (2) 8-OH-DPAT has similar effects in other species. This study investigates the impact of 8-OH-DPAT administration on feeding behaviour in non- and food-deprived NMRI and C57BL mice as well as transgenic L35 mice, characterized by an overexpression of postsynaptic 5-HT_{1A} receptors. Additionally, the microstructure of feeding, water intake and home cage activity were examined. 8-OH-DPAT increased food intake in non-deprived NMRI but not in C57BL mice and induced a hypophagic effect in food-deprived NMRI and C57BL mice. Preliminary data indicate neither a difference in feeding behaviour in L35 mice after 8-OH-DPAT administration nor a variety in the other parameters measured compared to control mice. These results suggest a central role for the 5-HT_{1A} receptor on feeding behaviour in mice, in which the hyperphagic effect of 8-OH-DPAT is most likely mediated by 5-HT_{1A} autoreceptors. Understanding the neurophysiology role of the 5-HT system on food intake may help to achieve new insights in disturbances of eating and body weight disorders.

P5.07

EARLY LIFE STRESS SEX-DEPENDENTLY AFFECTS TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE PRELIMBIC CORTEX AND STRIATUM DURING DIFFERENT STAGES OF ONTOGENESIS

Przyborowska A., Dudys D., Majcher I., Chocyk A., Wędzony K.
Laboratory of Pharmacology and Brain Biostructure, Institute of Pharmacology PAS, Krakow, Poland.

Clinical studies indicate that early life adversity increases the risk for psychiatric disorders, like e.g., depression, schizophrenia and addiction. Moreover, behavioral data imply that early life stress has enduring impact on dopaminergic neurotransmission. The mechanisms engaged in the above effects are poorly understood. Our recent study has revealed that maternal separation (MS) in rats, a model of early life stress, affects the number of midbrain neurons expressing tyrosine hydroxylase (TH), a rate-limiting enzyme in dopamine synthesis. In the present work, we investigated the impact of MS on TH expression in brain regions containing dopaminergic axonal terminals, i.e., in the prelimbic cortex (PLC), dorsal striatum (caudate-putamen, CPu) and nucleus accumbens (NAc) of juvenile, adolescent and adult rats of both sexes. Specifically, we applied immunohistochemical method and quantified the length density of TH-immunoreactive (IR) terminals in the PLC (by stereological estimation) and TH expression in the CPu and NAc, using optical densitometry. It was found that MS affected the length density of TH-IR terminals in the PLC in males only. MS increased the length density of TH-IR terminals (in layers I and II-VI) in adolescent male rats, however the opposite effect (a decrease) was observed in a layer I of the PLC in adults. Optical densitometry revealed that, in juvenile male rats, MS reduced TH expression in the CPu, NAc shell and core. Interestingly, in juvenile females an increase in TH level was observed as an effect of MS. In adolescence, MS did not affect optical density of TH immunoractivity in the CPu and NAc. Though, in adulthood, MS selectively increased TH expression in the NAc core in males only. Our results indicate that early life stress, sex- and age-dependently, disturbs density of dopaminergic innervation of the PLC, CPu and NAc. Moreover, they imply how early adversity affects synaptic plasticity and evokes dysfunction of dopaminergic systems.

P5.08

PROLONGED DOCOSAHEXAENOIC ACID SUPLEMENTATION IMPROVES STRESS-EVOKED COGNITIVE IMPAIRMENT IN RATS

Trofimiuk E., Braszko J.J.
Department of Clinical Pharmacology, Medical University of Bialystok, Bialystok, Poland

Omega-3 polyunsaturated fatty acids (FAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may slow cognitive decline. DHA plays an important role in neural function. Decreases in plasma DHA are associated with cognitive decline in healthy elderly adults and in patients with Alzheimer's disease. In this study we tested a hypothesis that DHA - active constituent of cod liver oil alleviates negative impact of prolonged restraint stress on cognititive functions of male Wistar rats. Specifically, we attempted to characterize the preventive action of long-lasting treatment with DHA (daily dose 300 mg/kg, p.o. for 21 days) in comparison with positive control (fluoxetine: 10 mg/kg daily, p.o. for 21 days) against an impairment caused by chron-

ic restraint stress (2 h daily for 21 days) on recognition memory tested in an object recognition task and on the spatial working memory tested in Morris water maze (MWM). We found that DHA administration statistically significantly prevented deleterious effects of chronic restraint stress both on recognition ($p<0.01$) and the working spatial memory ($p<0.001$). In conclusion, the present study demonstrated that prolonged treatment with a standardized high-concentration DHA preparation reduced stress-induced spatial reference and working memory and recognition memory impairments as measured in the MWM and object recognition task. The present findings not only corroborate the sparse literature concerning the behavioral effects of DHA but also demonstrate for the first time that the use of DHA facilitates functional recovery after stress evoked cognitive brain damage with potency comparable with fluoxetine. This effect appears to be sustained over time.

P5.09

EFFECTS OF POSTNATAL MATERNAL SEPARATION STRESS ON LONG-TERM POTENTIATION IN THE RAT LATERAL AMYGDALA

Danielewicz J.[1], Zieliński Z.[1], Hess G.[12]

[1] Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Krakow, Poland; [2] Department of Physiology, Institute of Pharmacology PAS, Krakow, Poland.

Stressful experiences during the early stages of life can distort normal brain development. In humans, mother-infant interactions may represent a key factor for disease susceptibility which can manifest itself as cognitive and behavioral disorders later in life. The maternal separation (MS) procedure of rat pups represents a useful experimental paradigm to study disturbances in brain function that might occur in response to adverse events during development. MS-subjected animals, as adults, express behavioral and neuroendocrine signs of elevated stress reactivity and cognitive deficits. However, mechanisms by which early life stress exerts its impact on the development and maturation of the brain are poorly understood. This study was aimed at finding the efects of repeated MS on long-term potentiation (LTP) in the lateral amygdala (LA) of adolescent rats. Wistar dams with their offspring were housed under 12:12 h light/dark cycle with food and water available ad libitum. On each of postnatal days (PNDs) 1 - 21 the dams were removed from the maternity cages for 3 h and placed individually in holding cages, while the litter stayed in homecages. Then, the dams were returned to the maternity cages. MS animals, as well as control, animal facility reared rats, were weaned at PND 28 and then housed in groups (4 - 6 animals). For electrophysiological experiments rats between PND35 and PND 50 were used. They were anesthetized and coronal brain slices (450 μm thick) containing the lateral amygdala were cut. Field potentials (FPs) were evoked in LA by the stimulation of the external capsule. LTP was induced using repetitive theta burst stimulation (TBS) protocol. While in slices prepared from control rats FP amplitude, 90 min after TBS, amounted 185.9 ± 17.04 % of baseline, in slices obtained from MS animals LTP was significantly weaker (117.7 ± 7.7%; $p=0.0002$). These preliminary results indicate that MS stress impairs LTP in the lateral amygdala of adolescent rats.

P5.10

EFFECT OF ACUTE AND REPEATED SOCIAL STRESS ON HIPPOCAMPAL TRANSCRIPTOME IN MICE

Stankiewicz A.[1], Zabielska J.[2], Milewczyk A.[2], Korowin M.[1], Gąsiorkiewicz I.[1], Swiergiel A.[1], Juszczak G.[1], Lisowski P.[3]

[1] Department of Animal Behavior, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland; [2] Department of Animal Physiology, University of Gdansk, Gdansk, Poland; [3] Department of Molecular Biology, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland.

The aim of the study was to investigate dynamics of transcriptomic changes in hippocampus during stress in correlation with behavioral and physiological symptoms of acute and chronic stress response. Male Swiss Webster mice were subjected to repeated social stress and decapitated after 1, 4, 8, and 13 days of repeated encounters with other male mice. There was also a group of mice subjected to 13 days of social stress and then left without stress for 5 days. Acute stress induced decrease in food intake and in body weight. Repeated stress induced thymic involution progressing with increasing duration of stress and significant increase in spleen weight, was observed after 13 days of stress. In mice subjected to stress and then left for 5 days for recovery the spleen weight did not differed from control mice and there was partial recovery of thymic weight. During the recovery period there was also increased food intake compared with control mice. Using microarray and quantitative real-time PCR technologies we found that social stress affected hippocampal transcription of genes involved in pathways of insulin secretion, intracellular signaling and cellular transport. Among them, during subsequent time points of social stress we observed progressive upregulation of Ttr gene coding transthyretin involved in amyloidosis, seizures, or dementia and prolactin receptor – Prlr, involved in anxiolyting effects at brain level. The results show that repeated stress provokes major changes in hippocampal physiological pathways. Effects of stress on expression of genes involved in insulin signalling and cellular trans-

port indicate that stress may affect the CNS structure and produce the long-term and irreversible changes in the CNS functions.

P5.11

THE JET LAG SYNDROME IN EPILEPTIC RATS: SEIZURE RESPONSE TO 8 H PHASE-SHIFT

Smyk M.[1], Coenen A.[2], Lewandowski M.H.[1], van Luijtelaar G.[2]
[1] Chair of Animal Physiology, Department of Neurophysiology and Chronobiology, Institute of Zoology, Jagiellonian University, Krakow, Poland; [2] Department of Cognition and Behaviour, Donders Institute for Brain, Donders Centre for Cognition, Radboud University Nijmegen, Nijmegen, The Netherlands.

Biological rhythms are synchronized to a 24 h day mostly by the presence of the light-dark cycle. Sudden changes in the timing of the light phase are known to cause disturbances in circadian physiology manifested as internal desynchronization and/or the jet lag syndrome, fatigue and impaired performance after rapid changes of time zones. The occurrence of absence seizures, characterized by reduced consciousness and the presence of 3 - 4 Hz (humans) and 7 - 11 Hz (rats) spike-wave discharges (SWD) in EEG, shows daily rhythmic fluctuations. The rhythm of SWD in WAG/Rij rats, a validated, genetic model of absence epilepsy, is generated endogenously by the circadian timing system and shaped by the light-dark cycle, motor activity and a momentary state of vigilance. In constant dim light condition, the rhythmicity in the occurrence of SWD is still present, however, internal desynchronization from the rhythm of motor activity is observed (Smyk et al. 2011). The main goals of the study were to verify the role of the light-dark cycle in the synchronization of the rhythm of SWD in WAG/Rij rats and to deliver evidence for the presence of distinct mechanisms controlling rhythms of SWD and motor activity. Chronic EEG and motor activity recordings were made in adult WAG/Rij rats kept in 12:12 light-dark cycle. After 4 baseline days, the onset of light was delayed by 8 h and the recordings were made during 10 consecutive days. An immediate effect of the phase shift on both rhythms was observed. On the 1st post-shift day the acrophase of the rhythms was advanced of about 7.5 h. After that, it gradually returned to the baseline level, however, the course of the resynchronization of the two rhythms was different. In conclusion, an important role of the light-dark cycle in the resynchronization of the rhythm of absence seizures has been confirmed. The phase shift caused an aggravation of epileptic activity and uncoupling between rhythmic occurrence of SWD and motor activity.

P5.12

EFFECTS OF REPEATED ADMINISTRATION OF CANNABIDIOL ON BODY WEIGHT GAIN, FOOD INTAKE AND ACCUMULATION OF ADIPOSE TISSUE IN RATS

Ignatowska-Jankowska B., Jankowski M., Torczynska A., Kurrek A., Swiergiel A.
Department of Animal Physiology, University of Gdansk, Gdansk, Poland

Cannabidiol (CBD) is a major non-psychotropic compound derived from Cannabis that has wide therapeutic potential. In our previous studies we observed decreased body weight gain in rapidly growing rats (10 weeks of age at the start of study) treated with CBD for 14 consecutive days. The present study aimed to evaluate the effects of repeated CBD administration on body weight gain, food intake and accumulation of abdominal adipose tissue in rats fed standard (SD) and high fat (HFD) diet. Adult male Wistar rats (n = 36) weighing approximately 400 g at the beginning of the experiment (14 weeks of age), fed with SD only, or having access to free choice HFD (60% kcal from fat, 10% kcal from sucrose) received intraperitoneal injections of CBD or vehicle for 14 consecutive days (5 mg/kg/day). Body weight gain as well as food and water intake were measured daily. Total amount of intra-abdominal adipose tissue was assessed by a dissection method. In contrast to previous observations in younger rats, repeated CBD administration induced significant increase in body weight gain of rats fed SD, but no significant change in food or water intake was observed. CBD produced tendency for decreased body weight gain in rats fed with HFD, but it did not reach level of significance. CBD did not affect total energy intake in rats that had access to HFD, but slightly increased preference for SD. CBD administration reduced intra-abdominal adipose tissue accumulation in rats with access to HFD, but not in rats fed with SD only. The results suggest that CBD may produce different effects on body weight gain depending on age or the metabolic state of the animal. Moreover, the results indicate that CBD may decrease accumulation of intra-abdominal adipose tissue in animals under HFD.

P5.13

EFFECTS OF ACUTE ADMINISTRATION OF CANNABIDIOL ON FOOD INTAKE IN DEPRIVED RATS

Ignatowska-Jankowska B., Jankowski M., Torczynska A., Kurrek A., Swiergiel A.
Department of Animal Physiology, University of Gdansk, Gdansk, Poland.

Cannabidiol (CBD) is a major non-psychotropic constituent of Cannabis. The endocannabinoid system, which can be modulated

by administration of CBD, is known to play an important role in control of food intake and energy balance. In recent years, numerous studies have revealed various pharmacological effects of CBD such as neuroprotective, analgetic, anxiolytic, antipsychotic and anti-inflammatory properties, and also antitumor potential. Surprisingly, very few reports concern effects of CBD on feeding behavior. Our previous studies revealed decreased body weight gain following repeated CBD administration in rats. The present study aimed to assess acute food intake in deprived rats under standard (SD) and high fat diet (HFD). Adult male Wistar rats (n=40), weighing approximately 350 ± 30 g were food deprived for 24 h and 30 minutes before return of food rats received intraperitoneal injections of CBD (5 mg/kg) or the vehicle. Rats were fed with SD (12% kcal from fat) only, or had additional access to HFD (60% kcal from fat, 10% kcal from sucrose). Food and water intake was measured 1 h, 2 h, 3 h and 24 h after animals were allowed ad libitum access to their assigned diet. Administration of CBD induced significant decrease in food intake of deprived rats within first 3 hours after food was returned in rats fed with SD only. Total energy intake within 24 h was not significantly altered in rats fed with SD. No change in total energy intake or preference of SD and HFD was observed in rats that had access to free choice HFD. CBD did not affect water intake or defecation. The results indicate that acute dose of CBD may decrease food intake in food deprived animals fed with SD, but not in rats having access to HFD. The data indicate that effects of acute CBD administration in deprived rats depend on type of diet.

P5.14

MODIFICATION OF DAILY FOOD INTAKE INDUCED BY CONCURRENT CHANGES IN GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AND CANNABINOID CB1 RECEPTOR ACTIVITY IN THE RAT

Radziszewska E., Bojanowska E.
Department of Behavioral Pathophysiology, Medical University of Lodz, Lodz, Poland.

Feeding behavior is closely related to the circadian activity of humans and animals. The endocannabinoid system is known to affect the circadian changes in energy balance and gastrointestinal peptides were found to change the expression of CB1 receptor in vagal terminals in the alimentary tract. Therefore, we have examined whether the anorectic action of CB1 receptor antagonist, AM 251, may be modified by activation or blockade of GLP-1 receptor. Male Wistar rats were housed in individual cages and maintained on a 12:12 hour light-dark cycle with free access to standard, pelleted rat chow and water. Each rat received a preweighed amount of food every day throughout the experiment. The animals were injected intraperitoneally either with a CB1 receptor antagonist, AM251 (2 mg/kg bw.), followed 15 min later by a GLP-1 antagonist, exendin (9-39) (160 µg/kg bw.), or AM 251 (1 mg/kg bw.) followed 15 min later by a GLP-1 agonist, exendin 4 (1.5 µg/kg bw.). All injections were made 1 – 1.5 hour before lights off. 24-hour food intake was recorded two days before and two days after the injection. AM 251 at a dose of 2 mg/kg significantly reduced daily food intake and concomitant injection of exendin (9-39), at a dose found previously to prevent the action of other anorectic agents, had no effect on a AM 251-induced decrease in food consumption. On the other hand, either 1 mg/kg AM 251 or 1.5 µg exendin-4 administered alone had no significant effect on 24-hour food intake. When, however, these drugs were co-injected, a marked reduction in 24-hour food intake occurred. These results indicate that (1) the anorectic action of CB1 receptor antagonist is not mediated by GLP-1 and (2) the CB1 receptor antagonist and GLP-1 receptor agonist act synergistically to reduce the daily food consumption in the rat when injected before of the nocturnal feeding phase. *This work was supported by the National Centre for Science (grant No. 0056/B/P01/2011/40).*

P5.15

EFFECT OF UNILATERAL STRIATAL 6-HYDROXYDOPAMINE LESION ON VENTILATORY RESPONSE TO HYPOXIA IN THE RAT

Budzińska K., Andrzejewski K.
Department of Respiratory Research, Mossakowski Medical Research Center PAS, Warsaw, Poland.

Motor disorders present in patients with Parkinson's disease (PD) can be mimicked in animals by striatal injection of 6-hydroxydopamine (6-OHDA) that causes a destruction of the dopaminergic nigrostriatal neurons. Since respiratory disturbances accompany PD, the aim of this study was to investigate whether 6-OHDA lesion in the striatum could elicit changes in ventilation and in ventilatory response to hypoxia. Experiments were performed on adult rats that received a microinjection of 6-OHDA or vehicle into the right striatum stereotaxically. Before and 14 days post striatal injection conscious rats were placed in whole body plethysmograph to measure ventilatory parameters as tidal volume, frequency of breathing and minute ventilation during eupnoea and ventilatory response to 3 minutes breathing with 8% hypoxic gas mixture. The hypoxic test was preceded by a behavioral cylinder test to check whether the 6-OHDA lesion was effective in evoking a motor deficit. After the 6-OHDA injection the cylinder test demonstrated a clear preference of the use of the forelimb ipsilateral to the 6-OHDA lesion during rising on the wall of cylinder. The pattern of lung ventilation after the lesion resembled that of the control animals. Breathing with 8% oxygen

evoked characteristic hypoxic hyperventilatory response; however in 6-OHDA-lesioned animals mean tidal volume increased more in response to hypoxia than in controls and attained significantly higher amplitude during the first minute of the hypoxic exposure. In conclusion, studies revealed that an increase in chemical drive for respiration due to hypoxia changed the hypoxic ventilatory response in 6-OHDA model of PD. Since dopamine is thought to exert an inhibitory influence on respiratory hypoxic response, an increase of hypoxic hyperventilation following 6-OHDA injection might result from a deficit of dopamine due to degeneration of dopaminergic neurons evoked by 6-OHDA.

P5.16

MORPHOLOGICAL CHANGES IN THE MIDBRAIN DOPAMINERGIC CELLS IN A RESPONSE TO NEW ENVIRONMENTAL AND AFTER ELECTRICAL STIMULATION OF THE VENTRAL TEGMENTAL AREA IN RATS

Jerzemowska G., Plucinska K., Kulikowski M., Wrona D.
Department of Animal Physiology, University of Gdansk, Gdansk, Poland.

The aim of the study was to test whether increased activity of the telencephalic dopaminergic systems found in rats differing in locomotor activity to novelty (high responders; HR or low responders; LR) is associated with differences in the morphology of cells containing the enzyme tyrosine hydroxylase (TH) in the main group of the brain dopaminergic neurons. The morphology of cells were analyzed in conditions of exposure to a new environment and after chronic electrical stimulation of the ventral tegmental area (VTA). Two groups of male Wistar rats were used: subjected to a new environment (moving from vivarium to the experimental room) and subjected to a 14-day unilateral electrical stimulation of the VTA, which produces behavioral signs of psychomotor activation. After termination of the stimulation procedure, all rats were subjected to the immunohistochemical and immunofluorescent staining of neurons expressing TH (TH+ cells). We analyzed the whole cell: measure area (μm^2), perimeter, major and minor axis length (μm) and circularity factor ($\mu m^2/\mu m^2$). We found that chronic electrical stimulation of the VTA causes significant changes in the morphology of TH+ cells as manifested by changes in their normal shape and distribution of pigment (immunohistochemical labeling: in the rats after stimulation of the VTA in comparison to the naive gropus grayscale value >70% vs >50%), as well as increasing the size of cells in both HR and LR rats (immunofluorescence labeling: area $p<0.01$, perimeter $p<0.001$, major axis length $p<0.05$ and circularity factor $p<0.05$ in the HR and LR rats after stimulation of the VTA in comparison to the HR and LR naive rats). The results obtained suggest that individual behavioral and neurochemical differences which are correlated with increased susceptibility to stress and propensity to develop drug addictions, which characterize HR animals, may be connected with alterations in the morphology and activity of the dopaminergic systems.

P5.17

LESION AND STIMULATION OF THE MESOLIMBIC MOTIVATIONAL DOPAMINE SYSTEM INFLUENCE BLOOD PERIPHERAL LYMPHOCYTE PERCENTAGE IN RATS DIFFERING IN LOCOMOTOR ACTIVITY

Plucinska K., Grembecka B., Glac W., Myslinska D., Jerzemowska G., Wrona D.
Department of Animal Physiology, Gdansk University, Gdansk, Poland.

The mesolimbic dopamine system, that plays a pivotal role in emotionally motivated behaviours, originates in the ventral tegmental area (VTA) and sends major projections to the nucleus accumbens (Acb). In the present work, we assessed the effects of manipulation in the mesolimbic dopaminergic system identified with the "brain reward system" on the percentage of peripheral blood lymphocytes in freely moving rats differing in locomotor activity to novelty (high responders; HR or low responders; LR). T and B lymphocyte populations were determined by flow cytometry (CD3-FITC/CD45RA-PC7). Peripheral blood was collected from rats exposed to chronic 14 day VTA electrical stimulation to obtain feeding or exploration response (constant current 0.1 ms duration, cathodal pulses, 50 Hz, 30-min daily session) and with a lesion electrode aimed at Acb: 3 weeks after electrode implantation, after 2 weeks of chronic VTA stimulation, on the 2^{nd} day after the Acb lesion (2mA for 15s) and on the 14th VTA stimulation day following the Acb lesion. As compared to the respective sham animals, chronic stimulation of the VTA caused significant ($p<0.05$) decrease in T cell percentage in both HR (33.32 ± 5.39% vs 48.04 ± 7.45%) and LR (44.99 ± 2.94% vs 51 ± 3.57%) animals. In addition, significantly ($p<0.05$) lower level of T cell percentage in HR animals (40.13±3.64% vs 50.94 ± 5.87%) rats were observed. On the 14^{th} VTA stimulation day following the Acb lesion, significant ($p<0.05$) decrease in B cell percentage in both HR (12.47 ± 3.55% vs 19.45 ± 1.58%) and LR (7.09 ± 2.48% vs 16.5 ± 3.46%) animals was observed. On the other hand, chronic stimulation of the VTA (20.99 ± 3.38% vs 17.65 ± 0.49%) and Acb lesion (25.14 ± 2.48% vs 20.62 ± 0.85%) caused significant increase ($p<0.05$) in B cell percentage in HR animals. The results emphasize the importance of individual differences (HR vs LR) in the influence of the mesolimbic motivational system on blood lymphocyte distribution.

P5.18

THE EFFECTS OF RESTRAINT STRESS ON GABAergic AND GLUTAMATERGIC INPUTS TO THE PARAVENTRICULAR NUCLEUS OF THE RAT

Tokarski K., Kusek M., Bobula B., Gądek-Michalska A., Hess G.

Department of Physiology, Institute of Pharmacology PAS, Krakow, Poland.

Chronic stress and resulting from it, prolonged hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis and elevated level of glucocorticoids in the circulatory system, have been linked to the pathophysiology of depressive disorders. Dysregulation of the HPA axix is characteristic for individuals with depression. The paraventricular nucleus of the hypothalamus (PVN) is a major regulator of stress responses acting via the release of corticotropin releasing hormone (CRH) to the pituitary gland. This study was aimed at establishing the effects of restraint stress on GABAergic and glutamatergic inputs to the paraventricular nucleus of the rat. Male adult rats were subjected to restraint in metal tubes lasting for 10 min (2 times daily, repeated for 3 consecutive days). Control animals were kept in home cages. Twenty-four hour after the last stress session rats were decapitated, their brains were removed and slices containing a part of PVN (420 μm thick) were cut using a vibrating microtome. Neurons were visualized using Zeiss Axioskop upright microscope using Nomarski optics, a 40× water immersion lens and an infrared camera parvocellular neurons of PVN were selected based on the morphology of the soma and on the response to depolarizing current pulses. Spontaneous IPSCs were recorded from parvocellular neurons which were voltage clamped at 0 mV and sEPSCs were recorded at -76 mV. The amplitude and the frequency of sIPSCs and sEPSCs were recorded. Obtained data indicate that the restraint stress resulted in an increase in the mean frequency of sEPSCs while the mean amplitude of sEPSCs was not altered. The restraint stress had no effect on frequency and amplitude of sIPSCs. Thus, restraint stress enhances glutamatergic, but not GABAergic, inputs to the paraventricular nucleus of the rat. *Grant: POIG 01.01.02-12-004/09-00 financed by European Regional Development Fund.*

POSTER SESSION II

Disorders of the Nervous System [P6]

P6.01

MOLECULAR AND BEHAVIORAL PATTERNS ASSOCIATED TO STRESS RESPONSIVITY AND PTSD RISK

Szklarczyk K., Smutek M., Korostyński M., Solecki W., Gołda S., Przewłocki R.

Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland.

Post-traumatic stress disorder (PTSD) is a chronic anxiety condition that develops as a result of a terrifying event. Clinical studies show that only about 10% of trauma-exposed people suffer from PTSD. Our research was focused on endophenotypes and molecular biomarkers of PTSD in an animal model. Differences in response to stress among inbred mouse strains (C57BL/6J, DBA/2J, SWR/J and 129P3/J) were compared: a single intense footshock was applied and ultrasonic vocalization during/after the stress was measured. Long-lasting effects were assessed 4-6 weeks after the traumatic event: conditioned and sensitized fear, social withdrawal, depressive-like behavior and susceptibility to drug addiction. SWR/J strain displayed the lowest conditioned fear, whereas sensitized fear was increased over time in C57BL/6J mice. Moreover, C57BL/6J strain exhibited increase in depressive-like behavior, while DBA/2J strain displayed increased social withdrawal. In addition, it was observed that exposition to traumatic stress increased sensitivity to rewarding properties of morphine in 129P3/J mice. Diverse long-lasting behavioral consequences of exposition to stress were associated with changes in basal and stress-induced profile of glucocorticoid receptor-dependent (GR) genes (e.g., Fkbp5 and Tsc22d3) in amygdala. Furthermore, our research showed that administration of GR antagonist disrupted reconsolidation of the traumatic event memory. Our research supports a model in which genetic factors are important for phenotypic variation in responsivity to stress. These genes may provide novel insight into mechanisms of stress-related disorders. *This work was supported by Polish MSHE grants NN405 274137, N405 143238, IUVENTUS Plus and POIG De-Me-Ter 3.1.*

P6.02

NMDA RECEPTOR ANTAGONISTS – A NOVEL APPROACH AT THE PHENOMENON OF NEUROPROTECTION

Makarewicz D., Duszczyk M., Kuszczyk M., Lazarewicz J., Salinska E.

Departament of Neurochemistry, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Alternative methods of the therapy in the brain ischemia such as preconditioning seem more interesting because of the lack of the

clinical applicable effective pharmacological neuroprotection. The role of NMDA receptor activation in triggering of this phenomenon was suggested, but it is not clear. Our recent *in vitro* studies (Kuszczyk et al. 2010) disclosed tolerance to the excitotoxic challenge by preconditioning with different NMDA receptor antagonists including MK-801 and memantine. The aim of the present study was to check if NMDA receptor antagonism induces also brain tolerance *in vivo* in different models of experimental brain ischemia. They included hypoxia-ischemia (H-I) in 7-day-old rats and 3-min global forebrain ischemia of Mongolian gerbils. In the neonatal rats exposure to 7% O_2 in N2 was used for hypoxic preconditioning (H-P) as a positive control, while for pharmacological preconditioning two NMDA receptor antagonists MK-801 (3 mg/kg) and memantine (5 mg/kg) were injected i.p. Gerbils were pretreated with MK-801. The animals were preconditioned 24, 48, 72 and 96 hours before the insults, and the brain damage or deficit of CA1 pyramidal neurons was evaluated two weeks later. Our results demonstrated that MK-801 administered in all studied time points almost completely reduced brain damage compared to the H-I group, while H-P and preconditioning with memantine were less effective. In gerbils MK-801 was effective only 24 h before global ischemia. These data demonstrate for the first time ischemic tolerance induced by MK-801 and memantine preconditioning *in vivo*. Known neuroprotective effects of the NMDA receptor antagonists in various models of brain ischemia may be partially ascribed to induced tolerance. We consider a role of mild oxidative stress and enhanced production of trophic factors in its mechanisms. *Supported by the MNiSW grant #0664/B/P01/2010/38.*

P6.03

INFLUENCE OF MINOCYCLINE AND VALPROIC ACID ON GLIAL RESPONSE TO INTRACEREBRAL HEMORRHAGE

Majak K., Kowanski P., Domaradzka-Pytel B., Spodnik J.H., Morys J.

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

Intracerebral hemorrhage (IH) is a devastating form of cerebrovascular disease. Several studies points at glial response as one of the key factors in processes related to brain damage and repair after IH. The aim of the present study was to evaluate the influence of administration of minocycline and valproic acid on glial response after IH. 56 adult male Wistar rats (290 - 410 g) were used for the study. In 48 rats IH was induced by injection of 200 µl of autologous blood into the temporal lobe structures. Next animals were divided into three groups that received either minocycline (3 × 200 mg/kg), or valproic acid (2 × 45 mg/kg) or 0.09% saline (2 ml) for 7 consecutive days. 8 sham-operated animals served as controls. Animals from each group were successively sacrificed at 2, 4, 24 and 48 weeks after the hematoma induction. The intensity of glial response was based on qualitative and semi-quantitative evaluations of immunostained sections. We observed that during the course of IH glial cells exhibit time-dependent changes in morphology and intensity of staining. 2 and 4 weeks after IH induction activated forms of astro- and microglia were observed near the border of hematoma as well as neighboring structures, while 24 and 48 weeks later they were present mainly around the glial scar and in the degenerating white matter. White matter structures also contained NOGO-A-immunoreactive oligodendrocytes. Administration of minocycline or valproic acid decreased the number of activated astro- and microglial cells in the white matter of hemisphere contralateral to site of injury at all time points. The number of oligodendrocytes was influenced only by minocycline. The obtained results indicate that minocycline and valproic acid administration modifies the number of glial cells in the white matter of hemisphere contralateral to the site injury. *The study was financially supported by Polish Ministry of Science and Higher Education grant nr 3419/B/P01/2008/35.*

P6.04

INTRAPERITONEAL DELIVERY OF EX VIVO ACTIVATED MACROPHAGES RESULTS IN DEPRESSION-LIKE BEHAVIOR OF MICE

Roman A., Kuśmierczyk J., Klimek E., Nalepa I.

Department of Brain Biochemistry, Institute of Pharmacology PAS, Krakow, Poland.

Excessive pro-inflammatory activity of macrophages is regarded as one of pathomechanisms of depressive disorders. Administration of lipopolysaccharide (LPS) induces potent activation of whole immune system and is used as an animal model of depression. In this study we investigated whether macrophages, previously activated ex vivo and delivered to mice, are able to induce the behavioral changes related to depression. The experiment was carried out on male C57BL/6J mice. Peritoneal macrophages were stimulated *in vitro* with LPS for 3 h. Then they were stained with 5(6)-carboxyfluorescein diacetate N-succinimidyl ester and injected intraperitoneally at the dose of 2×10^6cells per mouse. Control group was administered likewise with non-stimulated cells. Twenty hours later the recipients were subjected to tests of depressive-like behavior including the locomotor activity, the tail suspension test and the forced swimming test (FST). Presence of injected cells in various compartments of the body was assessed 28 h after administration using flow cytometry and fluorescence microscopy. We found that the administration of ex vivo activated macrophages caused a decrease of initial exploratory activity of

the mice (by 26%) and decreased struggling behavior in the FST (by 65%) in comparison to animals administered with non-stimulated cells. Majority of administered macrophages went away from the peritoneum. They were absent in spleen, in lymph nodes and in pleural cavity but were present in blood. Activated macrophages were more mobile than non-stimulated cells (below 1% and about 5% of peritoneal cells, respectively). These results suggest that *ex vivo* activated macrophages are able to change some parameters of recipients' behavior in depressive-like fashion. The fate of activated cells varies as they are more mobile in the recipients' body as compared with non-activated cells. *Supported by a grant POIG.01.01.02-12-004/09-00 financed by European Regional Development Fund.*

P6.05

EFFECT OF ADVANCED PATERNAL AGE ON ULTRASOUND VOCALIZATION IN MICE

Zacchini F.[1], Sampino S.[2], Swiergiel A.[3], Modlinski J.[2], Loi P.[1], Ptak G.[1]

[1] Department of Comparative Biomedical Sciences, University of Teramo, Teramo, Italy; [2] Department of Experimental Embriology, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland; [3] Department of Animal Behaviour, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland.

Delayed parenthood is a growing phenomenon in western countries due to modern lifestyle. In particular, delayed fatherhood has been described as a factor of risk for several mental disorders and intermediate phenotypes such as poor cognitive or social functions. In this work we want to investigate whether and how advanced paternal age can affect development of offspring. We evaluated UltraSound Vocalization (USV) activity on 4-day old mice, born from both aged and young (control) father, after removal from their nest. We found that the total number of USV in the 5 minutes of testing was significantly higher in pups from aged mice than control (p<0.002) and that the main difference was present during the first minute (p <0.0004). Also, our data showed that the number of high intensity USV was increased in pups from aged mice (p<0.002). We also evaluated the righting reflex ability of 6 and 10-days-old mice. We did not find any difference in righting reflex ability between groups. All together our data show that delayed fatherhood affect communication skills and anxiety like-behavior but no innate righting reflex ability. In conclusion, advanced paternal age affects the development of offspring and these effects are detectable already in first days of post-natal life and they could be first signs of brain diseases in adult life. Future analysis will be necessary to define the long term effect of advanced paternal age and the mechanism underlying developmental alterations in offspring.

P6.06

EFFECTS OF COCAINE ON THE DISTRIBUTION OF LYMPHOCYTE SUBSETS IN THE SPLEEN OF RATS: POSSIBLE INVOLVEMENT OF DOPAMINE RECEPTORS

Jankowski M., Ignatowska-Jankowska B., Glac W., Torczyńska A., Kurrek A., Świergiel A.

Dpartment of Animal Physiology, University of Gdansk, Gdansk, Poland.

The expression of dopamine, dopamine receptors and dopamine active transporters in peripheral lymphoid tissues and lymphocytes suggest that increased dopaminergic activity induced by cocaine may be involved in regulation of the distribution of lymphocyte subsets in the spleen. Adult male Wistar rats implanted with jugular vein catheter received cocaine hydrochloride (3 × 5 mg/kg/ml, i.v., at 30 min intervals) or saline infusions. Animals were pretreated with haloperidol (1 mg/kg/ml, i.v.) or vehicle 10 min prior to the first cocaine infusion. After each cocaine infusion the locomotor-activating effects of cocaine were measured. The spleens were collected 30 min after the third cocaine infusion and total splenocyte numbers and percentage numbers of leukocyte subpopulations were assessed using a morphological method. Three-color immunofluorescent antibody staining procedure (CD3-FITC/CD45RA-PC7/CD161A-APC and CD3-FITC/CD4-PC7/CD8-APC) was used for determination of T, B, NK, T CD4+ and T CD8+ lymphocyte subsets. Plasma corticosterone and serum concentrations of IL-4, IFN-gamma and cocaine were assessed. In the spleen, administration of cocaine after pretreatment with haloperidol decreased numbers of splenocytes, lymphocytes and T CD4+ and B lymphocytes, significantly in comparison to rats treated with cocaine alone. The proportions between lymphocyte subsets and CD4/CD8 ratio in the spleen were not affected. Cocaine or/and haloperidol increased plasma corticosterone concentration. Serum cocaine concentration indicated the possibility of accumulation of cocaine in the applied schedule of administration. Serum concentrations of IL-4 and IFN-gamma were not altered. Haloperidol abolished locomotor response induced by cocaine infusions. In conclusion, dopaminergic activity following administration of cocaine is involved in retention of T CD4+ and B lymphocytes in the spleen of rats.

P6.07

MORPHINE DEPENDENCE IN RATS: EFFECT OF LOW-LEVEL LEAD EXPOSURE DURING PERINATAL PERIOD

Listos J.[1], Listos P.[2], Baranowska-Bosiacka I.[3], Chlubek D.[3], Fidecka S.[1]

[1] Departament of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland; [2] Department of Pathological Anatomy, University of Life Sciences in Lublin, Lublin, Poland; [3] Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland.

In the present experiments the effect of perinatal lead exposure on development of morphine dependence in rats was examined. Female rats were gavaged daily with 0.1 % lead acetate solution. Lead ions were administered through pregnancy and two months through lactation. In the same time control animals received water. The development of morphine dependence was obtained by administration of increasing doses (10, 20, 30, 40, 50, 50 mg/kg, ip) of morphine for six consecutive days in lead intoxicated rats and in control animals. 20 min after the last injection of morphine the withdrawal signs were induced by application of naloxon at dose of 2 mg/kg, ip. Secondly, the development of morphine tolerance to antinociception activity was studied in the tail-flick test in lead intoxicated rats. Then rats received morphine (10 mg/kg, ip) for seven consecutive days and test was performed on 1st and 7th day of the experiment. The experiments have shown that in lead intoxicated rats morphine withdrawal signs (jumping, wet dog shakes) were significantly potentiated than in control animals. The morphine tolerance was also more expressed in lead intoxicate rats. The obtained results have shown that prenatal lead exposure intensify the effects of chronic treatment with morphine in rats.

P6.08

EFFECTS OF BUPROPION ON THE REINSTATEMENT OF NICOTINE-CONDITIONED PLACE PREFERENCE BY DRUG PRIMING IN RATS

Budzynska B., Biala G., Kruk M.

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland.

A common element in the phenomenon of addiction is poly-drug abuse. Behavioural responses related to the relapse to drug taking can be measured in various animal models e.g., in the conditioned place preference (CPP) paradigm. In the present experiments, we employed CPP paradigm including the establishment, extinction, reinstatement and cross-reinstatement procedures, to study mechanisms of nicotine seeking behaviour and interactions between nicotine and cannabinoids and ethanol. First, we revealed that nicotine produced a place preference to the initially less-preferred compartment paired with its injections during conditioning (0.175 mg/kg, base, i.p.). Once established, nicotine CPP was extinguished by repeated testing. Following this extinction phase, nicotine-experienced rats were challenged with CB1 receptor agonist – WIN 55,212-2 (0.5 mg/kg, i.p.) or ethanol (0.5 g/kg, i.p.). These priming injections of both drugs reinstated a marked preference for the compartment previously paired with nicotine. Furthermore, we evaluated the efficacy of atypical antidepressant drug - bupropion (5, 10 and 20 mg/kg, i.p.) in blocking reinstatement of nicotine CPP provoked by WIN 55,212-2 and ethanol. Our results demonstrated that bupropion (exept for dose 10 mg/kg used in reinstatement induced by WIN 55,212-2) attenuated the reinstatement of nicotine-conditioned response induced by both drugs. Results obtained in the present studies may contribute to better understanding of the neurochemical mechanisms underlying nicotine addiction and reciprocal relationships between nicotine, cannabis and ethanol. As reinstatement of drug-seeking is a factor for the development of dependence, bupropion may be useful in the relapse-prevention phase of addiction treatment.

P6.09

EFFECTS OF CHOLINERGIC RECEPTOR LIGANDS ON MEMORY-RELATED RESPONSES MEASURED IN THE ELEVATED PLUS MAZE TEST IN MICE

Budzynska B., Biala G., Kruk M.

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland.

The aim of present experiments was to investigate the influence of cholinergic receptor's ligands on memory-related behavior in mice, using the elevated plus maze (EPM) test. This test allows examining different processes of memory (acquisition and consolidation), depending on the time of drug treatment. The time necessary for mice to move from the opened arm to the enclosed arm (i.e. transfer latency, TL) was used as an index of memory. We revealed that in both processes of acquisition and consolidation, nicotine (0.035 and 0.175 mg/kg, free base, sc) shortened TL on the second day of experiment (TL2), improving memory processes. In contrast, scopolamine (0.3 and 1.0 mg/kg, ip) significantly increased TL2 values, impairing cognitive processes. In the following experiments, we evaluated the influence of a drug currently used in smoking cessation in humans, bupropion, on memory-related behavior induced by nicotine and scopolamine. Interestingly, the acute injection of bupropion (10 and 20 mg/kg), prior to injections of both nicotine (0.035 mg/kg) or scopolamine (1.0 mg/kg), significantly prevented nicotine-induced memory improvement or scopolamine-induced memory impairment. Bupropion can diminish not only the rewarding (dependence-producing) effects of nicotine, but also its cognitive effects related to addiction. Our studies further indicated the great involvement of the cholinergic system in memory and allow development of more effective pharmacotherapies for memory impairment-like treatment of human disorders in which cholinergic pathways can be implicated.

P6.10

EFFECTS OF OREXINS ON SURVIVAL OF RAT C6 GLIOMA CELLS. A COMPARISON TO THE PRIMARY RAT ASTROCYTE CELL CULTURES

Biegańska K.[12], Sokołowska P.[2], Namiecińska M.[2], Urbańska A.[2], Sobczak E.[1], Zawilska J.B.[12]

[1] Department of Pharmacodynamics, Medical University of Lodz, Lodz, Poland; [2] Laboratory of Chronobiology, Institute of Medical Biology PAS, Lodz, Poland.

Orexin-A and orexin-B, also named hypocretin-1 and hypocretin-2, are hypothalamic neuropeptides which are important regulators of sleep-wake cycles, reward-seeking, and body energy balance. These neuropeptides bind to two specific, membrane-bound receptors: OX1R and OX2R, members of the GPCR superfamily. A potent proapoptotic activity of orexins has recently been demonstrated in colon cancer cell lines and human colorectal tumor. In our studies we investigated effects of orexins on survival of rat C6 glioma cells, an experimental model for studies on glioblastoma multiforme, and compared them with those exerted on cultured astocytes from rat cerebral cortex. Orexins A and B decreased the number of surviving C6 glioma cells after 48 h of treatment (MTT test), and reduced [3H]thymidine incorporation into proliferating C6 cells. On the contrary, 48 h incubation of cultured astrocytes with orexins increased astrocyte viability and moderately stimulated their proliferation. Taken together, our results suggest that effects of orexins on cell survival depend on the cell type (normal versus cancer). It can be speculated that orexins may serve as potential anticancer factors in therapy of brain tumors. *Supported by MNiSW (grant No 4254/B/PO1/2010/38) and InterMolMed (grant No POIG.01.01.02-10-107/09).*

P6.11

THE PARTICIPATION OF SIGLEC-F RECEPTOR IN MICROGLIA-GLIOMA INTERACTIONS

Wielgat P., Braszko J.J.

Department of Clinical Pharmacology, Medical University of Bialystok, Bialystok, Poland.

Gliomas are highly invasive brain tumors with the occurence of numerous microglial cells arround the tumor. The density of these cells positively correlates with the malignancy, invasiveness and grading of gliomas. Sialic acid-binding immunoglobulin superfamily lectins (Siglecs) are members of immunoglobulin superfamily that recognize sialic acid residues of glycoproteins. Siglecs have intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIM), implicating them in the suppression of immunoreceptor signaling. Siglec-F is a CD33-related Siglec that binds to 2.3-, 2.6- and weakly 2.8-linked sialic acid. We analysed distribution and function of sialic acids and their receptor, Siglec-F, in glioma-microglia interactions. We observed Siglec-F gene transcription and Siglec-F protein expression in cultured embryonic stem cells derived microglia as well as high level of sialic acids in the mouse glioma cell line GL261. Flow cytomety analysis showed that sialylated structures expressed at the plasma membrane of glioma cells are recognized by recombinant mouse Siglec-F/Fc chimera. Enzymatic desialylation of the glioma cells with endoneuraminidase and α-neuraminidase significantly decreased binding of Siglec-F protein. Our data demonstrate that activation of immunosuppressive Siglec-F receptor by sialic acids can modulate microglia activity and immune response against malignant cells.

P6.12

REPEATED ADMINISTRATION OF CANNABIDIOL DECREASES SPLENIC LYMPHOCYTE NUMBER VIA CB2 RECEPTORS

Ignatowska-Jankowska B., Jankowski M., Torczyńska A., Glac W., Swiergiel A.

Department of Animal Physiology, Institute of Biology, University of Gdansk, Gdansk, Poland.

Cannabidiol (CBD) is a major non-psychotropic compound of cannabis that has been reported to exert neuroprotective, antipsychotic and anxiolytic effects. CBD has promising anti-inflammatory properities, but despite therapeutic potential its mechanisms of action remain poorly understood. Our previous study revealed decrease in number of lymphocytes B and T in peripheral blood following repeated CBD administration. The present study aimed to assess effects of repeated administration of CBD on distribution of lymphocyte subsets in the spleen and the involvement of CB2 receptors. Adult male Wistar rats (n=35, 10 weeks of age at the start of study) received intraperitoneal injections of CBD at a dose of 5 mg/kg/day, or the vehicle, for 14 consecutive days. Total and relative numbers of lymphocyte T (T CD4+, and T CD8+), B, NK subsets were determined by flow cytometry. The selective CB2 receptor antagonist AM630 (1 mg/kg) was administered 15 min before CBD (or the vehicle) in order to block CB2 receptors. Repeated administration of CBD decreased total leukocyte number resulting from decreased numbers of lymphocytes B and T (both T CD8+ and T CD4+) in the spleen. Pretreatment with CB2 receptor antagonist partially inhibited CBD-induced decrease in lymphocyte number that was most pronounced in case of T CD8+ lymphocytes. AM630 itself produced slight decline in lymphocyte number that did not reach statistical significance. Observed effects were accompanied by a decrease in body weight gain, which was prevented by pretreatment with CB2 antagonist. The results indicate that CBD reduces lymphocyte number in the spleen, as it does in peripheral blood and that CBD has ability to affect the lymphocyte number via CB2 receptor.

P6.13

CO_2, ISOFLURANE, AND SEVOFLURANE: HUMANE INDUCTION OF EUTHANASIA IN MICE?

Marquardt N., Fink H., Bert B.
School of Veterinary Medicine, Institute of Pharmacology and Toxicology, Freie Universität Berlin, Berlin, Germany.

Carbon dioxide (CO_2) is commonly used for euthanasia in mice but this method has been strongly criticised concerning animal welfare. Alternatives have not been sufficiently tested. Here, we investigate distress induced by exposure to CO_2, isoflurane (Iso), and sevoflurane (Sevo). NMRI mice were exposed to 100% CO_2 with different filling rates (20% (CO_2 20), 60% (CO_2 60), CO_2 100% (CO_2 100) of chamber volume/min) or Iso and Sevo in different concentrations (Iso 2%, Iso 5%, Sevo 4.8%, Sevo 8%). Control animals received airflow. We measured changes in behaviour, and vocalisations during induction until surgical tolerance (ST) or during 5 min of air exposure. Then, mice were decapitated and blood glucose, plasma adrenaline (A) and noradrenaline (NA) were measured. ST was reached fastest after exposure to CO_2 100, followed by CO_2 60, <Iso 5% <Sevo 8%. Thirty-seven and a half percent of the mice did not reach ST within 5 min while exposed to Iso 2% and Sevo 4.8%. Treatment with CO_2 20 did not induce ST in 75% of the mice. Compared to control animals, changes in behaviour were apparent with regard to grooming, arousal, escape behaviour and excitatory phenomena. No audible or ultrasound vocalisations were detected. Glucose concentrations were risen in Iso 2%, Iso 5% and Sevo 4.8% groups compared to control. A and NA concentrations were increased in CO_2 60 and CO_2 100 treated mice compared to all groups. Even though CO_2 60 and CO_2 100 induce narcosis faster than Iso and Sevo, the increases of A and NA point towards a higher perception of distress. However, further investigations (histopathology analysis of respiratory tract) are in progress to conclusively determine if Iso and Sevo in higher concentrations can be recommended for the induction of euthanasia in mice. *Support by BfR-ZEBET.*

P6.14

EFFECTS OF REPEATED CORTICOSTERONE ADMINISTRATION ON SYNAPTIC TRANSMISSION AND PLASTICITY IN RAT FRONTAL CORTEX

Wabno J., Bobula B., Hess G.
Department of Physiology, Institute of Pharmacology PAS, Krakow, Poland.

Alterations in the functions of glutamatergic and GABAergic systems, as well as disturbances in synaptic plasticity related to cognitive functions have been linked to the patophysiology of mood disorders. High level of glucocorticoids in the circulatory system observed after prolonged exposure to stress is considered as major cause of depression. Repeated corticosterone administration represents an animal model to study the effects of non-adaptative stress. The aim of the present study was to examine the influence of repeated corticosterone administration on excitatory and inhibitory synaptic transmission as well as long-term potentiation (LTP) in ex vivo slices of the frontal cortex. Male Wistar rats were treated with corticosterone (10 mg/kg s.c.; suspended in 1% Tween 80) twice daily, for 7 or 21 days. Frontal cortical slices were prepared 48 hours after last drug administration. Whole-cell recordings of spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) were made from layer II/III pyramidal cells at the holding membrane potential of -78 mV and 0 mV, respectively. Extracellular recordings of field potentials evoked by stimulation of layer V were made from layer II/III. We observed an increase of the mean sEPSCs frequency in slices prepared from animals treated with corticosterone for 7 and 21 days while the mean sEPSCs amplitude remained unchanged. In contrast, no corticosterone-induced changes in parameters characterizing sIPSCs were evident. After 7 and 21 days of stress hormone administration LTP was reduced. These data demonstrate that repeated corticosterone treatment enhances basal glutamatergic transmission and concurrently attenuates LTP. In contrast, GABAergic transmission remained unaffected by corticosterone.

P6.15

DIFFERENT IMMUNE RESPONSE OF BRAIN AREAS AND THE INVOLVEMENT OF INFLAMMATORY CYTOKINES (IL-1, IL-6, TNFα) IN SPATIAL LEARNING AND MEMORY DEFICITS AFTER MPTP INJURY IN MICE

Zaremba M.[1], Kurkowska-Jastrzębska I.[2], Cudna A.[2], Piechal A.[1], Członkowski A.[1]
[1] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland; [2] Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland .

Parkinson's disease (PD) is a neurodegenerative disorders causing not only motor dysfunction, but also cognitive disturbance. The pattern of cognitive deficits in PD often includes: executive impairments, episodic memory deficits and visuospatial dysfunctions. It also became evident that inflammatory processes play an important role in the pathophysiology of PD. Neurodegeneration intense brain immune activation and "cytokine storm" which might induce hyper-excitability of neuronal circuits and might reduce neuronal plasticity and cause impairments in learning and memory abilities. The role of cytokines in regulation of inflammatory responses in different brain regions during PD is unclear. It still remains to be fully understood as to how cytokines partici-

pate in the molecular and cellular mechanisms of deficits in learning, memory and cognition in PD. Loss of dopaminergic neurons in acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models is associated with massive and prolonged glial response and increased production and release of inflammatory mediators. To assess the inflammatory response following MPTP intoxication, we measured the IL-6, IL-1β and TNFα gene expression by real-time quantitative RT-PCR following the Morris water maze behavioral test that was provided at 7 days, 3 and 6 months from the intoxication. Our results indicate that neuroinflammatory activity in MPTP model was not restricted to the nigrostriatal system but also involved hippocampal and cortical areas, regions there are essential for cognitive functions such as working and long - term memory, not only in mice. To evaluate spatial learning and memory abilities of mice the mean latency of reaching the platform, the swimming distance, the time spent in the goal quadrant and crossing parameters were estimated. We found that these parameters correlated with level of mRNA expression of cytokines in hippocampus and cortex.

P6.16

ROLE OF IL-1β IN PRODUCTION OF TNF-α AND IL-6 BY PERIPHERAL MONONUCLEAR CELLS FOLLOWING SUBARACHNOID HEMORRHAGE IN RATS

Larysz-Brysz M., Jędrzejowska-Szypułka H., Olakowska E., Lewin-Kowalik J.
Department of Physiology, Medical University of Silesia, Katowice, Poland.

Subarachnoid hemorrhage (SAH) develops when extravasated arterial blood enters subarachnoid space and mixes with cerebrospinal fluid. This leads to numerous pathologies, including increased synthesis of proinflammatory cytokines, like interleukin-1β (IL-1β). Through broken blood-brain barrier, IL-1β may stimulate peripheral leukocytes. These peripheral mononuclear cells (PMC) may be an additional source of other cytokines and migrating to the brain they can enhance or reduce the pathologies resulting from SAH. We examined the effect of neutralization of IL-1β on secretion of TNF-α and IL-6 by PMCs in adult rats following SAH. SAH was produced by injection of 150 μL of autologous arterial blood into cisterna magna. In 50% of animals, IL-1β activity was inhibited by intracerebroventricular administration of anti-rat IL-1β antibodies. Control group consisted of sham-operated rats. Ninety minutes or 24 hrs following surgery, blood samples were collected from the extraorbital plexus and centrifuged to separate leukocyte subpopulations. Isolated PMCs (monocytes and lymphocytes) were cultured for 24 hrs and TNF-α and IL-6 concentrations in the supernatants were assessed with ELISA. SAH led to the increase of production of both TNF-α and IL-6 by PMCs. Neutralization of IL-1β activity significantly reduced the concentration of both cytokines 90 min as well as 24 hrs after SAH. The results indicate an important role of IL-1β in the activation of peripheral mononuclear cells in the course of subarachnoid hemorrhage.

P6.17

ROLE OF IL-1β IN STIMULATION OF PERIPHERAL MONONUCLEAR CELLS TO SECRETE ET-1 AND IN DEVELOPMENT OF VASOSPASM FOLLOWING SUBARACHNOID HEMORRHAGE IN RATS

Jędrzejowska-Szypułka H., Larysz-Brysz M., Woszczycka-Korczyńska I., Lewin-Kowalik J.
Department of Physiology, Medical University of Silesia, Katowice, Poland.

Subarachnoid hemorrhage (SAH) develops when extravasated arterial blood enters subarachnoid space and mixes with cerebrospinal fluid. As a result, much pathology develops, including arterial vasospasm leading to neuronal ischemia resulting in neurological deficits. Cerebral arteries may be constricted both directly by cytokine influence on arterial smooth muscles and indirectly, through increase of endothelin-1 (ET-1) synthesis or adhesion molecules production. Interleukin-1β (IL-1β) is secreted following SAH and through broken blood-brain barrier it may lead to the stimulation of peripheral leukocytes. These peripheral mononuclear cells (PMC) may be an additional source of ET-1 and migrating to the brain they can enhance the vasospasm. We examined effect of neutralization of IL-1β on secretion of ET-1 by PMCs as well as on basilar artery vasospasm in rats following SAH. SAH was produced by injection of 150 μL of autologous arterial blood into cisterna magna. In 50% of animals, IL-1β activity was inhibited by intracerebroventricular administration of anti-rat IL-1β antibodies. Control group consisted of sham-operated rats. Ninety minutes or 24 hrs following surgery, blood samples were collected from the extraorbital plexus and centrifuged to separate leukocyte subpopulations. Isolated PMCs (monocytes and lymphocytes) were cultured for 24 hrs and ET-1 concentration in the supernatants was assessed with ELISA. In brain stem specimens, vasospasm was determined. SAH led to the strong vasospasm and increase of production ET-1 by PMCs. Neutralization of IL-1β activity significantly reduced the ET-1 level in both time-points, but led to decrease of vasospasm only after 24 hrs. The results suggest that ET-1 does not influence the vasospasm in the acute phase but is involved in this process 24 hrs after subarachnoid hemorrhage.

P6.18

EFFECTS OF BLASTOMERE BIOPSY OF PREIMPLANTATION EMBRYOS ON EXPRESSION OF IMPRINTED GENES AND ANXIETY- AND DEPRESSION-LIKE BEHAVIOURS IN MICE

Sampino S.[1], Zacchini F.[1], Swiergiel A.[2], Modliński J.[3], Loi P.[1], Ptak G.[1]

[1] Department of Comparative Biomedical Sciences, University of Teramo, Teramo, Italy; [2] Department of Animal Behavior, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland; [3] Department of Embryology, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland.

Prenatal developmental period is a critical window, where any perturbation of embryonic/fetal environment can lead to behavioural abnormalities and diseases in adult life, such as depression and stress disorders. The mechanisms underlying long term effects, induced by early perturbation, are not well described, but an epigenetic origin has been suggested. In this work we wanted to investigate whether and how blastomere biopsy on 8-cells stage embryos can have long-term effects on behaviour. One-month-old mice derived from the biopsed embryos were subjected to a battery of behavioural tests. The animals displayed an increased locomotor and exploration activity ($p<0.05$) and increased anxiety-like behaviour. Interestingly, the depression-like behaviour in the tail suspension test was observed only in female offspring ($p<0.001$). In addition, to investigate the epigenetic mechanism underlying these behavioural alterations, we analyzed expression of imprinted genes Snrpn, Peg1 and Ube3a in blastocysts obtained after biopsy. These imprinted genes are highly expressed in pre-implantation embryos, where their epigenetic programming is defined, and in brain, shaping the behavioural phenotype of offspring. Real-Time PCR analysis revealed significant down-regulation of Peg1 ($p<0.05$), Snrpn and Ube3a ($p<0.01$) in blastocysts derived from the biopsed embryos, compared to controls. The results suggest that blastomere biopsy causes an altered expression of imprinted genes in preimplantation embryo. The reduction of expression of these transcripts can cause anxiety- or depression-like behaviours and alteration of locomotory activity in offspring obtained following biopsy of early embryos.

P6.19

THE LOW-FREQUENCY OSCILLATION MODEL OF HALLUCINATIONS IN NEURODEGENERATIVE DISORDERS AND IN DELIRIUM

Juszczak G.

Department of Animal Behavior, Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland.

Visual hallucinations in neurodegenerative disorders and in delirium are composed of fully developed objects of normal shape and size whereas fragmented or distorted objects and geometric patterns are uncommon. Usually, patients hallucinate other people or frightening animals whereas emotionally irrelevant objects are rarely hallucinated. Commonly, the same image repeats itself on different occasion and patients hallucinate one or very limited kinds of objects. The characteristic of hallucinations suggest that they result from activation of preexisting and shaped by experience neuronal representation of external objects. A consistent EEG finding in Parkinson's and Alzheimer's disease, dementia with Lewy Bodies, and in delirium is increased power in delta and theta frequencies with the degree of EEG slowing paralleling the frequency of occurrence of hallucinations. Therefore, I propose a theoretical model of hallucination that is based on current concepts in neuroscience and on electrophysiological findings in clinical and basic research. According to the proposed model the prolonged depolarization, associated with low-frequency oscillations, randomly activate neurons which, in turn, send impulses by forward and backward connections. It is expected that emotionally relevant objects are represented by networks with strongest synaptic connections and that the stronger object representation the higher probability that random activation of neurons will activate the entire network by means of reciprocal connections. Activated representation is further reinforced by attentional processes and enters the content of consciousness leading to hallucination. The proposed model explains the phenomenology of hallucinations occurring in neurodegenerative disorders and in delirium. The model can be also relevant for hypnagogic hallucinations experienced during the sleep onset and in schizophrenia.

P6.20

EXPRESSION OF METHYL-CPG-BINDING DOMAIN PROTEIN 3 (MBD3) IN THE EXPERIMENTAL MODEL OF THE TEMPORAL LOBE EPILEPSY IN RAT

Bednarczyk J., Sliwa A., Lukasiuk K.

Laboratory of Epileptogenesis, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

MBD3 is a member of the family of methyl-CpG binding domain containing proteins. It is a component of a multisubunit complex involved in nucleosome remodeling and histone deacetylation. MBD3 does not bind to DNA, but recruits histone deacetylases and DNA methyltransefases and can act as transcriptional repressor. The aim of this study was to verify if MBD3 is involved in epigenetic mechanisms leading to epilepsy development. We have studied MBD3 mRNA and protein expression in the brain of rats undergoing epileptogenesis evoked by amygdala stimulation induced status epilepticus ($n=6$) and time-matched sham controls

(*n*=6). mRNA and proteins were isolated from the dentate gyrus, hippocampal CA1-CA3 and extrahippocampal temporal lobe tissue. mRNA expression was studied with quantitative RT-PCR. Differences in the MBD3 mRNA expression in the dentate gyrus and extrahippocampal temporal tissue did not reach statistical significance. Two bands representing MBD3 were detected on western blot. Densitometric analysis revealed decrease of the intensity of the upper band in the extrahippocampal temporal lobe 14 days after status epilepticus (0.42 ± 0.18 fold of control, p=0.001). No difference between groups was observed in the dentate gyrus and CA1-3. Decrease in the expression of MBD3 protein in the temporal lobe during epileptogenesis may lead to derepression of transcription of genes crucial for epilepsy development. *Supported by Polish Ministry of Science and Education grant N N301 162135.*

6.21

EFFECTS OF OLANZAPINE, RISPERIDONE AND FLUOXETINE IN THE FORCED SWIMMING AND ELEVATED PLUS-MAZE TESTS IN RATS

Rogóż Z., Kabziński M., Sadaj W.

Department of Pharmacology, Institute of Pharmacology PAS, Krakow, Poland.

Several clinical reports have suggested a beneficial effect of the addition of a low dose of an atypical antipsychotic drug, e.g., olanzapine (OLA), risperidone (RIS) to the ongoing treatment with antidepressant drugs, particularly of the selective serotonin reuptake inhibitors (e.g., fluoxetine, fluvoxamine and paroxetine) in the treatment of drug-resistant depression. In anxiety disorders, like post-traumatic stress disorder, some studies found that atypical antipsychotics improved certain symptoms, while others failed to reach the same conclusion. Preclinical evidence on the intrinsic anxiolytic-like property of atypical antipsychotics is also inconclusive. In the present study we examined the effect of treatment with OLA or RIS, given separately or jointly with fluoxetine (FLU) in the forced swimming test (FST, an animal model of depression) and in the elevated plus-maze test (an animal model of anxiety) in male Wistar rats. The obtained results showed that treatment with OLA or RIS (0.05 and 0.1 mg/kg) and FLU (10 mg/kg) did not change the immobility time of rats in the FST. Moreover, co-treatment with OLA or RIS and FLU produced antidepressant-like activity in the FST, and that serotonin 5-$HT1_A$ receptors might play some role in these effects. RIS (0.1 and 0.3 mg/kg), OLA (1 mg/kg) and FLU (5 and 10 mg/kg) induced anxiolytic-like effect in the elevated plus-maze test. In contrast, co-administration with OLA or RIS and FLU was ineffective in that test. This finding indicates that low doses of OLA or RIS enhances the action of FLU in an animal model of depression, and they may be of particular importance to the pharmacotherapy of drug-resistant depression. In contrast, OLA or RIS and FLU may each be clinically effective in treating anxiety disorders, but their effects may be attenuated in the combination treatment with both medications. *This study was supported by grant POIG 01.01.02-12-004/09-00 from European Regional Development Fund.*

P6.22

THE INFLUENCE OF AAV-MEDIATED GENE TRANSFER OF HUMAN INTERLEUKIN 10 ON THE NEURODEGENERATIVE PROCESS IN THE MURINE MODEL OF PARKINSON'S DISEASE

Wawer A.[1], Joniec-Maciejak I.[1], Schwenkgrub J.[1], Sznejder A.[1], Ciesielska A.[2], Bankiewicz K.[2], Członkowska A.[3], Członkowski A.[1]

[1] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland; [2] Department of Neurosurgery, University of California San Francisco, San Francisco, USA; [3] Second Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland.

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. It is characterized by a progressive loss of dopaminergic neurons, which occurs mainly in the substantia nigra (SN), resulting in the loss of nerve terminals, accompanied by a decreased concentration of dopamine (DA) and its metabolites in the striatum. Concurrently with neurodegeneration, a chronic inflammation occurs in the affected regions of the brain. Mechanisms and etiology of the neurodegeneration are still unknown. One potential strategy for therapy of PD is to reduce the neurodegeneration by inhibiting the inflammatory reaction. Nowadays, there are high hopes for the gene therapy based treatment. This involves using a noninfectious virus administered directly into the brain. In this study a transfer of AAV vector containing the complementary DNA for human interleukin 10 (IL-10) into the nigrostriatal pathway was used. IL-10 is one of the major anti-inflammatory cytokines. The aim of the present study was to examine the expression of human IL-10, administered into striatum by viral vector AAV2-hIL-10 and investigate the influence of this cytokine on neurodegenerative process in the murine model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). One year old male C57Bl mice were used in this study. The expression of human IL-10 was measured by enzyme-linked immunosorbent assay (ELISA). To evaluate the influence of the human IL-10 on neurodegeneration concentrations of striatal DA, 3,4-dihydroxyphenyl acetic acid and homovanillic acid were measured by high performance liquid chromatography (HPLC). The findings demonstrate human IL-10 secretion in the mouse brain after striatal infusion of AAV2-hIL-10. This study suggests that human IL-10 delivered by an AAV2 vector preserves nigrostriatal function after MPTP intoxication (lower decrease in DA concentration). IL-10 can play a vital role in inhibition of inflammatory reaction or surviving cells stimulation.

P6.23

COMMON MECHANISMS OF INTRACELLULAR CALCIUM RELEASE BY TBBPA AND THAPSIGARGIN IN CULTURED NEURONS

Zieminska E., Stafiej A., Lazarewicz J.
Department of Neurochemistry, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Tetrabromobisphenol A (TBBPA) is a brominated flame retardant and the environmental toxin affecting the brain. The molecular mechanisms of the TBBPA-induced neurotoxicity are still unclear although recent studies suggest a role of calcium imbalance. It has been hypothesized that TBBPA may act as an intracellular calcium releaser from the stores in endoplasmic reticulum. To verify this hypothesis in the present study we examined changes in the intracellular calcium homeostasis induced by TBBPA, and their pharmacological modulation. Experiments were performed using an *in vitro* model of the primary cultures of rat cerebellar granule cells at 7th day *in vitro*. To evaluate TBBPA neurotoxicity, the cells were exposed for 30 min to TBBPA, and neuronal viability was tested after 24 h with propidium iodide staining. Changes in calcium homeostasis were characterized using the calcium-sensitive fluorescent probe fluo-3. The results demonstrated that TPPBA in concentrations exceeding 5 μM triggered rise in the intracellular calcium level, which was sensitive to inhibitors of ryanodine receptors 2.5 μM bastadin 10 with 200 μM ryanodine, but not to 2ABP, which inhibits IP_3 receptors. The same features were disclosed for the effects of thapsigargin, that is a recognized inhibitor of the calcium pump SERCA and a well known calcium releaser. TPPBA in the concentration-dependent manner in the range of 2.5 - 100 μM induced severe neurotoxicity. The toxic effect of TPPBA in concentrations up to 10 - 15 μM was insensitive to antagonists of ryanodine receptors, bastadin 10 with ryanodine. Collectively, these results indicate that TBBP-A like thapsigargin is a calcium releaser destabilizing the ryanodine receptors, however this effect does not explain the mechanism of TBBPA neurotoxicity. *This work was supported by the MNiSW grant N N401 024635.*

P6.24

THE EFFECT OF HUMAN INTERLEUKIN-10 ON THE NITRIC OXIDE SYNTHASES EXPRESSION IN MPTP-BASED MODEL OF PARKINSON'S DISEASE

Schwenkgrub J.[1], Joniec-Maciejak I.[1], Ciesielska A.[2], Sznejder A.[1], Wawer A.[1], Bankiewicz K.[2], Członkowska A.[3], Członkowski A.[1]
[1] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland; [2] Department of Neurosurgery, University of California San Francisco, San Francisco, USA; [3] Second Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland.

Parkinson's disease (PD) is a progressive degenerative disorder, which etiology and pathogenesis remains unknown. *Post mortem* analysis of PD brain and studies on neurotoxic animal models of PD have provided evidence to support the involvement of oxidative stress and neuroinflammatory processes in the pathogenesis of PD. The high level of nitric oxide (NO) is produced by iNOS during the neuroinflammatory process caused by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment. Under pathological condition NO can easily react with superoxide to form peroxynitrite ($ONOO^-$), which is a strong oxidant. In the present study was examined the influence of the increased concentration of IL-10 (an anti-inflammatory cytokine) on the NOS expression in mouse model of PD induced by MPTP. One year-old male C57Bl mice were used in this study. An adeno-associated viral vector expressing the gene for human interleukin-10 (hIL-10) was used to transduce striatal cells 4 weeks prior to MPTP intoxication. Mice were sacrificed at the different time intervals: 1, 7 and 21 days after MPTP injection. Immunohistochemical and western blot analyses provide evidence for the protective properties of AAV2-hIL-10 in the MPTP-induced model of PD. There were reduction in the dopaminergic neuron quantity in SNpc and tyrosine hydroxylase protein in the striatum after MPTP injections, whereas in the group additionally treated with AAV2-hIL-10 neuroprotection was observed. Treatment with AAV2-hIL-10 suppressed the MPTP-induced increase in iNOS and 3-nitrotyrosine (3-NT) expression in the midbrain.

P6.25

THE INFLUENCE OF IL-10 ON THE INFLAMMATORY REACTION CHANGES IN THE MICE AFTER 1-METHYL-4-PHENYL-1,2,3,6-TERTAHYDROPYRIDINE (MPTP) TREATMENT

Sznejder A.[1], Joniec-Maciejak I.[1], Ciesielska A.[2], Schwenkgrub J.[1], Wawer A.[1], Bankiewicz K.[2], Członkowska A.[3], Członkowski A.[1]
[1] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland; [2] Department of Neurosurgery, University of California San Francisco, San Francisco, USA; [3] Second Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland.

Parkinson's disease (PD) is one of most frequent neurological disorder characterized by the loss of dopaminergic neurons in substantia nigra and striatum. The typical reaction of central neural system (CNS) on neurodegenerative processes is microglia activation and the inflammatory reaction. Microglia activation stimulates astrocytes response, playing important role in neuroimmune reaction. Microglia cells secrete two types of mediators of the inflammatory process: anti- and pro- inflammatory. In the

first stage of Parkinson's disease, pro-inflammatory cytokines have important meaning. We investigated the effect of an adeno-associated viral vector (AAV2) containing the complementary DNA (cDNA) for human interleukine 10 (hIL-10). The aim of the present study was to examine the evaluation of inflammatory reaction changes following increased concentration of hIL-10 in the murine model of PD induced by MPTP. Male C57BL mice 12 month-old were used in this study. AAV2 vector was bilateraly administered into striatum at 7, 21, 28 days prior to MPTP intoxication. We observed changes in the morphology of microglia cells, infiltration of lymphocytes T (population of CD3+, CD4+ and CD8+) and some differences in the level of one of the most important pro inflammatory cytokines – IL-1α. Our study showed that IL-10 is strongly involved in the inflammatory reaction in the murine model of Parkinson's disease induced by MPTP. After MPTP intoxication we observed the increase of activated microglia cells, infiltration of lymphocytes T and higher level of IL-1α mRNA. AAV2-hIL-10-treated mice displayed a significant decrease in the activated microglia cells, elevated expression of IL-10 receptors observed on glia cells, strong infiltration of lymphocytes T (mainly CD4+ and CD3+, less CD8+) and minor expression of IL-1α mRNA. Further research must be conducted to provide more evidence of protective role of IL-10 in Parkinson disease.

P6.26

INVESTIGATION OF THE POTENTIAL CORRELATION BETWEEN THE COGNITIVE STATUS AND THE LEVELS OF BRAIN FATTY ACIDS IN YOUNG AND AGED C57BL/6 MICE

Jakubowska-Dogru E.[1], Yetimler B.[1], Ulusoy G.[2], Celik T.[2]

[1] Department of Biological Sciences, Middle-East Technical University, Ankara, Turkey; [2] Department of Medical Pharmacology, Gulhane Millitary Academy of Medicine, Ankara, Turkey.

The aim of the present study was to investigate the possible relationship between the levels of various brain fatty acids and learning indices in aged (22-23 months old) and young (2-3 months old) female C57BL/6 mice classified as "good" or "poor" learners basing on their performance in a spatial learning task: the Morris water-maze. The levels of several fatty acids including palmitic, stearic, oleic, linoleic, arachidonic (AA), and docosahexaenoic acid (DHA) were measured by gas chromatography in brain tissue samples from four different brain areas: hippocampus, cortex, striatum and hypothalamus. The results of behavioural tests confirmed a decline in learning skills with age. However, a great individual variation was revealed in learning scores between aged subjects indicating that biological aging is not always parallel to chronological aging. The relative levels of palmitic, stearic, oleic, linoleic, arachidonic, and DHA acids in the four examined brain structures were very similar. Interestingly, except hypothalamus, no significant relation has been found between the brain levels of DHA omega-3 acid and the animal's age or cognitive status. This finding contributes to the current debate on the value of DHA supplementation as an effective protective treatment against aging and dementia. The only significant correlation between learning performance and the brain fatty acid levels was found for arachidonic acid in the young mice hippocampus, structure known to be critical for spatial learning and memory. AA level was significantly lower in young "good learners" as compared to both young "poor learners" and old "good learners" with young "good learners" showing significantly better performance than the two other groups. These results are discussed in the context of recent reports about elevated AA levels in Alzheimer's dementia.

P6.27

TDP-43 TRANSGENIC RAT - ANIMAL MODEL TO STUDY ALS AND FTLD-U

Koza P.[1], Klejman A.[2], Kaczmarek L.[1]

[1] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Laboratory of Cell Engineering, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin inclusions (FTLD-U) are characterized by mislocalisation and aggregation of predominantly nuclear TDP-43 protein. Our aim was to generate transgenic rats overexpressing wild type of human TDP-43 gene, fused with EGFP and under the control of synapsin promoter. Using lentiviral vectors as a tool for transgenesis, three founders carrying human TDP-43 gene were generated. The incorporation of transgene into genome was confirmed by specific PCR reaction and transgene copy number was determined by Real-Time quantitative PCR. Microscopic visualization and western blotting technique showed an abundant expression of GFP - fused TDP-43 protein in the brains of 3 months old animals. Notably, at this age no gross degeneration of neurons was detected. RotaRod test conducted on 5 months old transgenic rats showed no distinct motor deficits. At present we carry out behavioral analyses to verify whether animals exhibit cognitive impairments, which could possibly appear before any motor disorders. As neurodegeration occurs in ALS and FTLD-U patients in age-dependent manner, we plan to run further morphological and behavioral analyses on aging transgenic rats.

P6.28

THE INFLUENCE OF LY354740 - AN AGONIST OF GROUP-II METABOTROPIC GLUTAMATE RECEPTORS (mGLuR II) ON NEURONAL APOPTOSIS IN PRIMARY NEURONS

Jantas D.[1], Gręda A.[1], Pilc A.[2], Lasoń W.[1]

[1] Department of Experimental Neuroendocrinology, Institute of Pharmacology PAS, Krakow, Poland; [2] Department of Neurobiology, Institute of Pharmacology PAS, Krakow, Poland.

A number of studies have shown neuroprotective effects of agonists of group-II metabotropic glutamate receptors (mGluR II) in various experimental models of excitotoxicity. However, an influence of these compounds on neuronal apoptosis is less recognized. We tested the effect of nonspecific agonist of mGluR II, LY354740 ((+)-2-aminobicyclo[3.1.0]hexane-2,6dicarboxylate) on staurosporine and doxorubicin evoked cell death in primary pure neuronal and neuronal-glial cortical cells, as well in cerebellar granule cells (CGC). We found that LY354740 (0.01-10 microM) was protective against staurosporine-evoked cell death in both, pure cortical neurons and CGC with higher efficacy in 12 DIV in comparison with the 7 DIV ones. Moreover, the neuroprotective effect of LY354470 in neuronal-glial cells did not differ from that found in pure neurons. The protective effect of mGluR II agonist was not connected with attenuation the St-induced caspase-3 activity and DNA fragmentation, but this agent decreased the St-evoked necrotic cell death as measured by propidium ioide staining. LY354470 had no influence on doxorubicin-evoked cell death, but attenuated the glutamate-mediated neuronal cell damage. Our data suggest that neuroprotective effects of the mGluR II ligand are stimuli- and development-dependent and are rather connected with attenuation of necrotic-, than the apoptotic cell death. *The study was supported by grant No NN405611638 from the Ministry of Science and Higher Education, Warsaw, Poland.*

P6.29

HYPERBARIC PRECONDITIONING COMBINED WITH POST-ISCHEMIC HYPERBARIC THERAPY PREVENTS ISCHEMIA-EVOKED DAMAGE TO CA1 HIPPOCAMPAL NEURONS OF MONGOLIAN GERBILS

Małek M., Gamdzyk M., Duszczyk M., Ziembowicz A., Sobczuk A., Lazarewicz J., Salińska E.

Department of Neurochemistry, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Lack of the clinically effective pharmacological neuroprotection in different forms of brain ischemia increased the interest in alternative methods of therapy, like hypothermia or induction of brain tolerance by pre- and post-conditioning. The hyperbaric oxygen (HBO) therapy (2.5 atm) applied after ischemia or traumatic brain injury is one of the proposed but still controversial methods. The aim of this study was to find whether HBO and hyperbaric air (HBA) preconditioning followed by hyperbaric treatment applied for 60 min at different times after 3 min forebrain ischemia in gerbils will give a significant protection. The effects of both treatments on brain temperature and animal behaviour were also examined. A telemetric system to measure brain temperature was used and for behavioural observations a nest building test. The density of viable CA1 pyramidal neurons was also quantified. Our results show that HBO preconditioning combined with HBO postischemic therapy significantly reduced ischemia-evoked increase of brain temperature. HBA was also effective. Both treatments significantly increased gerbils' ability to build a nest in comparison to untreated animals. The best effect was observed when postischemic therapy was applied 1 h after ischemia, but it was also effective 3 h after ischemia. Morphological analysis showed that HBO preconditioning combined with HBO postischemic treatment applied 1 h after ischemia significantly reduced neuronal damage in CA1 region of hippocampus resulting in 85% of surviving neurons compared to 18% of surviving CA1 neurons in the brains of animals subjected to ischemia but not treated with HBO. Our results show that HBO preconditioning combined with HBO therapy after forebrain ischemia in gerbils gives morphological protection which is accompanied by good behavioral results. Apart from inducing tolerance mediated by mild oxidative stress, HBO may affect blood oxygenation and other factors instrumental in brain protection.

P6.30

CELLULAR LOCALIZATION AND ACTIVITY OF MATRIX METALLOPROTEINASE-9 IN MOUSE MODEL OF IN SITU THROMBOEMBOLIC STROKE

Gawlak M.[1], Szymańska M.[2], Michaluk P.[2], Kaczmarek L.[2], Wilczyński G.[1]

[1] Laboratory of Molecular and Systemic Neuromorphology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Laboratory of Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Tissue plasminogen activator tPA is used for treatment of ischemic stroke patient. This thrombolytic agent resurrect blood supply to brain tissue, that is sensitive to oxygen and glucose deprivation. Administration of tPA is the only approved treatment and might be conducted up to 3 or 4.5 hours after ischemic stroke symptom onset. The benefits of it are time dependent and there is need for an improvement the timeliness of reperfusion. Some side effects of tPA is caused likely by its matrix metalloproteinase-9 (MMP-9) activation. Source and localization of MMP9 is still uncertain. This is investigated with

high resolution in situ zymography. We use mouse model of in situ thromboembolic stroke and reperfusion as a clinically relevant. In this experimental set we identify oligodendrocytes and neurons but not astrocytes and microglia as a cellular source of gelatynolytic activity. Oligodendrocytes activity is most prominent. This is in opposition to literature. Activity of leukocytes is high however number of them suggest that their contribution to overall activity is negligible.

P6.31

VARIATIONS IN EXCITATORY AND INHIBITORY PRESYNAPTIC PROTEINS EXPRESSION IN MOUSE SOMATOSENSORY CORTEX WITH RESPECT TO AGING AND PLASTICITY

Liguz-Lęcznar M., Zakrzewska R., Kossut M.
Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Aging is associated with deficits in cognitive function that can, in part, be explained by changes in neural plasticity.Present data indicate that molecular changes in the synapse inducing the imbalance of the excitatory versus inhibitory neurotransmitter systems may be the cause of age-related plasticity decline. Research on synaptic proteins during aging has focused primarily on postsynaptic proteins. Here, with semiquantitative western blot analysis we investigated the age related changes in expression of presynaptic proteins connected to inhibitory and excitatory neurotransmission in mouse somtosensory cortex. Subsequently, we linked those changes with the impairment in sensory learning, during which animals were subjected to classical conditioning, where tactile stimulation of one row of whiskers was paired with an aversive stimulus. Such procedure evoked functional plasticity in the young animals, expressed as a widening of the functional cortical representation of the conditioned row. Learning-related plasticity was vulnerable to aging and plastic change was not detectable in aged (one year-old) animals, even though they acquired the behavioral response (minifreezing behaviour). Western blot analysis revealed decreases in both glutamatergic-associated (vesicular glutamate transporters: Vglut1, Vglut2) and GABA-ergic associated (glutamic acid decarboxylases: GAD-65, GAD-67, vesicular GABA transporter VGAT) proteins. However, the slope of those declines was more dramatic for glutamatergic-associated proteins expression, what suggests the shift of the excitation/inhibition balance toward the inhibition in the somatosensory cortex of aged animals. Taking into account that associative sensory training increases inhibitiory transmission in the conditioned row of whiskers, we assume that age associated increase of inhibiton woud add to the effect of training and prevent the learning-induced reorganization of cortical network. *Supported by grant N N401 098739.*

P6.32

CHANGES OF Gα(q), Gα(11) and Gα(12) mRNA EXPRESSION LEVELS IN RAT PREFRONTAL CORTEX AND AMYGDALA AFTER REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR

Zelek-Molik A.[1], Kreiner G.[1], Filip M.[2], Wydra K.[2], Nalepa I.[1]
[1] Department of Brain Biochemistry, Institute of Pharmacology PAS, Krakow, Poland; [2] Laboratory of Drug Addiction Pharmacology, Institute of Pharmacology PAS, Krakow, Poland.

Inhibition of monoamine transporters by a psychostimulant, cocaine, increases the monoamines availability at synaptic cleft and leads to the enhanced stimulation of monoaminergic postsynaptic receptors. G proteins represent the down-stream connectors from receptors to intracellular signalling. The aim of the study was to assess the expression of Gα(q), Gα(11) and Gα(12) mRNAs following reinstatement of cocaine-seeking behaviour in the prefrontal cortex (PFC) and the amygdala (AMY) of male Wistar rats using a "yoked" procedure and RT-PCR technique. We found that phases of cocaine-seeking behavior differently influenced the Gα subunits depending on the brain structure analyzed. Cocaine self-administered for 18 days induced a significant increase of mRNA levels of all Gα subunits (by ~23% for Gα12 and Gα11, and by 46% for Gαq vs yoked) in the PFC. After 10 days withdrawal from cocaine when no change in G proteins was observed, reinstatement induced by priming dose of the drug decreased G12 and Gq. The effect was more pronounced after combination of the cocaine with cue previously associated with cocaine self-administration and was noticed in G11 as well. In AMY, changes in the expression of Gα mRNAs induced by cocaine self-administration dependent on environmental cues paired with cocaine. Cocaine self-administration decreased (by ~24%) all G protein mRNAs while opposite effect was observed when cocaine self-administration was paired with cue stimulus. Withdrawal from cocaine induced 2-fold increase in mRNA level of three G proteins. On the contrary, the reinstatement induced by the cue decreased significantly Gα mRNAs to the same degree as did its combination with cocaine-priming. Our study provides the first evidence that alterations of G proteins mRNA expression can be conditioned by environmental stimuli paired with cocaine administration. *Supported by statutory funds of the Institute of Pharmacology PAS.*

P6.33

HIPPOCAMPAL AND CORTICAL NEUROINFLAMMATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IS NOT ACCOMPANIED BY DEFICITS OF SPATIAL MEMORY IN A LATE PHASE OF THE DISEASE

Kurkowska-Jastrzębska I.[1], Świątkiewicz M.[2], Zaremba M.[23], Piechal A.[2], Cudna A.1, Oderfeld-Nowak B.[3]

[1] Second Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland; [2] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland; [3] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Cognitive dysfunctions are common features of multiple sclerosis. The exact mechanism of their appearance is unknown. The disconnection of some parts of the cortex reflecting an axonal loss, neuronal damage and alterations in synaptic transmission, have been postulated. In the present study, an autoimmune encephalomyelitis (EAE), a common model of multiple sclerosis, was induced passively by lymphocytes transfer, to evoke a one-phase disease in Lewis rats. The inflammatory reactions and neural injury in the hippocampus and frontal cortex were investigated. We found a decrease of the number of CA1 and CA4 pyramidal neurons by about 25% on 30 dpi and by about 50% in CA1 region on 90 dpi. This was accompanied by prolonged astroglial activation and by a rise of the pro-inflammatory cytokine mRNA expression (IL-1β, IL-6 and TNFα). A significant rise of NGF and BDNF was also found. In the frontal cortex, neural degeneration was not so evident. A slight astrocyte activation and a strong increase of expression of IL 6 on 30 dpi and IL1β and TNFα on 90 dpi was seen. Learning and memory abilities (Morris water-maze tests) were also evaluated 30 and 90 dpi. The mean latency of reaching the platform, the swimming distance, the time spent in the goal quadrant and crossing parameters were estimated. The reaction of animals suffering from EAE was not different from that of the control group, in any of the tasks except 20% higher chance for reaching the platform on 30 dpi. We demonstrated therefore the lack of correlation between strong neuroinflammation in the hippocampus and cortex and the deficits in memory and learning ability at a late phase of the disease. However, the severity of motor impairment during earlier stages of the disease made difficult identification of any early cognitive deficits. The possibility exists that early deficits could be later compensated due to simultaneously occurring compensatory processes involving activity of neurotrophic factors.

P6.34

THE INVOLVEMENT OF CHOLINERGIC SYSTEM IN NICOTINE SENSITIZATION AND CROSS – SENSITIZATION BETWEEN NICOTINE AND MORPHINE IN MICE

Staniak N.[1], Biała G.[2]

[1] Department of Public Health, University of Information, Technology and Menagement of Rzeszow, Rzeszow, Poland; [2] Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Lublin, Poland.

The mesolimbic dopamine system, the main neural system mediating sensitization, seems to overlap this mediating reward. Given some data indicating that dopamine neurons are effected by cholinergic neurotransmitters, the aim of present study was to determine the influence of varenicline, a partial α4β2 nicotinic receptor agonist (0.5, 1 and 2 mg/kg) and mecamylamine, a nonselective nicotinic receptor antagonist (0.5, 1 and 2 mg/kg) on behavioural sensitization and cross – sensitization induced by nicotine (0.175 mg/kg, base) and morphine (5 mg/kg) in mice. First, we revealed that repeated injections of nicotine (9 days, every other day) produced significant increase in locomotor activity in mice measured following 7-day withdrowal after injection of challange doses of nicotine and morphine. Subsequently, we found that varenicline and mecamylamine attenuated the acquisition and expression of nicotine sensitization as well as locomotor cross-sensitization between nicotine and morphine. Because they had no effects on naive mice, we concluded that the ability of both agents to block this results did not correspond to general supression of activity. The development of nicotine locomotor sensitization shows similarity to relapse described in ex-smokers and cross – sensitization seems to reflect the phenomenon of simultaneous abuse of several different drugs. Our results indicate similar nicotinic neurotransmission - probably through the α4β2 receptor subtypes - involved in the locomotor stimulant effects of nicotine and morphine in mice. This data suggest that cholinergic neurotransmission may be a potential target for developing pharmacotherapeutic strategies to prevent and treat nicotine and/or opioid addiction.

P6.35

INTRAPERITONEAL INJECTIONS OF ENDOTOXIN (*Proteus mirabilis*, O17 32/57) AND THE RATS ANXIETY-LIKE BEHAVIOR AND SEROTONERGIC SYSTEM ACTIVITY IN THE CHOSEN BRAIN AREAS

Wieczorek M., Koprowska M., Krotewicz M., Strzelczuk M.

Department of Neurophysiology, University of Lodz, Lodz, Poland.

Experimental results clearly indicate the existence of bidirectional communication between the nervous and immune systems. Changes in the activity of immune system produced by infections or any inflammatory event elicit neurochemical, endocrine and behavioral effects. But the details of those relationships are still not clear. It is hypothesized that afferent branches of nervous vagus may participate in signal transduction to the CNS in response to peripheral infections and/or inflammations, and then activate the hypothalamo-pituitary adrenal axis (HPA) and brain neurotransmitters system. It is commonly accepted that peripheral administration of cytokines or bacterial endotoxin (LPS) to rodents alters their behavior, increases body

temperature, activates the HPA axis and affects brain neurochemistry. In the present study we decided to use LPS from *Proteus mirabilis* (O17 32/57), a Gram-negative bacterium, which is mainly responsible for urinary catheter infection, inflammation of the urinary tract, but also for meningitis, to check it influences on the brain 5-HT system activity and rats anxiety-like behavior in the open field arena (OF). Obtained results indicate that intraperitoneally injected lipopolisaccharide from Proteus mirabilis (O17 32/57) influenced the brain serotonergic system activity. Also, this type of LPS slightly affects animals' behavior in the OF arena. However, our results are slightly differing from those, observed by other. In our experiment the peak responses in 5-HT system activity appeared around 240 minutes after endotoxin injections.

P6.36

DIFFERENTIAL EFFICACY OF MILD NORMOBARIC HYPOXIA IN INDUCING ISCHEMIC TOLERANCE IN TWO MODELS OF BRAIN ISCHEMIA *IN VIVO*

Duszczyk M., Makarewicz D., Małek M., Ziembowicz A., Lazarewicz J., Salinska E.
Department of Neurochemistry, Mossakowski Medical Research Centre PAS, Warsaw, Poland.

Induction of short ischemic episodes after the stroke can be neuroprotective. Hypoxia was also suggested as the factor producing neuroprotection in the animal brain. Therefore in our studies we aimed to test if normobaric hypoxia (10% of oxygen) induced after ischemia could prevent neuronal loss. The model of hypoxia-ischemia (H-I) in 7-days old rats and the model of global forebrain ischemia in Mongolian gerbils were used. 7-days old rats were subjected to H-I and the first of three episodes of postconditioning hypoxia was induced 1, 3 or 6 hours after H-I episode. After ischemia gerbils were subjected to three trials of 1h hypoxia applied every 24 hours. The first episode was induced immediately, 2.5 h or 6 h after the ischemic insult. The morphological and behavioral effects of the postconditioning were evaluated. In the model of H-I on rats, the assessment of brain mass deficit revealed that normobaric hypoxia induced signifficant neuroprotection when applied 1 h or 6 h after H-I but not 2.5 h. In the global forebrain ischemia model normobaric hypoxia itself was harmless and the number of pyramidal neurons evaluated in CA1 region was the same as in the sham group. The neuroprotective effect of normobaric hypoxia postconditioning was observed when hypoxia was induced immediately after ischemia but not 2.5 or 6 hours after the insult. The behavioral evaluation showed only small improvement in nest-building test in postconditioned animals. Presented data show that normobaric hypoxia postconditioning produces the neuroprotective effect, however the therapeutic window of this treatment varies according to the model of brain ischemia. *Supported by MSHE grant NN401003935.*

P6.37

AGED SOMATOSENSORY CORTEX DISPLAYS INCREASED DENSITIES OF PERINEURONAL NETS THAT ARE NOT ASSOCIATED WITH GAD-POSITIVE NEURONS.

Nowicka D., Karetko-Sysa M., Skangiel-Kramska J.
Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Ageing of the brain results in some degree of decline in a number of functions, one of which is neuronal plasticity. Recently, perineuronal nets (PNNs), a structuralized form of the brain extracellular matrix observed around some neurons (often GABAergic) have been postulated as a molecular brake, actively preventing plasticity in the adult brain. Removal of PNNs in visual cortex results in the restoration of ocular dominance plasticity in adult mice, and knockout mice with attenuated PNNs retain juvenile levels of ocular dominance plasticity. Little data, however, exist on the expression and function of PNNs in aged animals. We examined whether the decrease in neuronal plasticity during ageing could be the result of gradual increase in PNN density. To this aim we investigated PNNs across the layers of the somatosensory cortex of the adult (3 months) and aged (1 year) mouse. To visualize PNNs we employed Wisteria floribunda (WFA) lectin, binding to the sugar epitopes of PNNs, and CAT-315 antibody recognizing a specific glycoform of PNN constituent, aggrecan. GAD and parvalbumin (PV) antibodies were used to stain GABAergic neurons. Detailed microscopic analysis revealed that in aged animals the density of WFA-positive PNNs increased significantly in layers IV and V-VI. At the same time the density of CAT-315-positive PNNs decreased slightly, thus suggesting changes in PNN composition. Co-localization studies revealed that the observed increase was mostly confined to WFA+/CAT-315 neurons that did not contain PV. Moreover, this increase in enveloping was characteristic for neurons that were GAD-negative. Ageing had no influence on the density of GAD-positive or PV-positive cells. Our results suggest that in aged animals some additional set of neurons was enveloped by PNNs, possibly contributing to further limiting of cortical plasticity. Interestingly, these neurons are not necessarily GABAergic.

P6.38

INTRAPERITONEAL INJECTIONS OF LPS (*Proteus mirabilis*, O17 32/57) AND THE NORADRENERGIC AND ADRENERGIC SYSTEMS ACTIVITY IN THE CHOSEN RATS' BRAIN AREAS

Wieczorek M., Krotewicz M., Koprowska M., Strzelczuk M.
Department of Neurophysiology, University of Lodz, Lodz, Poland.

In 1975 Besedovsky's team as a first reported that hypothalamo-pituitary-adrenal axis (HPA) can be activated by immune stimuli. In his seminal experiment he noted that plasma corticosterone was elevated after peripheral immune challenges, such as horse and sheep red blood cells. Subsequently very many experimental data has been collected and confirmed his idea. Also many investigators have noted that immune challenges increased plasma concentrations of ACTH and glucocorticosteroides, although the effects appear almost immediately – within a few hours after injections. Up to now, the mechanisms by which HPA is activated by cytokines or LPS have not been fully established. In many previous experiments, endotoxin (from E. coli) administration to animals induces many physiological and behavioral effects, as shivering, fever, reduced locomotion and other behavioral changes, along with activation of the HPA-axis and brain noradrenergic (NE) and adrenergic (A) systems. During the present study we decided to use LPS from *Proteus mirabilis* (O17 32/57), a Gram-negative bacterium, which is responsible mainly for urinary tract inflammation and for meningitis to check it influences on the brain NE and A systems activity after stressful stimuli in the open field arena. LPS administration increased plasma concentrations of corticosterone. These increased concentrations were somewhat different than those observed after E. coli endotoxin treatment. Also, LPS administration increased hypothalamus concentrations of norepinephrine, epinephrine, their main metabolites; MHPG and MT as well as MHPG/NE and A/MT ratios, which reflects the neurotransmitter system activity, in all brain regions analyzed. Each of these responses was marked 90 minutes after injections with peak concentration at 240 minutes. These results indicate that i.p. injected LPS from P. mirabilis (O17 32/57) influenced the brain noradrenergic and adrenergic systems activity and HPA-axis too.

P6.39

OVEREXPRESSION OF P2X7R AND EARLY ACTIVATION OF CEREBRAL MICROGLIA DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Grygorowicz T.[1], Sulejczak D.[2], Rafałowska J.[3], Lenkiewicz A.[1], Strużyńska L.[1]

[1] Laboratory of Pathoneurochemistry, Mossakowski Medical Research Centre PAS, Warsaw, Poland; [2] Department of Experimental Pharmacology, Mossakowski Medical Research Centre PAS, Warsaw, Poland; [3] Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre PAS, Warsaw, Poland

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease which is one of the most frequent reasons of disabilities of young adults and a serious problem for modern medicine due to the unknown etiology. Experimental autoimmune encephalomyelitis (EAE) is a commonly used rodent model of MS. EAE is evoked by immunization of female Lewis rats with homogenate of guinea pigs' spinal cord combined with complete Freund's adjuvant and inactivated *Mycobacterium tuberculosis*. It is well known that during the development of MS and EAE, the immune system sensitizes against self myelin and the permeability of blood-brain barrier (BBB) increases what enables an inflow of immune cells into the central nervous system. The immune system attacks and destroys myelin in the brain and the spinal cord what further leads to degeneration of neurons. The aim of the study was to investigate the time-window of microglial activation, the level of proinflammatory cytokines (IL-1, IL-6, TNFα) and the status of BBB in the early stages of EAE. We correlated the results with the microglial and endothelial expression of purinergic P2X7 receptor which is known to play a role in inflammation due to a release of proinflammatory mediators. The results of microscopic analysis revealed the increased permeability of BBB. At day 2 and 4 p.i. we also observed decreased expression of claudin5 protein which is an important marker of BBB tightness. However, starting from day 6 p.i. we noticed significant upregulation of this protein expression. The early activation of microglial cells at day 4 post immunization (dpi), in asymptomatic phase of the disease, together with an increased level of proinflammatory cytokines were observed. These changes correlated temporary with overexpression of P2X7R which was noticed on microglial cells and pericytes of blood vessels in brains of EAE rats. The results suggest the critical role of this receptor in early events during EAE development.

P6.40

ENDOGENOUS AMPK IS INVOLVED IN AUTOPHAGY IN ASTROCYTE EXPOSED TO COMBINED OXYGEN-GLUCOSE DEPRIVATION

Gabryel B.[1], Kasprowska D.[1], Kost A.[2], Machnik G.[3], Wiaderkiewicz R.[2]

[1] Department of Pharmacology, Silesian Medical University, Katowice, Poland; [2] Department of Histology, Silesian Medical University, Katowice, Poland; [3] Department of Internal Medicine and Clinical Pharmacology, Silesian Medical University, Katowice, Poland.

AMPK is a highly conserved heterotrimeric serine/threonine kinase involved in the regulation of cellular metabolism and energy distribution. AMPK is an intracellular metabolic sensor which, through the reduction of adenosine-5′-triphosphate (ATP)-consuming processes and stimulation of ATP-generating pathways, maintains cellular energy homeostasis. AMPK activation

occurs through phosphorylation at a specific threonine residue (Thr172) on the α subunit. It was proved that neuronal AMPK has been implicated in pathology of cerebral ischemia. However, whether AMPK activation in astrocytes is responsible for intensification autophagy contributing to their ischemic injury remains to be determined. The aim of the present study was to investigate the time-dependent activation of AMPK as well as to evaluate the autophagy induction in astrocytes exposed to combined oxygen glucose deprivation (OGD). It was shown that exposure of cultured astrocytes to OGD (0.5 – 24 h) causes an increase in AMPK expression and activity. The role of endogenous AMPK in the process of autophagy activation was also demonstrated. *The work was supported by grant N N401 072139 from the Ministry of Science and Higher Educations (BG), Warsaw, Poland.*

P6.41

REDUCTION OF MICROGLIA RESPONSE AFFECTS OLIGODENDROCYTE PRECURSOR CELL DIFFERENTIATION IN A MODEL OF DEMYELINATION/REMYELINATION

Wylot B., Sielska M., Kaminska B., Zawadzka M.
Department of Cell Biology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Remyelination in the CNS is a regenerative process carried by oligodendrocyte precursor cells (OPC), which are recruited to the demyelination site and differentiate into mature oligodendrocytes to form a new myelin sheath. Macrophages were shown to support remyelination through myelin debris clearance as well as secretion of chemokines and growth factors stimulating OPC recruitment and differentiation. Moreover, several *in vivo* studies demonstrated that pharmacological macrophage depletion may impair remyelination. The role of macrophages in new myelin formation is not fully understood and the involvement of these cells in remyelination process has not yet been studied in a model of inherent macrophage reduction. Osteopetrotic (op/op) mice have a mutation in CSF1 gene leading to reduction in monocytes as well as microglia number. Therefore, they make a good model for studying the role of central- and peripheral-derived macrophages in regenerative processes of the CNS. The aim of the present study was to examine the influence of reduction in macrophages in op/op mice on remyelination process in a model of focal demyelination of the spinal cord. Osteopetrotic mice were injected with myelin toxin into the ventral and dorsal funiculus of the spinal cord to induce focal demyelination. Toluidine blue staining of semi-thin resin sections at 28 days post lesion (dpl) revealed impaired remyelination in op/op mice with the presence of extensive non-remyelinated areas in the lesion. Immunostaining of sections from op/op mice at 10 dpl showed severely reduced activity of macrophages at the lesion site as compared to control. OPC number in the lesions from op/op mice was not affected. Results of the present study provide further evidence for a crucial role of macrophages in supporting CNS remyelination. They also confirm usefulness of op/op mice as a model for remyelination studies.

P6.42

THE INFLUENCE OF CONDITIONAL INACTIVATION OF GR IN NORADRENERGIC SYSTEM ON STRESS RELATED BEHAVIOR IN MICE

Kreiner G.[1], Chmielarz P.[1], Kuśmierczyk J.[1], Parlato R.[2], Schuetz G.[2], Nalepa I.[1]
[1] Department Brain Biochemistry, Institute of Pharmacology PNS, Krakow, Poland; [2] German Cancer Research Center, Deptartment Molecular Biology of the Cell I, Heidelberg, Germany.

Depression is a mental disease affecting complex cognitive and emotional functions. Stress induced hyperactivity of hypothalamic-pituitary-adrenal system (HPA) is believed to be one of the major contributors to its pathology. The activity of HPA is controlled by glucocorticoid receptors (GR) which function may be impaired in depression, resulting in reduced GR-mediated negative feedback on the HPA-axis. Most of the compounds which modulate GR action also influence noradrenergic system by increasing noradreneline levels. The aim of this study was to investigate if conditional inactivation of GR in noradrenergic neurons of mice affects the animal behavior in stressful conditions. Selective ablation of GR in noradrenergic system was achieved using the Cre/loxP approach by crossing transgenic mice hosting the Cre recombinase under the dopamine beta-hydroxylase (DBH) promoter with animals harboring the floxed GR gene. Resulting GRDBHCre mutant mice were born at expected rates, viable and showed no obvious physical impairment regarding life span, weight gain and locomotor activity. Also plasma cortisol levels did not differ between mutant and control mice. Animals were screened for anxiety and depressive-like behavior in light/dark box test (LDT) and tail suspension test (TST). Male mutant mice did not unveil any differences from their control littermates in basal state nor after acute restraint stress (2 hrs). However, both tests performed after chronic restraint stress (14 days, 2 hrs/day) revealed that GRDBHCre mice were resistant to this type of experimental procedure showing similar anxiety status and immobility time as non-stressed controls. Our mutant mice may represent an interesting tool to study the role of stress in depression in context of noradrenergic system which is important target for antidepressant therapy. *This study was supported by grant POIG.01.01.02-12-004/09 (DeMeTer) financed by European Regional Development Fund.*

Sensory and Motor Systems [P7]

P7.01

CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF MOTONEURONES INNERVATING RAT MEDIAL GASTROCNEMIUS IN RESPONSE TO THE MUSCLE OVERLOAD

Haluszka A., Bączyk M., Celichowski J., Krutki P.
Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland.

Various models of chronic muscular activity, as workload training, tendon transfer, or paresis and peripheral nerve damage are examples leading to muscle overload, which may induce measurable effects in motor units. The aim of this study was to investigate whether 5-week overloading of muscles connected with their voluntary activation in a running wheel and by a treadmill training change electrophysiological properties of their motoneurons. Rats were subjected to chronic overload of the medial gastrocnemius (MG) muscle by cutting the lateral gastrocnemius, soleus and plantaris muscles from the Achilles tendon and sewing them directed proximally to the skin. As the result of this operation, only the MG muscle was able to evoke a foot plantar flexion during the daily locomotor activity. After one week of convalescence, rats were subjected to extensive voluntary activity on a running wheel and additionally to a training program on a treadmill (1 hour daily with a speed of 27 cm/s) for 5 weeks, 5 days a week. The acute experiments were carried out on the MG motoneurones in deeply anaesthetized animals. Intracellular recordings were performed from MG motoneurones located in L4-L5 spinal segments using glass micropipettes filled with 2 M potassium citrate solution. The results were compared to the control group of normally active, intact animals. Parameters of antidromic action potentials were measured and effects of intracellular injection of rectangular pulses of depolarization current were analyzed. The basic electrophysiological properties were considerably modified by the overloading either in fast and slow motoneurones. Moreover, we observed changes in their rhythmic properties, as the increased maximum steady-state frequencies of motoneuronal firing resulting in changes in the course of the steady-state frequency-current curves in the overloaded animals. The results of this study may help understand neuromuscular mechanisms of plasticity of overloaded muscles.

P7.02

DIFFERENCES IN THE PONTO-CEREBELLAR PROJECTION TO THE CEREBELLAR CAUDAL VERMIS AND HEMISPHERE: A RETROGRADE DOUBLE LABELING STUDY IN THE RABBIT

Zguczyński L.[1], Bukowska D.[2], Mierzejewska-Krzyżowska B.[1]
[1] Department of Anatomy and Anthropology, University School of Physical Education in Gorzow Wlkp., Gorzow Wlkp., Poland;
[2] Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland.

Following paired unilateral injections of fluorescent tracers FB (Fast Blue) and DY (Diamidino Yellow) into the the rostral and caudal paramedian lobule (rPML, cPML) as well as the pyramis (Pr) and uvula (Uv), the distribution pattern of retrogradely labeled neurons in the pontine nuclei (PN) indicates that these two ponto-cerebellar projections are organized topographically. Both projections are bilateral. Projection to PML originates from the caudal two-thirds whereas that to the Pr and Uv - from the entire rostrocaudal extent of PN. Participation of individual PN nuclei in the projections differs. The strongest connections to rPML/cPML and Pr/Uv send the dorsolateral (44% and 56%, respectively) and paramedian (24% and 26%, respectively) nuclei. The involvement of the lateral (20% and 14%) and peduncular (12% and 4%) nuclei is weaker. The ventral pontine nucleus sends no fibers to PML or Pr and Uv. Moreover, following injections into the rPML and cPML, some regions where FB and DY single labeled neurons were intermingled, contained in addition small number of double labeled neurons. These neurons are parent for collaterals projections to both parts of PML. Differences in projections under study may arise from various functions of the PML (rPML and cPML receive afferent information from the forelimb and hindlimb, respectively) and the caudal vermal lobules (Pr receives spinal cord afferents related to innervation of axial and proximal-limb muscles, and Uv is interconnected with the vestibular nuclei).

P7.03

CHANGES IN THE CONTRACTILE PROPERTIES OF MOTOR UNITS IN FUNCTIONALLY OVERLOADED MEDIAL GASTROCNEMIUS MUSCLE OF THE RAT

Kryściak K., Drzymała-Celichowska H., Krutki P., Celichowski J.
Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland.

The study aimed at examining the contractile properties of motor units (MUs) in medial gastrocnemius muscle subjected to 3 months compensatory overload, induced by bilateral tenotomy of its synergists (lateral gastrocnemius, plantaris and soleus). To assure that operated muscles were regularly voluntarily activated, surgical procedures were followed by keeping animals in wheel-equipped cages and treadmill exercise. The final electrophysiological experiments were carried out on 106 MUs of the overloaded medial gastrocnemius muscle (OMG) and 88 MUs of the untreated, healthy gastrocnemius muscle (MG). Functionally isolated

MUs were examined by electrical stimulation of thin filaments of the L4 – L5 ventral roots. MUs classification was based on 20 Hz tetanus index which divided MUs into fast and slow, whereas the fatigue index distinguished fast fatigable (FF) and fast resistant (FR) MUs. Results showed that both OMG mass and its relation to the body weight were higher in comparison to MG. MUs proportion was modified in response to altered functional demand and in OMG higher percentage of S and FF simultaneously with lower contribution of FR MUs were observed. Changes in MUs contractile properties of OMG in comparison to MG included: shorter half-relaxation time and lower twitch force in all types of MUs and in parallel higher (FR and S) or unchanged (FF) tetanus force. Changes in force parameters in OMG resulted in lower values of the twitch-to-tetanus ratio in all three types of MUs. Higher post-tetanic potentiation was also noted for all MUs types in OMG. Changes in fatigue resistance were observed only in fast MUs: for FF type the mean value of the fatigue index was lower in OMG in comparison to MG, but for FR type this value was higher in OMG. In conclusion, the adaptation of the medial gastrocnemius muscle to overload included transformation of some MUs accompanied by changes in MUs contractile properties.

P7.04

DIVERGENT AXONS WITHIN OLIVOCEREBELLAR PROJECTION TO THE PYRAMIS AND UVULA REVEALED BY A RETROGRADE DOUBLE LABELING TECHNIQUE IN THE RABBIT

Bukowska D.[1], Zguczyński L.[2], Mierzejewska-Krzyżowska B.[2]

[1] Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland; [2] Department of Anatomy and Anthropology, University School of Physical Education in Gorzow Wlkp., Gorzow Wlkp., Poland.

The study was initiated to determine the projection pattern of the climbing fibers from the inferior olivary complex (IO) to the two lobules of the caudal vermis, both independent and by way of axonal collaterals. Different fluorescent tracers, Fast Blue and Diamidino Yellow, unilaterally injected into the pyramis and uvula in the seven rabbits, resulted in single (*n*=32020) and double (*n*=403) labeling of neurons in defined restricted regions of the contralateral IO. These neurons, parent for independent and collateral projections, respectively, clustered in two aggregations (lateral and medial) through the rostrocaudal extent of IO. IO consists of a complex of three nuclei: the dorsal (DAO) and medial (MAO) accessory olive, and the principal olive (PO). MAO is accompanied by the dorsomedial cell column (dmcc) and the β nucleus (β). PO is composed of the dorsal (dlPO) and ventral (vlPO) laminas and comprises the ventrolateral outgrowth (vlo) and dorsal cap (dc). Distribution pattern of single labeled neurons indicates that the strongest projection to the pyramis and uvula arises from the lateral region of entire extent of MAO and that to the pyramis is more intensive. Neurons in the lateral region of DAO send numerous fibers exclusively to the pyramis. The rostral part of β more frequently supplies the uvula whereas the caudal part - the pyramis. Neurons of dmcc in similar degree project to the two cerebellar targets, however, from the rostral part projection is stronger to the uvula. Weak connections come from vlo and vlPO, and from dc only to the uvula. Neurons participating in collateral projection were found in the lateral region of MAO, the entire extent of β, the rostral and caudal levels of dmcc, and in vlo. To sum up, (1) the IO-pyramis and IO-uvula projecting neurons vary in number and in distribution, and (2) there is a small population of the IO neurons which project by divergent axons.

P7.05

DIFFERENCES IN THE CONTRACTILE PROPERTIES AND ACTION POTENTIALS OF MOTOR UNITS OF THE MEDIAL GASTROCNEMIUS MUSCLE IN YOUNG AND ADULT MALE RATS

Dobrzyńska Z., Celichowski J.

Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland.

The growing up of organism is connected with considerable changes in neuromuscular and other systems. Numerous studies concerning a development of muscles and nerves have been carried out, especially within the embrional and neonatal life period although postnatal development of motor unit properties has not been studied. The aim of the present study was to document changes in contractile properties of skeletal muscles during a life period when embryonic and neonatal isoforms of myosin disappear but muscle mass is dynamically increasing. The two groups of animals, the young Wistar rats - 40 days old and the adult ones - 270 days old were investigated. The basic contractile properties and action potential of motor units (MUs) in the medial gastrocnemius muscle were analyzed. The body weight of young rats was three times lower than of adults (146.9 g vs 461.3 g), but the muscle mass was four times lower (0.313 g vs 1.246 g, respectively). The contraction time and half-relaxation time of fast MUs in 40-day-old rats were slightly longer than in adults. The twitch forces for the three MU types were two to three times lower in young rats, where the tetanus forces for all MU types were three to five times lower for 40-day-old animals. The twitch-to-tetanus ratio for all three types of MUs in young animals was significantly higher than in adult ones (0.43 vs 0.29 for FF, 0.31 vs 0.21 for FR, and 0.15 vs 0.08 for S MUs for young and adult rats, respectively). Moreover, considerable difference in the proportion of MU types was also observed. In adult rats higher participation

of fast MUs was observed (33.7% of FF MUs and 55.4% of FR MUs) than in young ones (30.1% of FF and 41.1% of FR MUs) whereas slow MUs constituted 10.8% and 28.8% of studied populations of MUs in adult and young rats respectively. No significant differences in motor unit action potential parameters between young and adult animals were noticed.

P7.06

VARIABILITY OF THE TWITCH RESPONSES TO INDIVIDUAL STIMULI AT RANDOM STIMULATION PATTERNS OF FAST AND SLOW MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS

Celichowski J.[1], Raikova R.[2], Krutki P.[1]

[1] Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland; [2] Centre of Biomedical Engineering, Bulgarian Academy of Sciences in Sofia, Sofia, Bulgaria.

The unfused tetani evoked by train of stimuli at variable interpulse intervals (IPIs) of fast fatigable (FF), fast resistant (FR) and slow (S) motor units (MUs) were recorded and then decomposed into trains of twitch-shape responses to successive stimuli. The mean stimulation frequency was matched for each MU to evoke tetani of a similar fusion degree (lower for slow MUs, higher for fast MUs), whereas the variability range of IPIs was 50 – 150% of the mean IPI value for a given MU. The decomposition was done using an experimentally verified mathematical algorithm, described previously. Ten MUs of each type were analyzed. For each MU the twitch force, the contraction time and the force-time area for the single twitch recording were calculated and compared to both, the strongest and the weakest of the responses to successive stimuli. For each of studied MUs considerable variability of twitch parameters for responses to successive stimuli was observed, although the largest range of variability characterized slow MUs. In general, the decomposed twitch responses were stronger and had longer duration than the single twitches, although especially for 9 FF and to a smaller degree for 6 FR MUs the smallest decomposed responses were weaker but not faster than the single twitches of these MUs. The mean value of a ratio of the strongest decomposed twitch force to the single twitch force amounted to 2.4, 1.8 and 5.5 for FF, FR and S MUs, respectively. The ratio of the strongest decomposed twitch force-time area to the single twitch force-time area amounted to 1.8, 2.2 and 9.5 for FF, FR and S MUs, respectively. Analogically calculated ratio for the contraction time amounted to 1.5, 1.5 and 2.2 for FF, FR and S MUs, respectively. In conclusion, the results evidence that during voluntary activity of muscles successive action potentials generated especially by motoneurons of slow MUs have considerable variability in relation to evoked contractile responses of muscle fibers.

P7.07

CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF HINDLIMB MOTONEURONES OF RATS SUBJECTED TO 5 WEEKS OF THE WHOLE-BODY VIBRATION

Bączyk M., Hałuszka A., Celichowski J., Krutki P.

Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland.

Whole-body vibration (WBV) has been known to induce motor unit contractions by activation of a stretch reflex loop and therefore WBV-evoked effects have been accepted for improving muscle strength. The influence of the WBV on contractile properties of motor units has been described in several reports, however the WBV influence on properties of spinal motoneurones is unknown. The aim of this study was to determine whether the 5-week WBV training induces alterations in the electrophysiological properties of motoneurons. Wistar rats were trained on the vibratory platform (Power Plate) for 5 weeks (4×30 s daily, at 50 Hz frequency, the peak-to-peak amplitude of 2.5 mm, the maximum acceleration 4.79 g) and results were compared to the control group of normally active rats. The acute experiments were performed on deeply anaesthetized animals. Intracellular recordings were performed from sciatic motoneurons located in L4 – L5 spinal segments using glass micropipettes filled with 2M potassium citrate solution. Parameters of antidromic action potentials were measured and effects of intracellular injection of rectangular pulses of depolarization current were analyzed. Considerable differences in the basic electrophysiological properties were revealed between the experimental and control groups, mainly in fast motoneurones. The major findings concerned alterations in rhythmic properties of motoneurones resulting in the increased slope of the steady-state frequency-current curves in the WBV group. The described changes are likely due to the chronic changes in ion channel conductance, and therefore alterations of the membrane excitability of motoneurones, as well as to alternations in the afferent synaptic inputs from muscle receptors. The presented results help understand physiological background of the previously reported changes in the motor unit properties after the WBV and may thus contribute to standardization of the vibration training programs.

P7.08

CHANGES OF MOTOR UNIT CONTRACTILE PROPERTIES AFTER LONG-TERM EXPOSURE TO THE WHOLE-BODY VIBRATION IN THE RAT

Krutki P., Kaczmarek D., Łochyński D., Celichowski J.

Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland

Whole-body vibration (WBV) evokes increased activity of motor units (MUs) what is attributed to the enhanced afferent feedback and descending drive to the motoneuronal pool. In comparison to voluntary contractions, high-threshold MUs are more effectively activated during WBV due to lower recruitment thresholds of MUs during reflex contractions induced by mechanical vibration. Therefore WBV has been proposed to be a specific training for fast-twitch muscle fibers. Indeed, a 5-week WBV has been previously shown to induce the considerable increase of twitch and tetanic forces of FF MUs. The aim of the present study was to determine long-term effects of WBV on MU contractile properties. Two groups of Wistar rats were trained on the vibratory platform (Power Plate, USA) for 3 months or 6 months (4×30 s daily, at 50 Hz frequency, the peak-to-peak amplitude of 2.5 mm, the maximum acceleration of the platform 4.79 g). Results were compared to two control groups of normally active rats (matched with respect to age and body weight). Contractile properties were measured from functionally isolated MUs of the medial gastrocnemius muscle. Contrary to effects of short exposure to WBV, the long-term WBV did not changed significantly force parameters of FF units, only minor tendencies for an increase of tetanic forces of FF units were found after 3 or 6 months of WBV. It seems that effects of the WBV are temporary in FF MUs that adapt to long-term vibration stimuli. On the other hand, lower twitch forces, higher tetanic forces and a significant decrease of the twitch-to-tetanus force ratios were observed for FR and S MUs. Moreover, changes in proportion of fast MUs were observed after 3 and 6 months of WBV: the increase of a relative number of FF and the decrease of FR units. This suggests that the long-term WBV induces deeper processes that influence mechanisms of MU contraction (e.g., altered myosin heavy-chain expression or changed properties of motoneurones).

P7.09

GENDER-RELATED DIFFERENCES OF MOTOREURONS MORPHOMETRIC PROPERTIES OF THE RAT MEDIAL GASTROCNEMIUS MUSCLE

Mierzejewska-Krzyżowska B.[1], Bukowska D.[2], Szydłowska J.[1], Drzymała-Celichowska H.[2], Celichowski J.[2]

[1] Department of Anatomy and Anthropology, University School of Physical Education in Poznan, Poznan, Poland; [2] Department of Neurobiology, University School of Physical Education in Poznan, Poznan, Poland.

Gender differences are a well-known phenomenon in animal kingdom and concern body mass and morphology. The aim of this study was to determine the gender differences in the number and size of alpha and gamma motoneurons in motor nucleus of the medial gastrocnemius (MG) in the rat. The retrogradely labeled cell bodies of motoneurons of the same age male and female Wistar rats were studied following a bath of the proximal stump of the transected MG nerve in the horseradish peroxidase solution. The number and the soma diameters of male and female MG motoneurons were determined from serial microscopic images of stained sections using the Sony CCD-Iris Camera and MultiScanBase System. It was shown that weight of the central nervous system was on average 19% higher in males than in females. The mean number of motoneurons was 11% higher in males than in females and amounted to 95 and 86 motoneurons, respectively. In each case, the average soma diameters of motoneurons in motor nucleus were distributed bimodally: motoneurons smaller than 32.5 micrometer were recognized as gamma and greater ones as alpha motoneurons. In the present material the percentage composition of gamma (22%) and alpha (78%) motoneurons were very similar in both sexes. The mean number of alpha motoneurons was 13% higher in males than in females, but differences between the mean soma diameter in the two kinds of motoneurons, gamma and alpha, in both sexes were not significant. It is concluded that a total number of motoneurons in the rat MG motor nucleus in males and females is different. Whereas in females the number of alpha and gamma motoneurons is smaller than in males, the size of motoneurons is similar. Therefore, the gender differences in weight of the central nervous system reflect rather a difference in a total number of neurons than in their size.

P7.10

INTRA- AND INTER-LIMB COORDINATION AFTER LOW THORACIC LATERAL HEMISECTION IN RATS

Leszczynska A., Cabaj A.M., Slawinska U., Majczynski H.

Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Lateral thoracic hemisection of the spinal cord in adult rats results in initial severe impairment of hindlimb movements followed by a relatively fast improvement of locomotor functions. There are data showing substantial or even complete recovery of locomotor performance within 21 – 30 days after spinal cord injury. In our previous study we showed that improvement of locomotor performance reached plateau about four weeks after the injury but animals did not show the full locomotor recovery. The aim of present study was to analyze the time course of relationships between activity of flexors and extensors of each of four limbs during locomotion after lateral hemisection of the spinal cord in rats. The locomotion was tested in freely moving animals walking at speed 0.4 – 1.0 m/s. Bipolar EMG electrodes for the chronic recordings were implanted in soleus and tibialis muscles of hindlimbs and biceps and triceps of forelimbs. The EMG recordings were performed once a week up to

six weeks after spinal cord injury. Our results showed that 7 days after the lateral hemisection of the spinal cord the relationship between flexor and extensor muscle (intralimb coordination) of hindlimbs was severely impaired. Moreover, analysis of interlimb coordination revealed that relationships between forelimbs and hindlimbs and hindlimbs themselves were also impaired. Two weeks after surgery the relationships between flexor and extensor muscle of right as well as left hindlimb returned to normal. Diagonal coordination between left forelimb and right hindlimb was also similar to that before the lesion. Only coordination between left hindlimb and remaining three limbs was impaired through the whole period of the study (six weeks after surgery). This results confirmed hypothesis, that after lateral thoracic hemisection of the spinal cord rats did not show the full locomotor recovery.

P7.11

ACTIVATED SCHWANN CELLS DELIVERED INTO THE SUBARACHNOID SPACE VIA CISTERNA MAGNA INDUCE REPAIR OF INJURED RAT SPINAL CORD WHITE MATTER

Lewin-Kowalik J., Marcol W., Jędrzejowska-Szypułka H., Ślusarczyk W., Smętek J., Sordyl R., Larysz-Brysz M.
Department of Physiology, Medical University of Silesia, Katowice, Poland

We investigated the influence of activated Schwann cells on regeneration of the spinal cord in rats. Young adult male Wistar C rats were used (*n*=36). Focal injury of spinal cord white matter at Th10 level was produced using our original non-laminectomy method by means of high-pressured air stream. Schwann cells obtained from 7-days-predegenerated rats' sciatic nerves (*n*=3) were cultured, transfected with GFP and injected into cisterna magna (S group, *n*=23) three times: immediately after spinal cord injury and 3 as well as 7 days later (300 000 cells/injection). Control animals (*n*=10) were subjected to the spinal cord injury only. Neurons in brain stem and motor cortex were labeled with FluoroGold (FG) delivered caudally from the injury site and transported via spinal ascending tracts a week before the end of experiment. Functional outcome (BBB scale, Sciatic Functional Index) and morphological features of regeneration were analyzed during 12-week follow-up. The lesions were characterized by means of MRI. Maximal distance of expansion of implanted cells in the spinal cord was measured and the number of FG-positive neurons was counted. Rats treated with Schwann cells presented significant improvement of locomotor performance when compared to the control group. MR images showed no cyst in the spinal cord in S group, while in the control group changes resembling typical post-traumatic syringomyelia were found. The sizes of lesions were also significantly smaller in S group. Distance covered by Schwann cells was 12 mm from the epicenter of injury. Number of brain stem and motor cortex FG-positive neurons in S group was significantly higher than in control group. Obtained data revealed that activated Schwann cells are able to induce the repair of injured spinal cord white matter. The route of cells application via cisterna magna appeared useful for their delivery into the injury area.

P7.12

DIFFERENTIAL EFFECTS ON LOCOMOTOR-LIKE MOVEMENTS OF PARAPLEGIC RATS PRODUCED BY ACTIVATION OF 5-HT2 OR 5-HT7 RECEPTORS

Slawinska U.[1], Miazga K.[1], Majczyński H.[1], Cabaj A.M.[2], Jordan L.M.[3]
[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Bionics, Institute of Biocybernetics and Biomedical Engineering PAS, Warsaw, Poland; [3] Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada.

There is considerable evidence from research in neonatal and adult rat and mouse preparations to warrant the conclusion that activation of 5-HT2 and 5-HT7 receptors leads to activation of the spinal cord circuitry for locomotion. Both types of receptors are involved in control of locomotor movements, but it is not clear how they are implicated in the responses to 5-HT agonists observed after spinal cord injury. Here we used different agonists that are known to be efficient in promoting locomotor recovery in paraplegic rats: 8-OHDPAT (acting on 5-HT7) and quipazine (acting on 5-HT2 receptors). Motor performance was tested before and 15 – 30 min after i.p. drug application in spinal rats placed with the forequarters on a platform above a treadmill while the hindlimbs were touching the moving treadmill belt. Tail pinching was used to induce hindlimb movements that were monitored using video recordings synchronized with simultaneous EMG recordings from the soleus and tibialis anterior muscles of both legs. The application of either 5-HT receptor agonist improved hindlimb plantar walking. Analysis of intra- and interlimb coordination confirmed that the motor performance was significantly better, but in slightly different ways, after application of either drug. Interlimb coordination (left-right coordination) was significantly better after 8-OHDPAT, and the activity of antigravity soleus muscle was significantly longer during locomotor-like movement enhanced by quipazine (an improvement in intralimb coordination). Our results suggest that 5-HT2 and 5-HT7 receptors both facilitate activity in the spinal circuitry controlling locomotion, but their effects are likely exerted on different populations of spinal neurons. These agonists also have affinity to other types of receptors (e.g., 8-OHDPAT acts also on 5-HT1a

receptors and quipazine also has affinity for 5-HT3 receptors), so further experiments are needed to substantiate the roles of 5-HT2a and 5-HT7 receptors.

P7.13

SUBCELLULAR LOCALIZATION OF GELATINASE ACTIVITY IN SKELETAL MUSCLE OF CONTROL AND TRAINED RATS

Yeghiazaryan M.[1], Żybura-Broda K.[2], Rylski M.[2], Slawinska U.[1], Kaczmarek L.[2], Wilczyński G.[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Matrix metalloproteinases (MMPs) are key regulatory molecules in the formation, remodeling, and degradation of extracellular matrix components in both physiological and pathological processes. However their intracellular presence and activity was also reported. The purpose of this study was to examine the expression and subcellular localization of the gelatinases MMP-2 and MMP-9 in skeletal muscle fibers of normal and physically trained rats. In control hindlimb muscle, the activity and expression of the gelatinases were barely detectable in muscle fibers. In contrast, 5 days after physical training, there was significant upregulation of gelatinolytic activity in myofibers, mainly in their nuclei, and to a lesser extent in sarcoplasm and sarcolemma, as assessed by high resolution in situ zymography. The nuclei of satellite cells did not contained the activity. Within the myonuclei, the gelatinolytic activity was distributed throughout the nuclear interchromatin area. Subcellular fractionation followed by gel zymography revealed that MMP-2, but not MMP-9, is the myonuclear gelatinase whose activation occurs upon training. Training activated and upregulated MMP-9 in the cytoplasm. By RT-PCR, there was significant increase in MMP-9 mRNA only. We conclude that training activates nuclear MMP-2, it also increases both the expression and activity of cytoplasmic/sarcolemmal MMP-9. We suggest that the gelatinases play roles in muscle adaptation to training; MMP-2 may be involved in the processes of nuclear gene expression.

P7.14

FUNCTION OF FIRST AND HIGHER-ORDER SOMATOSENSORY THALAMIC RELAYS VARIES WITH LEVEL OF AROUSAL

Sobolewski A., Kublik E., Świejkowski D.A., Kamiński J., Wróbel A.

Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Sherman and Guillery (2002) proposed that thalamic sensory nuclei might function as first- or higher-order relays. According to their hypothesis, first order relays (such as rat's ventral postero-medial nucleus, VPM) transmit sensory information from the periphery to the cortex, while the function of higher-order relays (such as rat's medial posterior nucleus, PoM) is to transmit information between cortical areas via cortico-thalamo-cortical connections. In our experiments on awake Wistar rats we recorded extracellular field potentials from VPM, PoM and barrel cortex, while mechanically stimulating the rats' vibrissae and manipulating their level of arousal by additional aversive stimuli, i.e. loud sounds, or electric shocks applied on the skin of the ear. We assessed functional connection strengths between the three structures during stimuli processing using cross-trial correlation (Sobolewski et al. 2010). Our results from quiescent rats corroborate Sherman and Guillery's proposition. At low arousal level (no aversive stimuli) the sensory signal was primarily relayed from VPM to the barrel cortex and from there to PoM. However at high arousal level this network scheme was short-circuited and the barrel cortex also received input directly from PoM. We show that sensory pathways,form a dynamic system, which is capable of reconfiguring the sensory signal's route to cortical areas in step with the animal's behavioral context. *This research was supported by the Polish National Science Centre grant N N401 533040.*

P7.15

THE EFFECT OF HIGH-FREQUENCY, LOW-THRESHOLD ELECTRICAL STIMULATION OF PERIPHERAL NERVES ON THE POOL OF NEUROTROPHIN 3 IN THE LUMBAR SPINAL CORD AND SOLEUS MUSCLE OF THE RAT

Gajewska O., Piasecka J., Kasicki S., Skup M., Czarkowska-Bauch J.

Department of Neurophsiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Locomotor exercise, sufficient to increase expression of brain-derived neurotrophic factor and neurotrophin 4 in the lumbar spinal cord, does not affect protein level of neurotrophin 3 (NT-3), as we have shown previously. The effect of 7 days of direct, low-frequency electrical stimulation of the tibial nerve on expression of NT-3 was also negligible although this stimulation was addressed to low-threshold muscle afferents expressing the NT-3 and its high-affinity receptor trkC. To verify whether upregulation of NT-3 requires stronger stimulation, we maximized efficiency of electrical stimulation. Hoffmann reflex, recorded from the soleus muscle, allowed controlling low-threshold stimulation

delivered by cuff electrode implanted over the tibial nerve. Electrodes were implanted bilaterally. The nerve was stimulated unilaterally for 7 days, starting 3 weeks after surgery. The contralateral limb served as a control. Series of 3 rectangular pulses of 200 μs duration and 4 ms inter-pulse intervals were applied every 25 ms in four 20 min sessions daily. NT-3 was evaluated in supernates of homogenates from L1 – L2 and L3 – L6 segments of the spinal cord and in the soleus muscles with ELISA. In intact rats (n=4) NT-3 concentration amounted to 225 pg/mg of protein in the soleus muscle and about 60 pg/mg in lumbar segments of the spinal cord. NT-3 increased by 77% in the soleus muscle on stimulated and by 18% on non-stimulated side, comparing to intact rats. In L3 – L6 segments of the spinal cord the NT-3 was raised by 35% and 15 % on stimulated and non-stimulated side, respectively. In L1 – L2 segments there was bilateral increase of NT-3 by about 30%. We show that high-frequency low-threshold stimulation of the tibial nerve, by means of chronically implanted cuff electrodes, is capable to activate NT-3 protein both in the soleus muscle and in the caudal lumbar spinal cord indicating that also NT-3 expression is regulated in activity-dependent manner. *Supported by MSE grant N N401 0480 33.*

P7.16

HOW DOES THE FIRING RATE OF SUPERIOR COLLICULUS NEURONS CORRELATE WITH IMAGE OF BRAIN ACTIVITY?

Mochol G., Wypych M., Foik A., Wójcik D., Waleszczyk W.J.
Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Firing rate of the majority of cells from superficial layers of cat's superior colliculus (SC) is modulated in relatively long time scale. Such changes in spike generation do not depend on presented visual stimuli. To investigate whether these modulations of firing rate are related to changes in cortical states we analyzed visually evoked activity of SC neurons and electrocorticogram (ECoG) simultaneously recorded from the occipital lobe. The extracellular single unit activity was recorded from superficial, retinorecipient layers of the SC in anaesthetized and paralyzed cats. The level of anaesthesia was kept constant during recordings. As a visual stimulus we used light spot moving with different, randomly selected velocities. On average, each neuron was recorded continuously for 1 hour. Simultaneously we recorded ECoG from contralateral area 18 close to representation of the area centralis. The power spectra of ECoG data were calculated using fast Fourier transform in sliding windows. The firing rate of a given neuron was calculated in the same time windows and then correlated with the power in a given frequency band of ECoG. Most of the observed firing rate modulations were on the time scale from several to tens of minutes and were positively or negatively correlated with the changes in ECoG power in the band between 0.5 to 8 Hz, sometimes even to 13 Hz. For some neurons we also observed correlations between firing rate and power in the beta band (13 – 30 Hz) of ECoG and in most cases those correlations were opposite to correlations in lower bands. Rarely we observed also the relation between firing rate and the power of gamma band. Fast modulations of firing rate were not correlated with changes of ECoG power in any band. These results show that responsiveness of particular subpopulations of collicular neurons is differently related to the global state of brain activity. *Supported by Polish MSHE grant N N303 070234.*

P7.17

INTEGRATION OF GRAFTED EMBRYONIC MOTONEURONES INTO THE ADULT HOST SPINAL CORD: ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL ANALYSIS

Gál L.[1,2], Pajer K.[2], Nógrádi A.[2], Slawinska U.[1]
[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Ophthalmology, University of Szeged, Szeged, Hungary.

Transplantation of embryonic spinal motoneurones into an injured adult spinal cord results in reinnervation of the denervated hindlimb muscles via the avulsed and reimplanted L4 ventral root. The aim of our study was to investigate the integration of the grafted motoneurones into the host spinal cord circuits. Embryonic spinal cord pieces were grafted into the L4 segment of adult rats, the L4 ventral root was avulsed and reimplanted into the graft. In control animals the L4 ventral root was avulsed but they received no grafts. Six months after grafting EMG recordings were performed from the Extensor Digitorum Longus, Soleus and Tibialis Anterior muscles. After EMG recordings the anterograde tracer Phaseolus vulgaris was injected iontophoretically into the L4 segment, either into the graft or into the host cord near the graft. The EMG analysis of recordings showed that grafted motoneuron activity was related to rhythmic locomotor limb movement. In the EMG activity recorded in grafted animals during sitting 2 populations of motor unit action potentials (MUAPs) were distinguished. MUAPs in the first group were recruited for a long period of time and firing below 50 Hz, while MUAPs of the second group (with relatively higher amplitude) were recruited for short period of time (<200 ms) and firing between 50 and 200 Hz. This latter group may be characteristic for the grafted motoneurones. The morphological analysis of our anterograde tracing studies has shown that while processes of neurones of the host spinal cord are only able to enter the periphery of the graft, grafted neurones send great number of processes into the host spinal cord and establish functional connections with the host spinal cord circuits. These results indicate improved functional recovery induced by the grafted embryonic motoneurones following depletion of the host motoneurones and suggest that there is a functionally meaningful rewiring between the neurones of the graft and the host spinal cord.

P7.18

OSCILLATIONS IN THE ACTIVITY OF SUPERIOR COLLICULUS NEURONS: LOCKED OR NON-LOCKED TO VISUAL STIMULUS?

Foik A., Mochol G., Wypych M., Waleszczyk W.J.
Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

It is suggested that oscillatory activity of visual neurons plays an important role in encoding of information about stimuli. There are a number of publications on oscillations in the retino-geniculo-cortical pathway, but less is known about oscillatory activity in the extrageniculate pathway. We try to understand the role of oscillations in the processing of visual information in the superior colliculus (SC), the first, retinorecipient structure of the extrageniculate pathway, playing an important role in visual perception, spatial localization of an object of interest, saccadic eye movements and visually guided behavior. Extracellular single unit activity was recorded from superficial layers of the SC in anesthetized and paralyzed cats. Recordings were performed during periods without visual stimulation and also during visual stimulation with spot of light moving in a broad range of velocities or flashing at different locations of the receptive field in pseudo-random order. Autocorrelation function and Fourier transform were calculated for background as well as for evoked neuronal activity. Two variants of autocorrelation method revealed two kinds of oscillatory patterns: non-locked and locked to stimulus onset. First type of oscillations was found in the majority of analyzed cells during visually evoked activity and the frequency patterns of these oscillations were in many cases similar to those observed in background activity. The stimulus-locked oscillations were observed in about half of recorded cells and strength of these oscillations varied depending on firing rate, stimulus velocity and direction. Such oscillations were clearly visible in the case of fast changes in the receptive field of tested neuron. Since two types of oscillations occurred independently and sometimes simultaneously in the recorded activity, thus they may play different role in the processing of visual information by collicular neurons. *Supported by Polish MSHE grant N N303 070234.*

P7.19

ROLE OF INHIBITION IN SHAPING RELIABILITY: TRIAL-BY-TRIAL VARIABILITY OF VISUAL RESPONSES IN SUPERIOR COLLICULUS IS INFLUENCED BY GABA

Wypych M., Mochol G., Foik A., Waleszczyk W.J.
Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Previously we have shown that variability of visual responses of superior colliculus (SC) neurons depended on whether the main visual input to the cell was of Y- or W-channel origin (Mochol et al. 2010). To better understand the mechanisms underlying previous finding in this study we test whether GABAergic system influences the variability of SC visual responses. In acute experiments on anesthetized cats extracellular responses of single neurons to spot of light moving in broad range of velocities were recorded from retinorecipient, superficial layers of SC, allowing to determine the Y- or W-channel input. Simultaneously local iontophoretic injections of GABA (nonspecific GABA receptors agonist) or bicuculline ($GABA_A$ receptors antagonist) were performed. Trial-by-trial variability was assessed with Fano factor (FF; ratio of variance of spike counts to mean number of spikes in a given period of time). In the majority of cells application of GABA resulted in decrease of firing rate (FR) and changes of FF. These changes were consistent with previously found correlations between FR and FF. If major input to the tested neuron was of Y-channel origin and changes in FF correlated negatively with changes in FR, GABA-induced decrease of FR was accompanied by an increase of FF. In the case of major W-type input the result was opposite: FF followed changes in FR, consistent with positive correlation between the two in control trials. Injection of bicuculline however, which typically resulted in an increase of neural activity, did not lead to coherent changes of variability. The FF could change slightly or remain unchanged independently of the correlation of FF and FR in control trials. Our results show that GABAergic system may play different roles in shaping the reliability of visual responses in SC depending on the origin of visual input and types of GABA receptors involved. *Supported by Polish MSHE grant N N303 070234.*

P7.20

THE EFFECT OF AAV1/2-MEDIATED BDNF TRANSGENE OVEREXPRESSION ON $trkb^{FL}$ AND $trkb^{TK}$ RECEPTOR TRANSCRIPTS AND ON $TrkB^{FL}$ CELLULAR LOCALIZATION IN THE TRANSECTED SPINAL CORD OF THE RAT

Ziemlińska E.[1], Wewiór I.[1], Grygielewicz P.[1], Czarkowska-Bauch J.[1], Kügler S.[2], Skup M.[1]
[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Neurology, University of Goettingen, Goettingen, Germany.

Brain-derived neurotrophic factor (BDNF) regulates its full-length TrkB ($TrkB^{FL}$) receptor. BDNF administration to the brain or spinal cord after injury stimulates neuronal plasticity and brings some improvement of impaired functions, but a prolonged

exposure of neurons to BDNF *in vitro* and BDNF infusions to the brain downregulate $TrkB^{FL}$ protein and reduce its downstream signaling, thus limiting BDNF effectiveness. In our recent study we used AAV-mediated transfer of BDNF transgene to cause long-term delivery of BDNF to isolated spinal cord transected at Th11 – Th12 segments. Three groups of rats were used: intact, spinal PBS (spPBS), and spinal AAV-BDNF (spBDNF) injected. The treatment resulted in substantial improvement of treadmill locomotion at two weeks after spinalization, but its effect weakened in time (7 weeks). The mechanism underlying this effect may arise from decreased abundance and availability of $TrkB^{FL}$ and its truncated forms. To verify it, we compared levels of $trkb^{FL}/trkb^{TK}$ transcripts (qPCR) and evaluated $TrkB^{FL}$ segmental distribution (immunohistochemistry). Both transcripts decreased in the scar and in L1 – L2 segments in spPBS rats, but tended to increase in L1 – L2 in spBDNF rats ($p<0.07$). In L3 – L6 segments no group differences in transcripts were found. Comparison of $TrkB^{FL}$ and c-Myc labeling of transgene-derived BDNF revealed that: (1) caudally to the transection, $TrkB^{FL}$ was abundant in neurons and white matter oligodendroglia (2) c-Myc (+) or (-) neurons showed comparable intensity of $TrkB^{FL}$ labeling (3) neuronal $TrkB^{FL}$ labeling was higher in segments with BDNF excess. In summary, BDNF overproduction in isolated spinal network does not downregulate TrkB transcripts, either it alters cellular abundance and pattern of $TrkB^{FL}$ segmental expression. Data suggest that other aspects of TrkB-mediated signaling are responsible for weakening of functional effect of BDNF. *Supported by S007/Polish-German/2007/01 grant and EMBO fellowship (for EZ).*

P7.21

REGULATION OF SPINAL NOCICEPTIVE TRPV1/CB1 PROCESSING AS THERAPEUTIC OPPORTUNITIES IN A RAT MODEL OF NEUROPATHIC PAIN

Starowicz K.[1], Zychowska M.[1], Mrugala M.[1], Makuch W.[1], Korostynski M.[2], Slezak M.[2], Petrosino S.[3], Di Marzo V.[3], Przewlocka B.[1]

[1] Department of Pain Pharmacology, Institute of Pharmacology PAS, Krakow, Poland; [2] Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland; [3] Endocannabinoid Research Group, Istituto di Chimica Biomolecolare CNR, Pozzuoli, Italy.

Anandamide (AEA) has emerged as a multifunctional lipid mediator of various stimuli. Latest reports suggest a role for AEA as an endovanilloid ligand, however, no data exist on the potential role of endogenous AEA upregulation in the spinal cord in neuropathic pain model. Rats chronically implanted with intrathecal (i.t.) catheters underwent sciatic nerve ligation (CCI model). The effect of selective inhibitor of AEA enzymatic hydrolysis, URB597 and the involvement of TRPV1 or cannabinoid CB1 receptors, were investigated. Measurements of allodynia and hyperalgesia were made 7 days after CCI and the levels of AEA in the spinal cord of CCI rats were determined. The spinal endovanilloid/endocannabinoid system was studied by means of qRT-PCR and western blott analysis in CCI rats. Finally, the distribution of TRPV1 and endovanilloid degradation enzymes were compared in the rat lumbar spinal cord. Depending on the administered dose, URB597 (10 – 200 μg/rat) reduced pain via CB1 or TRPV1 receptors. URB597 (10 – 100 μg) dose-dependently enhanced spinal AEA levels. Surprisingly those were reduced by 200 μg of URB597 suggesting an indirect effect of an endovanilloid/endocannabinoid AEA action at TRPV1. Alterations in lypoxygenases (LOX) mRNA support the idea of alternative ways of AEA metabolism. LOX-mediated production of hydroperoxides was associated with increased phospholipase A2 acitvity. Finally, baicalein by blocking the 12-LOX activity reduced the URB597 (200 μg) analgesic effect in CCI rats. We suggest that i.t. AEA reduces neuropathic pain by acting as an endovanilloid, on the he spinal cord TRPV1/CB1 neurons. When endogenously up-regulated with URB597, AEA exerts analgesia via both receptors. Dependent on efficiency of FAAH a secondary route of AEA metabolism plays a role in CCI model. Moreover spinal lipoxygenase metabolites contribute to the AEA-mediated nociception in CCI model suggesting a complex interplay these systems *in vivo*. *Supported by 0152/B/2008/35.*

P7.22

IMMUNOCHTOCHEMICAL ANALYSES OF GAT-1 IN "TRAINED" BARREL HOLLOWS AFTER WHISKER-SHOCK TASK

Jasinska M.[1], Pyza E.[2], Hamed A.[34], Siucinska E.[5]

[1] Department of Histology, Jagiellonian University Collegium Medicum, Krakow, Poland; [2] Department of Cell Biology and Imaging, Jagiellonian University Collegium Medicum, Krakow, Poland; [3] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Warsaw, Poland; [4] Department of Neurochemistry, Institute of Psychiatry and Neurology, Warsaw, Poland; [5] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland.

Classical conditioning, which combines stimulation of a row of facial vibrissae (conditioned stimulus, CS) with a tail shock (unconditioned stimulus, UCS) expands the representation of "trained" whiskers and increases GABAergic measures in the hollows of "trained" barrels in the first somatosensory cortex (SI) of adult mouse. This study investigated how the appearance of the emotional effect of aversive learning (CS+UCS), i.e. aver-

sive conditioned vocalization, affected the expression of puncta of a prominent high-affinity GABA plasma membrane transporter GAT-1 in the barrel cortex of mice 24 h after learning. We detected that application of aversive stimulation to the tail evoked of 6 – 18 kHz audible vocalization episodes. The aversive vocalization conditioned responses (CRs) during the interval in anticipation of the UCS in the first session of CS+UCS training were the largest in number and longest in duration in CS+UCS mice. Learning led to increased expression (54%) of neuronal and astroglial (GAT-1) puncta in the "trained" barrel hollows compared to controls. The electron microscopic observations confirmed that immunoreactivity for GAT-1+ puncta was localized: in single synaptic terminals present on symmetric synaptic specialization, on symmetric synapses of double-synapse spines, and on astrocytic processes. Our data provide a causal link between vocalization conditioning, GAT-1 localized on GABAergic terminals and astrocyte networks and learning–dependent plasticity in the layer 4 of the adult SI cortex. *MNiSW grant NN401018833 to ES.*

P7.23

INTRASPINAL INJECTION OF AAV5_eGFP or AAV5_L1: EXTENT OF TRANSDUCTION AND INFLUENCE ON REGROWTH OF CST AXONS IN ADULT RATS AFTER SPINAL CORD TRANSECTION

Płatek R.[1], Korczyński J.[2], Czarkowska-Bauch J.[1], Skup M.[1]
[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Biochemistry, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Among approaches targeting restoration of function after spinal cord injury a promising one is to use L1 cell adhesion molecule, known to promote axon outgrowth, fasciculation, guidance and myelination in regeneration. L1 is upregulated after injury, manifesting requirements of the impaired networks for successful repair. Previously, our strategy to overexpress L1 gene in the lesion site was found to support reinnervation of the lumbar spinal cord after thoracic spinal cord compression in mice. Here we verified whether L1 overexpression induced caudal to complete spinal transection, may still have an impact on rostral segments affecting a regrowth of corticospinal tract (CST). AAV5 vector encoding eGFP or L1 protein under mCMV promoter was injected bilaterally into spinal L1 segment 30min after spinal cord transection at Th10/11. To label CST, rats were injected with anterograde tracer DiI to sensorimotor cortex, 1 week after spinalization. Effectiveness of transduction with AAV5 vector was evaluated based on distribution of eGFP protein at 7, 14 and 35 days. To evaluate CST regrowth, distances between lesion rostral border and tips of regrowing/sprouting single CST axons as well as the majority of axons were measured. eGFP expression occured throughout entire dorsoventral axis, in spinal gray and white matter, already 7 days postlesion and maintained up to 35. eGFP (+) fibers were traversing all segments caudally to lesion, some closing near its border but none seen in the lesion. It indicated that L1 transgene may be long-term available within segments below transection. CST tracing in AAV5_L1 revealed that in 3 out of 5 rats majority of CST axons reached the lesion border, whereas in AAV5_eGFP group only 1 out of 6 rats showed similar contact. In conclusion, AAV5_L1 overexpressed in segments caudal to complete transection may affect cellular milieu in transection proximity which results in better CST regrowth. *Support: S007/Polish-German/2007/01 grant.*

Cognition and Behavior [P8]

P8.01

SEARCHING BEHAVIOR AND CORTISOL CONCENTRATION IN SALIVA IN DRUG AND EXPLOSIVES DETECTING DOGS

Adamkiewicz E., Walczak M., Jóźwik A., Jezierski T.
Department of Animal Behaviour, Institute of Genetic and Animal Breeding PAS, Jastrzebiec, Poland.

Trained dogs are used for detection of drugs and explosives by law enforcement forces in many countries. There are, however, some gaps in our knowledge of behavior of certified detection dogs that may have impact on the results of their work. For example explosives detection dogs are expected to be less excitable and more cautious at work, not to cause the target material to explode. The aim of the study was to assess the most important behavioral characteristics during experimental searching (time to detect the target material, false alerts, style of searching, speed of movement, effective exploration time) in dogs of two breeds most commonly used for drugs and explosives detection. To assess the level of dogs' arousal during work the concentration of cortisol in saliva before and after searching was estimated. One hundred twenty-three drug detection dogs and 82 explosives detection dogs were investigated, out of which there were 106 German Shepherds (GS) and 99 Labrador Retrievers (LR). To analyze fluctuations in concentration of cortisol, saliva samples were collected before and 20 minutes after searching. The mean time elapsing to detect drugs during experimental searching was shorter (59 and 71 s for GS and LR dogs respectively) compared to the detection of explosives (101 and 91 s for GS and LR dogs respectively). Preliminary results show that mean cortisol concentration in saliva was slightly higher in explosives detection dogs compared to drug detection

dogs. For both specialties the mean cortisol concentration increased during searching. In the GS dogs the increase in concentration of cortisol during searching compared to the level before work was particularly evident, whereas an opposite tendency was observed in the LR dogs. These breed differences should be confirmed using more saliva samples.

P8.02

(FE)MALE EMPATHY: SEX-SPECIFIC NEURAL CORRELATES OF SOCIALLY TRANSFERRED FEAR

Mikosz M.[1], Knapska E.[1], Nowak A.[1], Nikolaev E.[2], Madrowski N.[2], Sadowska J.[1], Kaczmarek L.[2], Werka T.[1]

[1] Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Human studies demonstrate that women outperform men in standard tests of empathy. Recent data also suggest that women to a higher degree recruit mirror neuron-containing areas, which have been linked to processing of social emotional stimuli. However, it is not clear whether these differences are due to sex-specific mechanisms of empathy. Empathy, which in its simplest form can be defined as the capacity to be affected by the emotional state of a conspecific, is vital for survival, as it allows individuals to learn about potential danger from other members of their social group and thereby to adapt rapidly to environmental challenges. Viewed in this way, empathy is not a phenomenon limited to higher primates. With a goal of studying neural mechanisms of empathy, we have designed an animal model, in which a naive rat interacts with its cagemate, which was subjected to fear conditioning. We used this model of socially transferred fear to compare behavioral responses and pattern of neuronal activation in male and female rats. As multiple behavioral and molecular measures in females depend on current sex hormone levels, we also chose to monitor estrus cycle phase. We found that a brief interaction with a cagemate subjected to fear conditioning results in a robust activation of both medial and lateral divisions of central nucleus, as well as lateral and cortical nuclei of the amygdala and the prelimbic cortex in male rats. In female rats we found marked differences in c-Fos expression between estrus and diestrus. In diestrus, the activation patterns partially reflected the expression observed in males, whereas in estrus there were no significant differences between experimental groups. Collectively, our results support the existence of a neural circuit processing empathy. Moreover, our data show sex- and estrus cycle phase- specific activation patterns within the limbic system.

P8.03

LOSS OF mGluR5 RECEPTORS ON DOPAMINE D1-EXPRESSING NEURONS ABOLISHES NOVELTY-SEEKING BEHAVIOURS

Sikora M., Rodriguez J., Przewłocki R.

Department of Molecular Neuropharmacology, Institute of Pharmacology PAS, Krakow, Poland.

High novelty seeking behaviour has been suggested to be related to altered dopaminergic activity, however the underlying mechanisms are still poorly understood. Here we investigate how glutamatergic modulation of dopaminoceptive neurons affects novelty seeking in a mouse model with selective knock down of metabotropic glutamate receptor 5 (mGluR5) in neurons expressing D1 receptors. Mutant mice (mGluR5KD-D1) displayed normal habituation to a novel environment (time $F_{3,84}$=11.33; p<0.0001, genotype $_{F4,86}$=1.29; p=0.2661). When a novel object was placed in the middle of the open field apparatus mGluR5KD-D1 mice spent significantly less time interacting with it compared to wild-type controls (t=28.39; p=0.0095). Moreover, mGluR5KD-D1 mice showed no operant sensation seeking behaviour. While over subsequent training sessions wild-type animals gradually increased the number of operant responses associated with presentation of a light and noise, mutant mice showed no such behaviour (two-way ANOVA for active lever: genotype $F_{1,90}$=22.06; p=0.001; session $F_{10,90}$=4.5; p<0.0001; genotype × session $F_{10,90}$=1.963; p=0.04). Decreased operant sensation seeking was not assosiated with higher anxiety as assessed by the elevated plus maze. Finally, mGluR5KD-D1 mice exhibited normal operant responding for food as a reinforcer (two-way ANOVA for active lever: genotype $F_{1,45}$=0.93; p=0.359; session $F_{5,45}$=19.09; p<0.0001; genotype × session $F_{5,45}$=0.13; p=0.985). In conclusion, these results indicate mGluR5 receptors located on dopamine D1 receptor-expressing neurons are essential in novelty-seeking behaviour.

P8.04

SOCIAL MODULATION OF CONDITIONED FEAR EXTINCTION IN MICE

Nowak A., Werka T., Knapska E.

Laboratory of Defensive Conditioned Reflexes, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Fear-eliciting properties of a stimulus acquired through conditioning can be extinguished by repeatedly presenting the conditioned stimulus (CS) in the absence of the unconditioned stimulus. Such extinction process does not reflect unlearning of the original association, but results in a transient inhibition of fear. For example, extinguished fear responses may return after a change in context (i.e., renewal). The return of fear after extinction is a considerable

challenge for maintaining long-lasting fear suppression after exposure-based therapies. Until recently, the recovery phenomena were studied only in isolated animals. Social context is an important factor affecting behavior of the animals extensively interacting with their conspecifics, such as humans and mice. In the present study, we examined the influence a conspecific behavior on renewal of extinguished fear. Male C57BL/6 mice were housed in pairs, handled in order to minimize stress caused by the experimenter's presence, and then separately subjected to cued fear conditioning. Subsequently, one mouse of the pair was subjected to 6 sessions of fear extinction that significantly reduced the conditioned response (freezing) to the CS. Another mouse from the pair was merely exposed to the experimental cage for equivalent amount of time, therefore its freezing to the CS remained high. On the test day, the mice were tested either together or separately. The animals were trained and tested in a two-compartment cage, which was divided by a perforated transparent partition allowing the mice to see, hear and smell their neighbor, but not to contact them physically. We report that exposure to a fearful familiar conspecific results in renewal of conditioned fear in a mouse that was previously subjected to successfull extinction procedure. The animal model presented here can be a useful tool for studying neuronal basis of the social aspects of fear recovery after extinction.

P8.05

D-CYCLOSERINE AND MIDAZOLAM CHANGE THE EXPRESSION ALPHA-2 SUBUNITS OF GABA-A RECEPTOR AND GEPHYRIN IN THE LIMBIC STRUCTURES AS WELL AS GABA RELEASE OF HIGH AND LOW ANXIETY RATS

Lehner M.[1], Wisłowska-Stanek A.[2], Skórzewska A.[1], Turzyńska D.[1], Sobolewska A.[1], Płaźnik A.[12]

[1] Department of Neurochemistry, Institute of Psychiatry and Neurology, Warsaw, Poland; [2] Department of Experimental and Clinical Pharmacology, Medical University, Warsaw, Poland.

We examined the effects of midazolam and D-cycloserine on the release of GABA in the basolateral amygdala (BLA) of high (HR) and low (LR) anxiety rats during extinction session of a conditioned fear test. HR and LR anxiety rats were selected according to their behaviour in the contextual fear test (i.e., the duration of a freezing response was used as a discriminating variable). Administration of D-cycloserine (15 mg/kg, i.p.), significantly enhanced the inhibition of an aversive context-induced freezing response observed during the extinction session in HR and LR rats 7 days after contextual fear test. It was also found that midazolam and D-cycloserine facilitated the GABA release in HR rats under the influence of conditioned fear. HR rats pretreated with saline had higher expression of alpha-2 subunits of GABA-A receptor in BLA compared to LR rats. Administration of D-cycloserine and midazolam increased the expression of alpha-2 subunits in the BLA of HR rats compared to HR rats pretreated with saline, and to drug administered LR rats. Moreover, D-cycloserine enhanced the expression of alpha-2 subunits and gephyrin in the prefrontal cortex of HR rats. Together, these findings suggest that animals that are more vulnerable to stress differ in the expression of alpha-2 subunits of GABA-A receptor in amygdala and prefrontal cortex which is involved in the control of emotional behaviour. These animals might have innate deficits in forebrain systems that control the activity of the limbic structures including GABAergic innervation of the BLA. These data indicate also possible neurochemical mechanisms for individual differences observed in response to anxiolytic drugs among patients with anxiety disorders. The current results also suggest that activation of glutamatergic function can initiate neural changes which improve fear extinction and provide preclinical evidence in support of the clinical use of NMDA(R) modulators for the treatment of anxiety-related disorders.

P8.06

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF YOHIMBINE ADMINISTRATION INTO THE AMYGDALA OF RATS

Strzelczuk M., Dadura P., Krotewicz M., Wieczorek M., Koprowska M.

Department of Neurophysiology, University of Lodz, Lodz, Poland.

Yohimbine is an indol alkaloid obtained from the root of *Rauwolfia serpentina* or from the bark of *Pausinystalia yohimbe* tree. This alkaloid is extensively used to explore the role of alpha 2 – adrenoceptors in experimental and clinical studies. Yohimbine has been predominantly used as a pharmacological tool in treatment of men's impotence. Moreover, it was proved that yohimbine induces anxiety states and defense reactions. The amygdala is a structure in brain corresponding to the experience anxiety. It is suggested that intra-amygdalar administration of yohimbine might result in anxiogenic effect. In our experiment we have studied effects of yohimbine administration into the central nucleus of amygdala on locomotion, exploratory behavior and the noradrenergic system activity. Our study consisted of three parts: implantation of cannulas into the central nucleus of the amygdala (AMC), elevated plus maze test and biochemical analyses. We have examined the content of biogenic amines in the main emotional structures in the brain. We observed that animals preferred closed to open arms of the elevated plus maze. Additionally, the obtained data showed that changes in the concentration of noradrenaline in the AMC and hippocampus are in line with changes in the concentration of serotonin. This may confirm thesis that yohimbine has an influence on serotoninergic transmission.

P8.07

TEMPORAL TRAINING IMPROVES BOTH AUDITORY COMPREHENSION AND SPEECH PRODUCTION IN APHASIC PATIENTS

Oron A.[1], Moczulska A.[1], Lewandowska M.[1], Skolimowska J.[1], Pilczuk B.[2], Domitrz I.[2], Kwiecinski H.[2], Szelag E.[1]

[1] Laboratory of Neuropsychology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland; [2] Department of Neurology, Medical University of Warsaw, Warsaw, Poland.

Temporal information processing (TIP) is a well known neural process that underlies many aspects of human cognition, e.g., speech comprehension and speech production. The specific disorders of these language functions result from left hemispheric brain damage. A lot of literature data, as well as earlier experiments performed in our Laboratory have indicated that specific temporal training improves both TIP and auditory comprehension. The aim of this study was to test whether such a training in aphasic patients influences selectively speech reception, or also speech production. We compared performance of ten aphasic subjects before and after the training. Patients attended 16 training sessions (3 sessions a week, 45 minutes each). The training task was to indicate the temporal-order of two sounds presented to both ears in rapid succession. The task difficulty was adjusted adaptively, according to subject's performance. Before and after the training both TIP abilities (temporal-order threshold measuring for two monaurally presented clicks) and language skills were tested. Language tests comprised global auditory comprehension and phonemic hearing on word and sentence levels, moreover, naming and verbal fluency. The preliminary results showed that our temporal training improved both auditory speech comprehension and speech production. These results indicate that temporal mechanisms underlying both receptive and expressive language functions can be ameliorated by the specific temporal training in milliseconds time domain.

Supported by 507/1/N-DFG/2009/0.