NATURAL KILLER CELL - MEDIATED LYSIS OF NEURONS *E. Backström[1], B.J. Chambers[2], R. Mariotti[1], H.G. Ljunggren[2], K. Kristensson[1],* [1]Department of Neuroscience, [2]Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden.

Natural killer cells have been reported to be able to kill various transformed and virus infected target cells. It was recently observed that NK cells also could kill syngeneic dorsal root ganglia (DRG) neurons by a perforin-dependent mechanism. We demonstrate here that this phenomenon does not reflect a general ability of NK cells to kill neurons in culture. While DRG neurons of the peripheral nervous system were readily killed, ventral spinal cord neurons and hippocampal neurons of the CNS were resistant to lysis. The resistance to NK cell-mediated lysis of the latter neurons was not related to protection by MHC class I molecules, since similar $\beta_2$microglobulin neurons were equally resistant to lysis. While exploring other possible molecular mechanisms for the selective triggering of lysis of DRG neurons, we observed that the retinoic acid early inducible gene-1 (RAE-1), the product of which is a ligand for the NK cell activating receptor NKG2D, was expressed at high levels in the DRG neurons. In contrast, RAE-1 was not expressed, or only expressed at very low levels, in the resistant CNS-derived neurons. Blocking NK cells with anti-NKG2D antibodies inhibited NK cell-mediated killing of the DRG neurons. Thus, we demonstrate that NK cell-mediated lysis of DRG neurons correlates with the expression of RAE-1 and that this lysis is dependent on activation of NK cells via NKG2D. This observation explains the ability of NK cells to kill syngeneic neurons *in vitro*. The present findings are discussed in relation to the possible role of NK cells in neuroinflammatory disorders.

PATHOBIOLOGY OF INFLAMMATORY SIGNALING IN THE CENTRAL NERVOUS SYSTEM *M. Bentivoglio,* Department of Morphological and Biomedical Sciences, Faculty of Medicine, University of Verona, Verona, Italy.

In the nervous system, inflammation has mainly been studied up to now in the context of autoimmunity and infection. However, inflammatory mechanisms have been recently implicated in an array of brain responses to different kinds of damage. Especially interesting are findings pointing to a dichotomous role of the brain inflammatory response, which on the one hand can be aimed at reparative phenomena and on the other hand lead to neurotoxicity and neuronal dysfunction in the presence or absence of neurodegeneration. Thus, the outcome of an inflammatory response in the brain may be orchestrated by different parameters related to the affected individual (including genetic background, age, previous challenges, etc.), as well as by the local (neuronal and glial) microenvironment in the affected brain district. Very stimulating are also findings indicating that molecules involved in inflammatory signaling, such as cytokines and their receptors, are constitutively expressed in the normal brain, and may mediate neuronal functions distinct from their role in peripheral tissues. An overview will be presented of two different sets of experimental data, comparing the glial and neuronal responses during an inflammatory challenge and during damage potentially lethal for neurons. The first paradigm examined the response of different cell types to pro-inflammatory mediators circulating in the cerebrospinal fluid, a condition that characterizes neuroinflammatory diseases such as multiple sclerosis, but is also documented in typical neurodegenerative diseases. Intracerebroventricular administration of interferon-gamma and tumor necrosis factor-alfa to experimental rodents led to a complex timing of reactions of different glial cell populations, with sequential activation of microglia (implicating also induction of inducible nitric oxide synthase), astrocytes, damage to mature oligodendrocytes followed by stimulation of oligodendrocyte precursors, and induction of the anti-apoptotic protein Bcl-2 in glia and neurons. The other set of data is focused instead on determinants of neurodegeneration, and in particular the fate of motoneurons following peripheral challenges. The prompt activation of microglia in different paradigms of structural and functional alteration of the relationship between the motoneuron and its peripheral muscle target, the induction in motoneurons of neuronal nitric oxide synthase and a variety of other molecules, including Bcl-2, document how retrograde signaling and the interplay between neurons and surrounding glia may bring about inflammatory signaling and represent key factors in neuronal cell death.

EFFECT OF ACUTE AMMONIA INTOXICATION ON IN VITRO PROTEIN SYNTHESIS IN DIFFERENT RAT BRAIN REGIONS *J. Burda[1], V. Danielisová[1], C. Montoliu[2], M. Némethová[1], V. Felipo[2],* [1]Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic; [2]Departamento de Neurobiologia, Instituto de Investigaciones Citológicas, Valencia, Spain.

Striking similarities between metabolic pathways activated in the brain by ammonia toxicity and ischemia led us to hypothesize equal or similar changes in translational activity. This assumption is supported by the data of Bessman (1) and Schott (2) demonstrating substantial inhibition of protein synthesis after ammonia intoxication or presence of ammonia in incubation medium, respectively.
Mechanisms leading to the inhibition were poorly studied until now. Impaired energy metabolism can be used as an explanation for inhibition of protein synthesis in whole cells system - in vivo studies, slices or cell cultures, only. Reported data do not notice whatever about likely changes of the activity of translational machinery itself though these can be expected according to evidence that ammonia intoxication produces significant changes in NMDA activation, glutamate, calcium, NOS, NO and free oxygen radicals.
Acute ammonia intoxication was induced by intraperitoneal injection of ammonium acetate (1 - 7 mM/kg of body weight). Dose-dependent inhibition of protein synthesis as soon as 15 min after i. p. ammonium acetate administration with different sensitivity of brain regions was observed. Time-dependent (two-phase) inhibition with first minimum at 30 minutes after 5 mM/kg of ammonium acetate administration followed by transient improvement at 2 hours and next more profound inhibition characterised by marked inhibition of reinitiation ability 6 hours after treatment also with different sensitivity of brain regions. Study of the effect of ammonia toxicity on protein synthesis might help us to understand mechanisms not only ammonia-induced damage of CNS but comparison of obtained data could be useful to clarify mechanisms carrying postischemic death of neurons.
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TWO DIFFERENT MECHANISMS ARE ESSENTIAL FOR THE ACQUISITION OF ISCHEMIC TOLERANCE IN CA1 FIELD OF HIPPOCAMPUS: ROLE FOR PROTEIN SYNTHESIS *J. Burda[1], M. Hrehorovská[1], L. García Bonilla[2], V. Danielisová[1], D. Čížková[1], R. Burda[3], M. Némethová[1], J.L. Fando[4], M. Salinas[2],* [1]Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic, [2]Hospital Ramón y Cajal, Servicio de Bioqúimica, Madrid, Spain, [3]Faculty Hospital of L. Pasteur, Košice, Slovak Republic, [4]Universidad de Alcalá, Departemento de Bioquímica y Biologia Molecular, Spain.

Ischemic preconditioning of heart and brain is a well-documented neuroprotective phenomenon. However, the mechanism underlying the increased resistance to severe ischemia by a preceding mild ischemic exposure remains unclear. In this study we have determined the effect of ischemic preconditioning on ischemia-induced translation inhibition in the neocortex and hippocampus of rats. Different ischemic periods of 3, 4, 5 and 8 minutes, as well as distinct times between sublethal and lethal ischemia (30 min) were studied. In the different situations the rate of protein synthesis *in vitro*, the expression of HSP72 or HSP70, the levels of initiation factor 4G and the phosphorylated levels of initiation factor 2alpha as well as the cell death after 7 days of the last ischemic

episode were determined. Our results suggest that two different mechanisms are essential for the acquisition of ischemic tolerance at least in CA1 sector of hippocampus. First one embraces a highly significant reduction in translation inhibition after lethal ischemia, especially at early time of reperfusion, in both vulnerable and non-vulnerable neurons. This ischemic tolerance-induced recovery in translation is accompanied by a decreased eIF4G degradation without changes in the reperfusion-induced increased eIF2 (alphaP) levels. For the acquisition of full tolerance a second mechanism, highly dependent on the time interval between preconditioning (sublethal ischemia) and lethal ischemia is necessary; thus indicating the necessity of delayed synthesis of protective (anti-apoptotic) proteins, which prevents the death of vulnerable neurons.

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GENETIC DISRUPTION OF POLY(ADP-RIBOSE) POLYMERASE INHIBITS OXIDATION OF MACRO-MOLECULES AND DEPLETION OF NAD IN BRAIN DURING INFLAMMATION *M. Cąkała, G.A. Czapski, J.B. Strosznajder,* Medical Research Centre, Department of Cellular Signalling, Polish Academy of Sciences, Warsaw, Poland.

Poly(ADP-ribose) polymerase is a nuclear DNA-binding protein that participates in the DNA base excision repair pathway in response to genotoxic stress in mammalian cells. Rapid DNA single-stranded breaks are induced by free radicals, leading to over-activation of PARP and depletion of cellular energy resulting in mitochondrial free radical generation and cell necrosis. Recent studies have noted the participation of PARP in the inflammation. Inactivation of PARP gene improves in the animal the outcome of a variety of pathological conditions associated with inflammation.

The aim of our study was to investigate whether inactivation of PARP gene protects against oxidation of macromolecules in the lipopolysaccharide (LPS) and/or galactosamine-induced inflammation in the brain. The effect of PARP gene inactivation on proteins, lipids and DNA oxidation and NAD^+ concentration in the brain was investigated and it was compared with effect of specific PARP inhibitor 3 – aminobenzamide (3-AB). Mice C57BL/6 $PARP^{-/-}$ were injected i.p. with LPS at a dose of 1 μg/g b.w. or/and with D(+)-galactosamine at a dose of 700 μg/g b.w. for 6, 12, 24 or 48 h. Wild-type mice were injected i.v. with 3-AB at a dose of 30 mg/kg b.w., and after 1 h with LPS at a dose of 1 μg/g b.w. for 48 h. Lipid peroxidation was evaluated by measurement of thiobarbituric acid reactive substances (TBARS) concentration. Protein oxidation was estimated by carbonyl groups contents in proteins using 2, 4-dinitrophenylhydrazine. DNA fragmentation was evaluated using agarose-gel electrophoresis. Moreover NAD^+ was assayed using spectrophotometric enzymatic cycling assay. Our data presented that lipid peroxidation, protein carbonyl group content, DNA fragmentation and NAD^+ concentration was not significantly changed in PARP knock-out mice injected with LPS and/or galactosamine in comparison with control animals treated with PBS. This results indicated that inactivation of PARP gene is responsible for the resistance of central nervous system to inflammatory processes.

EFFECT OF BODY TEMPERATURE DURING NEONATAL ASPHYXIA ON PLASMA ACIDOSIS, PLASMA IRON RELEASE AND BEHAVIOURAL DISTURBANCES IN JUVENILE AND ADULT RATS

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Newborn mammals, showing reduced normal body temperature, might be protected against iron-mediated, delayed neurotoxicity of perinatal asphyxia. Therefore, we decided to study the effects of body temperature on plasma pH and iron levels in newborn rats exposed to a critical anoxia and on postanoxic development of behavioural disturbances in juvenile and adult rats. Neither pH nor plasma iron were affected by anoxia at normal neonatal body temperature of 33°C. However, acidosis and hyperferremia developed at body temperature adjusted to a level typical of healthy (37°C) or febrile (39°C) adults. Neonatal anoxia at 37°C and 39°C led to significant behavioural abnormalities in juvenile and adult rats. Both the physiological body temperature and postanoxic chelation of iron with deferoxamine prevented the disturbances. In conclusion, from comparative physiology viewpoint, the present study constitutes warnings to neonatologists (1) to prevent postnatal hyperferremia and (2) to stop treating mild (by 1-2°C) reduction in body temperature of asphyxiated babies as unequivocally harmful.

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POLY(ADP-RIBOSE)POLYMERASE INHIBITORS PROTECT BRAIN CORTEX LIPIDS AND PROTEINS AGAINST DAMAGE EVOKED BY OXIDATIVE STRESS *G.A. Czapski, M.A. Napierała, D. Kopczuk, J.B. Strosznajder,* Department of Cellular Signalling, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

Poly (ADP-ribose) polymerase (PARP; EC 2.4.2.30) is a nuclear enzyme involved in DNA repair, gene expression, cellular differentiation. However, PARP overactivation may lead to depletion of NAD and energy stores followed by cell death. PARP inhibition was shown to be a successful strategy in preventing cell death in brain, heart ischemia, and inflammation. Our last study indicated that PARP inhibitor, 3-aminobenzamide (3-AB), protects neurons in CA_1 layer of hippocampus against oedema and neuronal death after short transient forebrain ischemia. The aim of this study was to determine the molecular mechanism responsible for protective effect of PARP inhibitors in oxidative stress, to obtain the information about the specificity of these widely used compounds. The effect of PARP inhibitors on protein and lipid oxidation was investigated, and compared with the effect of antioxidants. Moreover, the ability of these compounds for scavenging of hydroxyl radicals was estimated using spin-trapping method combined with HPLC. Oxidative stress was induced during 15-min incubation of rat brain cortex homogenate at 37°C with 25 μM $FeCl_2$ and 10 μM ascorbic acid or with 25 μM $CuSO_4$ and 10 mM H_2O_2. Lipid peroxidation was evaluated by measurement of thiobarbituric acid reactive substances (TBARS) concentration. Protein oxidation was determined by using fluorescent probe TyrFluo to detect the level of protein dityrosine. PARP inhibitors: 3-AB, 1,5-dihydroxyisoquinoline (DHIQ) and 3,4-Dihydro-5[4-(1-piperindinyl)butoxy]-1(2H)- isoquinoline (DPQ), and antioxidants: Trolox, resveratrol and cyclohexylbisphenol, were tested in concentration range 0-5 mM. Our data indicated that 3-AB and DHIQ in spite of PARP inhibition, are also free radical scavengers. DHIQ decreased in concentration dependent manner both lipid and protein oxidation evoked by transition metals and hydrogen peroxide. At 2.5 mM concentration DHIQ decreased TBARS by about 65%. 3-AB suppressed dityrosine formation but had no effect on lipid peroxidation. DPQ is not a free radical scavenger and did not affect neither protein nor lipid oxidation. Resveratrol, Trolox and cyclohexylbisphenol at 0.05 and 1mM respectively diminished lipid peroxidation evoked by oxidative stress. Our results indicate that among the investigated compounds, DPQ seems to be the most specific PARP inhibitor. 3-AB expresses low, but DHIQ exerts very pronounced antioxidant properties, however with 25 times lower IC_{50} than resveratrol. This data suggest that combination of resveratrol with specific PARP inhibitor may offer a new therapeutic strategy.

MAPPING OF N-TYPE AND R-TYPE CALCIUM CHANNELS IN SPINAL CORD AND DORSAL ROOT GANGLION CELLS FOLLOWING SEGMENTAL NERVE LESION *D. Čížková, M. Maršala, T.L. Yaksh,* Department of Anesthesiology, University of California, San Diego, La Jolla, USA.

Pharmacological studies suggest a role of voltage-dependent Ca^{2+} channels in the development of neuropathic pain associated with nerve

injury. To distinguish the role of individual neuronal voltage-dependent Ca^{2+} channels (VDCC) in central nociceptive transmission, we defined the distribution of N-type (α_{1B} subunit) and R-type (α_{1E} subunit) Ca^{2+} channels in spinal cord (SC) and dorsal root ganglia (DRG) throughout L5-L6 segments of rats before and at 1-50 days after tight ligation of the left fifth and sixth lumbar spinal nerves. Immunostaining in control animals with specific antibodies recognizing α_{1B} subunit showed diffuse staining pattern in the dorsal horn, ventral horn as well as in the DRG. In contrast, the nerve ligation evoked a significant up-regulation of α_{1B} subunit represented by an intense granulation in the ipsilateral dorsal horn (laminae I-II) that appeared at 4-5 days, peaked at 12 days and almost completely disappeared at 50 days post ligation. Similarly, in the DRG, the majority of cell bodies and nerve fibers showed α_{1B} subunit up-regulation, which could be observed only in the small diameter neurons after 50 day survival. The expression of α_{1E} Ca^{2+} subunit in control spinal cord was characterized by combination of smooth somatic staining and dense punctate positivity along the variously orientated nerve processes and dendrites, with highest density in the laminae I-III and IX. However, the DRG cells revealed only weak and diffuse α_{1E} immunopositivity. The nerve ligation caused no changes of α_{1E} Ca^{2+} subunit in the dorsal horn, however down-regulation in the ventral horn and up-regulation in the DRG, showing intense diffuse and punctate staining of all cell types and nerve fibers at 12 days post surgery. The staining pattern was also suppressed by longer survival period. These data suggest that both N-type and R-type calcium channels are important for development of neuropathic pain and administration of selective N-type or R-type Ca^{2+} of channel antagonists might be effective in treatment of patients with chronic pain.

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ISCHEMIC PRECONDITIONING DEFENDS THE EXCESSIVE IRON DEPOSITION OF POST-ISCHEMIC RAT BRAIN

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It is well established that iron, which is of considerable importance for normal neurological function, is highly regulated in all organ systems. All organs including the brain contain iron, and the proteins involved in iron uptake (transferrin and transferrin receptor) and intracellular storage (ferritin). However, because the brain resides behind a barrier and has a heterogeneous population of cells, there are aspects of its iron management that are unique. Iron management, the timely delivery of appropriate amounts of iron, is crucial for normal brain development and function. Mismanagement of cellular iron can result not only in a decreased metabolic activity but an increased vulnerability to oxidative damage. Preconditioning of the brain with sublethal ischemia induces tolerance to subsequent lethal periods of ischemia (ischemic tolerance). In this study, we used of iron histochemistry to investigate the postischemic changes of iron deposition in the cerebral cortex, hippocampus and striatum in a rat model of cerebral ischemia and ischemic tolerance from 1 and 8 weeks after recirculation. Forebrain ischemia was induced by 4-vessel occlusion for 5 min as an ischemic preconditioning. Two days after the preconditioning or sham operation, second ischemia was induced for 20 min. The number of neurons in which the iron granules were found and the amount of granules per cell were higher if compared with sham-operated control after 1 week and reached maximum by 8 weeks after recirculation in layer V of the cortex. In the hippocampus iron accumulation appeared in the CA1 region by 1 week after recirculation and reached maximal levels by 4 weeks. At 8 weeks, the number of iron-containing cells was decreased, but several iron-laden clusters could be seen in the pyramidal cell layer of the hippocampal CA1 region. Neurons in the striatum pars dorsolateralis contained granular iron deposits and these granules also became more apparent with the increased postischemic time. When rats were exposed to 5 min ischemia 2 days before lethal 20 min ischemia the deposition of iron in the pyramidal cells in the layer V of the cortex was significantly decreased from 1 to 8 weeks after the recirculation. In the CA1 region of the hippocampus with ischemic tolerance iron deposited in pyramidal cells was seen until 4 or 8 weeks.

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^{1}H MAGNETIC RESONANCE SPECTROSCOPY STUDY HUMAN BRAIN TUMORS IN VITRO

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Metabolism of histologically different brain tumors is essentially similar to the parent tissue. On the other hand varied metabolic pathway flux rates and altered proportions of main metabolic components define many, if not all tumors. This can be resulted in the different MR spectra. Autopsies of the different human brain tumors were immediately frozen in liquid nitrogen and after samples collection the perchloric acid extracts were made. An external standard (3 aminopropionic acid) was added to each sample for quantification of different metabolites. Perchloric acid extracts of the human brain autopsy were studied using ^{1}H magnetic resonance spectroscopy (MRS) in vitro. Varian VXR 300 spectrometer was used for obtaining ^{1}H MR spectra. ^{1}H MR spectra of brain tumors reveals changes in the relative resonance intensities of chemical compounds normally observed in the spectrum (N-acetylaspartate, choline, creatine, lactic acid etc.). Meningiomas exhibited a characteristic prominent signal from alanine in each sample. The lactate elevation was also found due an anaerobic glycolysis during the removing sample from the brain. Lactate, normally undetectable in the brain, accumulates within necrotic tissues, cysts and especially in the active tumors because of a high rate of glycolysis even in aerobic tumors. Almost all samples exbibited essentially no signal from N-acetylaspartate (NAA) and decreased signals from creatine (phosphocreatine) and inositol. Gliomas exhibited typical elevation in the NAA, inositol and creatine signals. In the astrocytomas it was possible to detect the prominent signals from inositol and creatine. It seems that ^{1}H magnetic resonance spectroscopy will be suitable method for detection of different brain tumors and treatment efficacy study.

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COMPARATIVE STUDY OF NADPH-DIAPHORASE ACTIVITY IN THE HIPPOCAMPUS IN A RAT MODEL OF CEREBRAL ISCHEMIA AFTER TANAKAN PRETREATMENT

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The NADPH-diaphorase (NADPH-d) histochemistry provides a simple method to localize presence of nitric oxide (NO) produced by nitric oxide synthase (NOS). NO is a potent vasodilatator and neuromodulator. There is an overproduction of NO and other free radicals during ischemia and reperfusion. Potencial effects of Tanakan (EGb 761-Gginkgo biloba extract) as an scavenger of reactive species were studied on the rat forebrain model of ischemia. Experiments were performed on adult Wistar rats pretreated with Tanakan during 7 days. Forebrain ischemia was produced by the four-vessels occlusion model (1) for 30 min following 1 and 6 days of reperfusion. In the present study, we used NADPH-diaphorase histochemistry (2) to investigate the postischemic changes in NO production in the hippocampus. In the control sections scattered weak NADPH-d positive neurons were observed in the hippocampus. NADPH-d positivity was found in the wall of cross-sectioned blood vessels. In the group of rats after 30 min of ischemia and 1day of reperfusion NADPH-d activity was well pronounced in small arteriols. The number of NADPH-d positive neurons slightly increases in granular layer of the dentate gyrus (DG) and scattered NADPH-d positive neurons were also found in the CA_{1-3}

subfields. In the group of rats after 30 min of ischemia and 6 days of reperfusion rich blood vessels network was identified throughout the whole hippocampus. Dilated blood vessels were strongly stained. There were focal dense areas of NADPH-d positive pyramidal neurons in the CA_1 subfield. Increased number of scattered neurons were also found in the CA_{2-3} subfields and in the DG. Our findings suggest that Tanakan serves as a scavenger of free radicals in animals subjected to 30 min of forebrain ischemia and 1 and 6 days of reperfusion.
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DISTRIBUTION OF GEPHYRIN POSITIVE SYNAPSES ON FLEXOR DIGITORUM PROFUNDUS MOTONEURONS IN THE DEVELOPING RAT Z. *Fallah, G.J. Clowry,* Department of Anatomy and Pathology, Medical School of Shahed University, Teheran, Iran.

The development of fine motor control is in part dependent on acquiring the ability to independently activate some motoneuron pools whilst inhibiting activity in others. The present study tested the hypothesis that, during the development, increasing control of motor activity is brought about by an increasing density of inhibitory synapses to motoneurons. Hence, this study investigated the possible changes in inhibitory inputs to motoneurons during maturation of locomotion and onset of forepaw use in rats. Flexor digitorum profundus motoneurons were identified by retrograde labelling with Cholera Toxin B Subunit (CT-B). At postnatal ages P7, P14 and P30. Sections of cervical spinal cord were immunofluorescently double-stained for CT-B and for the protein gephyrin, a component of synaptic receptor for the inhibitory neurotransmitter glycine. Using daul Channel laser confocal microscopy, the numbers of clusters of gephyrin immunoreactivity per 100 μm of cell membrane were calculated for lengths of somatic, proximal and distal dendritic motoneuron membrane. All compartments showed a slight but not statistically significant increase in density with age. However, it was observed that at the youngest age smaller motoneurons had significantly fewer gephyrin clusters on their cell bodies, but not proximal dendrites, than larger motoneurons. This correlation disappeared with age. We also observed that the more distal dendrites had densities of gephyrin synapses comparable with proximal dendrites. These observations were confirmed qualitatively by immunoelectron microscopy.

SPECTRAL SHIFTS OF VERY SLOW BRAIN POTENTIALS IN LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX OF FREELY MOVING RATS IN RESPONSE TO DIFFERENT ILLUMINATION CHANGES *I.V. Filippov,* Yaroslavl State Medical Academy, Department Physiology and Biophysics, Yaroslavl City, Russia.

Previously, different forms of very slow oscillatory activity with frequencies below 0.5 Hz in the visual system of the brain have been described. However, the functional significance of very slow brain potential oscillations in the central mechanisms of vision remains poorly documented and mainly unknown. This study was undertaken to test the hypothesis that dynamical shifts of very slow brain potential (VSBP) oscillations in lateral geniculate nucleus (LGN) and primary visual cortex (V1) accompany environmental illumination changes. This research was carried out on the 5 freely moving adult albino rats with two gold electrode pairs implanted in LGN and ipsilateral V1 for long-term bipolar extracellular VSBP recordings. Experiments were performed when animals were exposed to different background illumination levels: darkness (0 lx), n=50 experiments and ambient light (2000-2500 lx), n=50 experiments. Data acquisition and analysis processes were performed using AC/DC amplifier connected to analog-to-digital converter and personal computer. VSBP oscillation recording segments were subjected to power spectral analysis. All recordings demonstrated the presence of different frequency domains of spontaneous VSBP oscillation patterns (second, multisecond waves, and seldom minute fluctuations) in LGN and V1 both during darkness and light exposure. In darkness (in LGN and V1), it was found the presence of oscillations in the range of seconds (0.1-0.25 Hz frequency domain), multisecond (0.0167-0.03 Hz frequency domain) and occasionally minutes (0.001-0.002 Hz frequency domain). Continuous illumination induced significant ($P<0,001$) dramatic VSBP changes in both structures only in the frequency domain of seconds, manifested by a remarkable decrease in the spectral power of 0.1-0.25 Hz oscillations. These data confirm the hypothesis that has been tested, and permit the conclusion regarding the possible relations between VSBP oscillations in the band of seconds and central mechanisms of vision (e.g. specific visual attention shifts). It is suggested that VSBP multisecond oscillations, obviously, reflect processes of global excitability of LGN and V1 neuronal networks during different illumination levels presumably mediated via inputs to these CNS structures from main brainstem nuclei (e.g. locus coeruleus, raphe nuclei, etc.).

PHOSPHOLIPASE A_2 AND LIPID MEDIATORS IN BRAIN INJURY *G. Goracci,* Department of Internal Medicine, Division of Biochemistry, University of Perugia, Perugia, Italy.

One of the first biochemical events observed at the onset of brain ischemia is the increase of free fatty acids (FFA), which are released from membrane phospholipids (1). Several reports have indicated that the phenomenon is consequent to the activation of phospholipase(s) A_2 (PLA_2) but other mechanisms such as degradation of diacylglycerols produced by phospholipase C or by the reversal of cholinephosphotransferase reaction (2) cannot be excluded. Although the molecular mechanisms involved in controlling PLA_2 activity in neural cells are still largely unknown, there is no doubt that these enzymes catalyse the limiting step for the production of lipid mediators (i.e. eicosanoids, platelet-activating factor (PAF), lysophosphatidic acid and others) which participate to physiological and pathological processes in brain. For instance, PAF plays a role as mediator in neurotransmission and synaptic plasticity but it becomes neurotoxic when its concentration increases as during ischemia. Various isoforms of PLA_2 are present in the nervous tissue differing for molecular weight, cellular and subcellular localisation, Ca^{2+} requirement and substrate specificity (3). Thus, the attribution of specific functions to each enzyme type is quite a difficult task. Particular attention has been devoted to two types of PLA_2 with respect to brain injury: Ca^{2+}-dependent cytosolic ($cPLA_2$ - type IV) and secretory, ($sPLA_2$ - type IIA). The former is activated by receptor-mediated mechanisms, by $[Ca^{2+}]_i$ increase and phosphorylation which cause the translocation from cytosol to membrane. Due to its specificity for arachidonic acid, it is considered the enzyme triggering eicosanoids production. $sPLA_2$ is secreted by inflammatory cells and is believed to have defence functions but also to be neurotoxic. $sPLA_2$ is also present in brain mitochondria and its activity increases following ischemia and reperfusion (4). We have recently reported that this enzyme is released from brain cortex mitochondria when membrane potential is reduced by deprivation of respiratory substrates or by uncouplers (5). Thus, the release of $sPLA_2$, similarly to that of cytochrome c, might participate to cell signalling as a response of mitochondrial dysfuntions and be involved in neurodegenerative processes.

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HYPOXIA INDUCIBLE FACTOR-1 AND NEURONAL CELL DEATH *J. Gross,* Molecular and Biological Research Laboratory, Department of Otorhinolaryngology, Charité Hospital, Humboldt University, Berlin, Germany.

In hypoxia the transcription factor hypoxia-inducible factor-1 (HIF-1) strongly contributes to the expression of genes involved in glycolysis, glucose transport, erythropoiesis, and angiogenesis (1). In addition, HIF-1 has been implicated in the regulation of neuronal cell death (2). HIF-1 is a dimeric transcription factor composed of a HIF-1 alpha and a HIF-1 beta subunit, also known as ARNT. Under hypoxia the HIF-1 alpha subunit is stabilized and together with ARNT forms the active HIF-1 heteromer which binds to hypoxia response elements (HRE) in the regulatory parts of the HIF-1 target genes. To better understand the relations between HIF-1 activity and cell death we compared the HIF-1 activity and the cell death rate of untreated and NGF treated, neuron-like PC12 cells. Apoptosis and necrosis, both are important mechanisms of cell death following hypoxia and ischemia. Cell damage was assessed by flow cytometry of double stained (Annexin V and propidiumiodide) cells, and by analysis of the overall death parameters LDH and mitochondrial dehydrogenase. In parallel, cells were transfected with a control and a three-hypoxia-responsive-elements (HRE) containing vector and HIF-1-driven luciferase activity was determined. Exposure of NGF-treated, neuron-like PC12 cells to hypoxia resulted in a higher cell death rate when compared to untreated controls. Triggers of cell death following oxygen deprivation include reduced ATP production by anaerobic glycolysis, increased intracellular production of reactive oxygen species and increased expression of pro-death genes. PC12 cells exposed for two days to NGF exhibited a decrease of HIF-1 activity up to a factor of ten. Possible mechanisms involved are decreased binding capacity to the promoter region of the target genes, increased degradation of HIF-1 by ubiquitination, or heterodimerization with partners of NGF induced transcription factors. The decrease of HIF-1 activity may contribute to the enhanced hypoxia-induced cell death via reduced expression of HIF-1 alpha regulated genes responsible for adaptation to hypoxia, like those for glucose transport proteins and enzymes of the glycolytic chain.

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END TO SIDE ANASTOMOSIS OF PERIPHERAL NERVE IN CLINICAL AND EXPERIMENTAL MODEL *P. Haninec, P. Dubový, F. Šámal,* Department of Neurosurgery 3rd Faculty of Medicine, Charles University, Prague, Department of Anatomy, Masaryk´s University, Brno, Czech Republic.

Despite of the progress in peripheral nerve surgery, the positive results in peripheral nerve reconstruction in some locations remain difficult to achieve (1-4). This is true mainly for repair of avulsed roots of the brachial plexus. The results of 13 patients with the new method of end to side anastomosis of axillary nerve to various donor nerves are presented. The results were evaluated by means of functional and electrophysiological methods. Fair and good results were achieved in neurotization of axillary nerve in 75% of patients with follow up longer than 24 months. Electrophysiology proved reinnervation in 100% of these patients. The average muscle strength, according to the muscle test, was 3.80. The functional recovery started most often 7-12 months (average 9 months) postoperatively. End to side anastomosis of the rat musculocutaneus nerve with the ulnar one was used in the experimental animal model. Behavioural, electrophysiological as well as morphological methods proved the reinnervation of biceps brachii muscle mainly through collateral reinnervation from ulnar nerve motoneuron pool (segments C7-Th1: 355 motoneurons). The motoneurons (62±14, x±SD) of the ulnar nerve motoneuron pool are able to send off collateral sprouts from their intact axon into musculocutaneus nerve. Other motoneurons of the same pool (21±11, x±SD) sent off the axons directly to the musculocutaneus nerve without collateral sprouting, probably due to the axonotemesis of these motor nerve fibres during the formation of perineural window. Our results demonstrate that the end to side anastomosis of peripheral nerve might be successfully used in reinnervation procedures of brachial plexus injury, mainly in the cases with insufficient sources of motor nerve fibres from neighboring motor nerves.

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CYCLOHEXYLBISPHENOL INHIBITS OXIDATIVE STRESS BUT NO cGMP LEVEL IN 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDRO- PYRIDINE (MPTP) MOUSE MODEL OF PARKINSON'S DISEASE *M. Chalimoniuk[1], A. Stępien[2], J.B. Strosznajder[1],* [1]Department of Cellular Signaling, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, [2]Department of Neurology, Aviation Institute of Medicine, Warsaw, Poland.

The pathogenesis of the neuronal degeneration in Parkinson's disease (PD) is untill now unknown. The previous studies suggest that the free radicals are responsible for neuronal degeneration in substantia nigra. Nitric oxide (NO) may be one of important reactive radical involved in cell death. Soluble guanylyl cyclase (sGC) is the cellular receptor for NO that regulates cGMP level. Excessive synthesis of NO and cGMP may alter wide range of physiological and biochemical processes in cells. The aim of our study was to investigate the effect of cyclohexylbisphenol on oxidative stress and cGMP level in MPTP animal's model of PD. Mice C57/BL have received three injection of MPTP in a dose 40 mg/kg b.w. at 3 hour intervals, control mice have received saline only. After last injection of MPTP the same group of animals received cyclohexylbisphenol in a dose of 40 mg/kg b.w. i.p. Mice were killed 7, 14 and 21 days after last injection. Free radicals were assayed using fluorescence method (DCF), lipid peroxidation was evaluated by determination of thiobarbituric acid reactive substances (TBARS). Moreover, cGMP and glutathione levels were determined in striatum, midbrain and hippocampus. Our results indicated that MPTP induced increase of oxidative stress (DCF, TBARS), cGMP level and depletion of glutathione at 3, 7 and 14 days after injection in striatum and midbrain. However, 21 days after injection of MPTP free radicals dependent processes and glutathione level were close to control value but cGMP level remained enhanced. Cyclohexylbisphenol, which expresses to have a strong antioxidant properties in vitro, inhibited free radicals formation, lipid peroxidation and protected glutathione level against depletion but had no effect on MPTP induced elevation of cGMP in striatum and midbrain. Our results suggest that cyclohexylbisphenol can be useful in the treatment of PD together with the other more specific compounds.
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NEUROPATHIC CHANGES OF RAT MOTONEURONS AFTER SCIATIC NERVE INJURY *S. Jergová, D. Čížková, M. Čížek[1],* Institute of Neurobiology, Slovak Academy of Sciences, Košice, [1]University of Veterinary Medicine, Košice, Slovak Republic.

Several peripheral nerve-injury models have been developed and are used to study the underlying mechanisms of neuropathic pain. Animals with neuropathic pain show varying degrees of deformity of the foot and postural changes but it is not clear whether the abnormal foot posture is a result of motor impairment or a form of guarding behaviour reflecting the level of pain. In the present study the retrograde axonal transport of FlouroGold (FG) to the motoneurons at the lumbar spinal cord level in the intact and injured rat sciatic nerve was examined. We have analysed possible alternations of FG labeling in regard to the

damage of motor axons caused by peripheral injury. In our experiment we have employed chronic constriction injury model, transection of sciatic nerve and tight ligation of sciatic nerve as well, all injuries at mid-thigh level and we have compared the differences in extent of motoneurons damage caused by involved neuropathic models. Two weeks after injury a fair amount of heavily fluorescent motoneurons was detected throughout L4 - L6 spinal cord segments. There were no significant changes in the number of FG-labeled motoneurons in the ventral horn between injured and control rats and also changes between involved nerve-injury models were not observed. This fact suggests that these peripheral nerve-injury models did not cause significant degenerative changes in the motoneurons and axons forming the corresponding ventral root and, therefore, it seems that foot and postural changes of neuropathic animals are result of a complex mixture of motor and sensory abnormality.
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POSSIBLE MECHANISMS OF NEUROTOXIC AND NEURO-PROTECTIVE EFFECTS OF GLUTAMATE ON NEUROENDOCRINE FUNCTION *D. Ježová,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Glutamate, the major excitatory neurotransmitter in the brain, is known to exert both neurotoxic and neuroprotective effects. Neurotoxic action is depending on the age and the amount of glutamate. Treatment of neonatal rats with high doses of glutamate is producing serious neuroendocrine and behavioural dysfunction by destroying neurons in the arcuate nucleus and other brain regions. Among other changes, increased gene expression of proopiomelanocortin (POMC) the precursor molecule of ACTH, in the pituitary of newborn as well as adult rats was observed (1). In humans, only very high doses of glutamate are inducing hormone release. On the other hand, glutamate neurotransmission seems to be involved in the control of hormonal responses during stress as evidenced particularly by pharmacological studies in rats (2). We have shown that stress stimuli are inducing changes in gene expression of ionotropic glutamate receptor subunits in several brain regions. Moreover, chronic stress situations were accompanied mainly by alterations in mRNAs coding for subunits of AMPA receptor subtype (2). Prolonged exposure to stressors may induce changes in subunit composition of glutamate receptors with consequent changes in receptor functional characteristics. Long-term changes in gene expression may participate in both neuroprotection and negative consequences of repeated stress exposure.
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MULTI-LINEAGE DIFFERENTIATION POTENTIAL OF NEURAL STEM CELLS IN DEVELOPING EMBRYOID BODIES *J. Karbanová, J. Mokrý*, Department of Histology and Embryology, Medical Faculty, Charles University, Hradec Králové, Czech Republic.

In developing organism, neural stem cells (NSCs) that are of neuroectoderm origin, give rise to neurons, astrocytes and oligodendrocytes. When NSCs are cultured in vitro in medium supplemented with serum, they differentiate into β-III tubulin$^+$ neurons, GFAP$^+$ astrocytes and O4$^+$ oligodendrocytes. The aim of our study was to assess whether NSCs retain their ability to generate neural cells even if they are co-cultured with „totipotent" embryonic stem cells or whether they change their properties. Embryonic stem cells are derived from the inner cell mass of murine blastocysts and therefore they can give rise to almost all cell types. NSCs were isolated from mouse E14 foetuses, cultured in serum free medium supplemented with basic fibroblast growth factor and epidermal growth factor, and labelled with exogenous β-galactosidase by incorporating *lacZ* gene using retroviral transfection. As alternative source of NSCs, we used cells isolated from the subependymal zone of adult transgenic „green mice" expressing eGFP (1). We mixed NSCs with undifferentiated ES cells in equal ratios and allowed them to aggregate in hanging drops, differentiate and form embryoid bodies (EBs). Chimaeric EBs were yielded at different time points from culture and the presence of cells derived from labelled NSCs was visualised with laser scanning confocal microscopy or histochemically (using X-Gal as a substrate). Pictures acquired from confocal microscope showed that NSCs preferentially settled down in the surface endoderm. The labelled cells co-expressed α-fetoprotein, an endodermal marker. Some nucleated X-Gal$^+$ blood elements were present in vascular lumina. Other labelled cells were identified among mesenchymal elements inside of embryoid bodies. Our results indicate that NSCs, which are normally predetermined to produce neural elements, may change their fate and participate in haematopoiesis, in formation of endodermal and other cells. These findings demonstrate that the neural stem cells may reveal an enormous plasticity when they are exposed to a suitable microenvironment.
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REGULATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) EXPRESSION DURING IN VITRO - INDUCED NEURONAL DIFFERENTIATION OF MULTIPOTENTIAL STEM CELLS *Z. Katarova, M. Schwirtlich, E. Gocza, P. Varjú, G. Szabó,* Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

We have investigated the temporal and cellular patterns of expression of the different forms of glutamic acid decarboxylase (GAD), and its product γ-amminobutyric acid (GABA) during the course of retinoic acid (RA) - induced, *in vitro* neuronal differentiation of the embryonic stem (ES) cells and the clonally derived immortalized cell line of neuroectodermal origin NE-7C2 by semi-quantitative RT-PCR, Western blotting and immunocytochemistry. Depending on RA concentration, the pluripotent ES cells can give rise to multiple cell types, including GABAergic neurons *in vitro* (1). RA-treatment of mitotically active NE-7C2 results in the generation of different types of neurons and glia (2), thus these cells can be regarded as prototype of multipotential neuronal stem cells. Despite some similarities, we observed marked differences in the expression profiles of the GAD forms and GABA between the two systems. The synthesis of embryonic GAD forms (inactive GAD25 and enzymatically active GAD44) always preceded that of adult forms (GAD65 and GAD67). Adult GAD forms were found exclusively in cells with neuronal morphology, expressing neuronal specific markers. This highly reproducible order of induction of different GAD forms is reminiscent of their temporal regulation during mouse embryonic development (3). However, only undifferentiated ES expressed the enzymatically active GAD44 and GABA, undifferentiated NE-7C2 cells contained only the inactive GAD25. The timing of induction and subsequent up-regulation of the adult GAD65 and GAD67 showed clearly distinct patterns in the two systems, which suggests that the developmental potential of ES cells differs from that of NE-7C2. We have also found that other components of the GABAergic signaling- GABA transporters and GABA-A receptor subunits are also present in undifferentiated and RA-treated ES cells and show characteristic expression patterns in the course of neuronal differentiation. Our data strongly suggest, that *in vitro*-induced GABAergic differentiation of ES and NE-7C2 cells follows a clearly distinct pattern characteristic for each cell type.

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ASTROCYTIC ACTIVATION AND NEURODEGENERATION/ NEUROGENERATION *N. Katsube, N. Tateishi,* Discovery Research Laboratories III, Minase Research Institute, Ono Pharmaceutical Co., Ltd., Osaka, Japan.

Astrocytes are known to support the migration, maturation and survival of developing neurons. In the mature mammalian brain, astrocytes constitute nearly half of the total cells, providing structural, metabolic and trophic support of neurons. Active, rather than supportive, and pathophysiological roles for astrocytes in the adult CNS have been proposed quite recently. We have previously shown that GABA-evoked chloride response in cultured astrocytes gradually decreased and was associated with loss of $GABA_A$ receptors, while S-100 and glial fibrillary acidic protein (GFAP) contents increased, as the culture prolongation (1). We have also found that in cultured astrocytes, astrocytic function alters during culture accompanied with increase in S-100beta content, decrease in the expression of glia specific glutamate transporters, GLT-1 and GLAST mRNA, and so forth. Therefore we postulated that cultured astrocytes are considered to be activated astrocytes that are observed in various neurodegenerative disorders including acute stroke. In the present study, in order to further characterize neurodegenerative role of astrocytic activation, effect of ONO-2506, a novel astrocyte-modulating agent, on astrocyte-related parameter changes (cell survival and cell functions) both in vitro and in vivo were examined. Expression of GLT-1 mRNA was investigated in cultured astrocytes and in focal ischemic rat brain. We also examined the correlation between glutamate neurotoxicity and GLT-1 expression using neuron astrocyte co-cultute system. In both cultured astrocytes focal ischemic rat brain, the reduction of GLT-1 mRNA was observed. ONO-2506 suppressed the reduction of GLT-1 mRNA and ameliorated the neurodegeneration both in vitro and in vivo. Microdialysis study also revealed that delayed, long lasting increase in extracellular glutamate levels is also due to the reduction of astroglial glutamate transporters. ONO-2506 treatment prevented both extracellular glutamate levels and subsequent formation of infarction and neurological dysfunction. Taken together, astrocytic activation is the primary event, preceding the neuronal degeneration in various insults, and activation of astroglial cell (functional changes in astrocyte) may play a key role for the neurodegeneration/ neurogeneration in various neurodegenerative diseases.

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NEUROTROPIC XENOBIOTICS AND MELATONIN EFFECT ON REACTIVE OXYGEN SPECIES FORMATION AND CATECHOL-AMINES CONTENTS IN THE PREOPTIC AREA OF HYPOTHALAMUS IN RATS *G.O. Kerkeshko, V.M. Prokopenko, M.G. Stepanov, A.V. Korenevsky, A.V. Arutjunyan,* Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, Saint Petersburg, Russia.

A neurotoxic effect of many xenobiotics is accounted for by reactive oxygen species (ROS) formation. Hypothalamic structures responsible for reproduction regulation, such as the preoptic area (PA) of hypothalamus where gonadoliberinergic neurone's perikarya are located, were shown to be some of the most sensitive ones to neurotoxic xenobiotics influence. This makes actual a task of searching for compounds that show a neuroprotective effect and have no side effects when used for a long period of time. A pineal gland hormone melatonin known to possess antioxidant properties (1) was used as a putative neuroprotector. The aim of the study was to estimate ROS formation in PA of hypothalamus of female rats in control and under influence of neurotoxic xenobiotics and melatonin. The estimation was assessed with H_2O_2-luminol-dependent chemiluminescence. Along with it, catecholamines, that when oxidized are a source of ROS in brain, were measured with HPLC. In the control group diurnal changes in ROS formation level have been found to be opposite to the diurnal dynamics of catecholamines contents in PA. Chronic inhalation of neurotoxic xenobiotic toluene (50 and 500 mg/m^3) resulted in disturbances of diurnal changes of ROS formation level as well as of dopamine and norepinephrine contents. Single intraperitoneal administration of 1,2-dimethylhydrazine possessing neutrotropic properties (21 mg/kg) enhanced ROS formation and disturbed catecholamines contents diurnal changes. The experiments carried out have shown that continued administration of water solution of melatonin (10 μg/ml) to the animals enhanced ROS formation and disturbed catecholamines contents diurnal changes in PA, too. The data obtained are in accordance with recent publications stating that melatonin administered at pharmacological doses can possess prooxidant properties (2). Melatonin-caused elevation of ROS formation may be causing a disturbing mechanisms resposible for diurnal changes of catecholamines content.

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ATTENUATION COLCHICINE ACTIVATED FOS EXPRESSION IN THE RAT DEEP CEREBELLAR AND VESTIBULAR NUCLEI BY DEXAMETHASONE *A. Kiss, Z. Pirnik,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

Light microscopic avidin-biotin-peroxidase immunohistochemistry was used to find out whether dexamethasone pretreatment may affect the induction of Fos protein in cell nuclei at 4 levels (anterior, prefastigial, postfastigial, posterior) of the cerebellar-vestibular neuronal complex (CVC) 48 h after i.c.v. central administration of colchicine. Male Wistar rats were pretreated with dexamethasone (2.5 mg/kg/day, s.c.) three times prior and 24 after an intracerebroventricular delivery of colchicine (60 μg/ 10 μl). Animals were sacrificed 48 h after colchicine treatment. In colchicine treated animals, which exhibited a large number of Fos-positive cells over the entire CVC, the dexamethasone administration resulted in a substantial reduction in the number of the Fos-immunoreactive cells in each structure of the CVC. Distinct dexamethasone dependent reduction (80-90 %) of Fos-immunoreactivity was observed in each of the deep cerebellar nuclei. Less number of dexamethasone-sensitive cells was recognized in the vestibular structures. From the vestibular nuclei, the maximal Fos-inhibition by dexamethasone was recognized in the medial vestibular nucleus. However, either in this case the number of suppressed cells did not exceed 50 %. The results provide for the first time evidence about the dexamethasone dependent reduction of Fos-immunoreactivity in the cells of the CVC in response to stimulation elicited by colchicine. The data also indicate that the glucocorticoids might be involved in the regulation of some functions of the CVC under stress conditions.
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MITOCHONDRIA AND IMMEDIATE REPERFUSION DAMAGE: IMPLICATIONS FOR THE ROLE OF ACIDOSIS *T. Kristián[1], P. Bernardi[2], G. Fiskum[1]*, [1]Depatment of Anesthesiology, School of Medicine, University of Maryland, Baltimore, USA; [2]CNR Unit for the Study of Biomembranes and Department of Biomedical Sciences, University of Padova, Padova, Italy.

Although the hypothesis that the mitochondrial permeability transition (MPT) plays a significant role in ischemic brain damage is not new, there is no compelling evidence that the MPT is actually induced within the brain *in vivo* or that the MPT is a primary cause of mitochondrial dysfunction that causes neuronal cell death. In stark contrast to what has been reported earlier for isolated mitochondria studied under non-physiological conditions, our recent results demonstrate that acidic pH actually promotes the MPT in isolated brain mitochondria when tested under more physiologically-relevant conditions. In de-energized

mitochondria the MPT was inhibited at acidic pH. However, mitochondria energized either with complex-I or complex-II substrates displayed opposite behavior, i.e. acidic pH promoted rather than inhibited the MPT. Since acidic pH and high calcium and phosphate levels accompanied immediate reperfusion conditions in post-ischemic brain, we studied the mitochondrial morphology and integrity of mitochondrial membranes during the first hours of recovery. Rats were subjected to 10 min of forebrain ischemia. Following different reperfusion periods, their brains were removed and sub-regions of the hippocampus were dissected and fractionated into cytosolic and mitochondrial compartments. Another group of animals was used to obtain brain samples for the corresponding processing for electron microscopy. Electron microscopic examination revealed swollen mitochondria in pyramidal cells of the CA1 sector of the hippocampus at 10 min of reperfusion. However, one hour after the start of reperfusion the mitochondrial morphology seemed to be normal. Furthermore, there was a significant translocation of cytochrome c from the mitochondria to the cytosolic compartment within the first few minutes of reperfusion following the ischemic insult. It is not likely that the cytochrome c release was mediated by pro-apoptotic Bax protein since there was no increase in the level of mitochondrial Bax at this time of reperfusion. Taken together, these data strongly suggest that there is an MPT induced in the brain, particularly during immediate reperfusion when the tissue pH is acidic and intracellular calcium and phosphate concentrations are elevated.

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CATECHOLAMINE SYNTHESIZING ENZYMES AND THEIR MODULATION BY IMMOBILIZATION STRESS

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Corticotropin-releasing hormone is known as a crucial factor for catecholamine production and release in the organism. Corticotropin-releasing hormone deficient (CRH -/-) mice can serve as an interesting model for studying mechanisms involved in the response of a hypothalamic-pituitary-adrenal axis to different stressors. Thus, comparison of gene expression and protein level of catecholamine synthesizing enzymes in adrenal medulla of Sprague-Dawley rats, CRH +/+ and CRH -/- mice was of our special interest. Particularly, we focused on: 1. comparison of the adrenomedullary tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltransferase (PNMT) on the levels of gene expression and protein in Sprague-Dawley rats, CRH -/- mice and their CRH +/+ mates, 2. investigation of the changes in TH, DBH (dopamine-beta-hydroxylase) and PNMT gene expression and protein level in CRH +/+ mice after single and repeated immobilization stress. Levels of TH, DBH and PNMT mRNA were determined by RT-PCR and quantified relatively to the housekeeper. The amount of corresponding proteins was determined by Western blot analysis.

We detected a clear signal of 645 bp for TH mRNA and of 260 bp for PNMT mRNA in adrenal medulla of Sprague-Dawley rats and CRH +/+ mice, with higher concentration in rats. The amount of TH and PNMT protein in rats was significantly higher compared to mice. On the other hand, we found significantly lower concentration of TH/PNMT protein in CRH -/- mice compared to CRH +/+ mates. After the single immobilization stress exposure we revealed no significant changes in adrenomedullary TH, DBH and PNMT gene expression and protein. However, all these investigated parameters were significantly increased after repeated immobilization stress exposure. Our results indicate differences not only among species, but also between CRH -/- mice and their CRH +/+ mates, which may reflect differences in the transcriptional regulation of these enzymes in adrenal medulla of rats and mice. We also demonstrate that repeated immobilization stress exposure increased TH, DBH and PNMT gene expression and protein level in adrenal medulla of mice similarly to rats.

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CHANGES OF NADPH DIAPHORASE ACTIVITY IN THE RETICULAR FORMATION OF THE MEDULLA OBLONGATA FOLLOWING SPINAL CORD HEMISECTION AT CERVICAL LEVELS

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Specific population of neurons containing reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) in the reticular formation (RF) produces a unique neurotransmitter, nitric oxide. In order to investigate the changes of NADPHd activity in neurons of the reticular formation in the rabbit an experimental model consisting in the hemisection of the spinal cord at cervical (C4-C6) levels was used. The sections of the experimental (n=8) and control (n=5) animals were treated in the solution containing 0.25 mg/ml of β-NADPH and 0.5 mg/ml of nitroblue tetrazolium for 3 hours at 37 °C. On 7th postsurgery day, a statistically significant increase of somatic and fiber-like (i. e. dendritic or axonal) NADPHd activity was found in the medullary midline structures, on both sides of the caudal and also rostral ventromedial two-thirds and in the caudal dorsolateral one-third of the RF in the medulla oblongata. The increase of NADPHd exhibiting neuronal activity in autonomic nuclei regulating respiratory (1), cardiovascular depressor (2) as well as pressor (3) reflexes might to be one possible explanation for autonomic hyperreflexia consisting in changes of breathing (4), orthostatic hypotention (5) and paroxysmal hypertention (6) following damage of the spinal cord at cervical level.

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LOCALIZATION OF AN EPILEPTOGENIC ZONE BY ^{1}H MAGNETIC RESONANCE SPECTROSCOPY

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Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy. In many cases, if the seizures cannot be controlled by antiepileptic drugs, surgical intervention may be necessary. The usual lesion causing TLE is hippocampal sclerosis which is sometimes difficult to detect by MRI (about 20% of TLE patients have normal MRI). However, the ratio of signal intensities of N-acetylaspartate (NAA) to those of creatine (Cr) and choline (Cho) was found to be reduced in atrophic hippocampus, as well as in the hippocampus with apparently no atrophy but with abnormal EEG features. Localized proton MR spectra were measured in a series of patients with TLE on a 1.5 Tesla Siemens Helicon scanner. The 8 cm^3 volumes of interest were selected in right and left temporal lobes covering at least parts of hippocampi. The echo time and the repetition time were 136 ms and 1.6 s, respectively. After collecting 256 scans, the data were zero filled to 2K, Fourier transformed and phase corrected. In most spectra, spline baseline correction was applied. Finally, the NAA/(Cr+Cho) ratios were calculated. It was found that a substantial part of the patients (53%) had the NAA/(Cr+Ch) ratio unilaterally reduced below 0.72 which is considered to be a sign of an epileptogenic focus. In one patient among them, bilateral reduction has been observed. In the rest of the patients, either normal (29%) or slightly reduced (18%) ratios was observed. In summary, the presented MR spectroscopic technique may help in localization of epileptogenic zones in TLE patients when MRI gives ambiguous results.

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ROSTRO-CAUDAL AND LAMINAR DISTRIBUTION OF NITRIC OXIDE SYNTHASE ACTIVITY IN THE GRAY AND WHITE MATTER OF THE SPINAL CORD *N. Lukáčová, D. Čížková, J. Pavel, J. Maršala,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Nitric oxide (NO) has been recognized as an important neuromodulator and neurotransmitter in the CNS. In this study catalytic NOS activity measured by radioassay, neuronal NOS immunoreactivity (NOS-IR) and NADPH diaphorase positivity was studied in dorsal horns (DH), intermediate zone (IZ), ventral horns (VH), dorsal, lateral and ventral columns (DC, LC and VC) in cervical, thoracic, lumbar and sacral segments of the spinal cord of the rabbit and dog. Catalytic NOS activity was unequally distributed along the rostrocaudal axis of the spinal cord with a comparatively low value in the thoracic but high in the lumbosacral segments. Similarly, disproportional distribution of both neuronal categories, with a significantly higher number of NADPHd-exhibiting rather than neuronal NOS-immunoreactive somata was found in the above mentioned segments. Comparing the gray matter regions, extremely high enzyme activity was noted in the DH of all segments studied in the rabbit and in cervical and lumbar segments of the dog. Due to the location of NADPHd-positivity and NOS-IR in laminae I-II, pericentral region and intermediolateral cell column of the thoracic and lumbar segments and high enzyme activity in IZ along the rostro-caudal axis of the spinal cord, NO is thought to regulate autonomic tone and sensory transduction. The majority of NADPHd-exhibiting axons identified in the DC of the white matter was concentrated in the deep portion of the gracile fascicle and along the cuneate fascicle in all segments studied. The highest enzyme activity was found in the DC of cervicothoracic segments, mainly in segment C8. Numerous, mostly thin NADPHd-positive axonal profiles were detected in the LC, but no positivity was found in the area consistent with the location of the dorsal spinocerebellar tract. Among spinal cord segments, extremely high enzyme activity was seen in LC of lumbar segments. NADPHd-exhibiting bundles containing thick axons of sulcomarginal fasciculus were found in the VC in all cervical and upper thoracic segments. A long propriospinal bundle showing prominent NADPHd positivity was localized in the VC throughout the lower thoracic and upper lumbar segments. Our results clearly demonstrate the presence of NOS in the gray and white matter of the spinal cord.

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BRAIN STEM ORIGIN OF DESCENDING PREMOTOR NITRIC OXIDE SYNTHASE IMMUNOREACTIVE PATHWAYS IN THE DOG *J. Maršala,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Almost half a century ago, Thomas and Pearse (1) demonstrated that NADPHd-exhibiting neurons are resistant to ischemia or anoxia. In addition, immunocytochemical staining for nitric oxide synthase (NOS) revealed that NOS immunostaining is almost identical with histochemical staining for NADPHd. So far, the occurrence and extent of NOS-immunoreactive axons involved in the white matter in general, and in the premotor pathways in particular, is largely marginal. To begin the study of long descending premotor pathways of brainstem origin, we used the double-labelling technique (fluorescent retrograde tracer Fluorogold and NOS-immunocytochemistry) to identify the regions in the medulla, pons and midbrain that project NOS-immunoreactive axons to different spinal cord segments. Altogether nine adult mongrel dogs (n=9) divided into three subgroups were used in this study. The details of the surgical procedure and application of the tracer (Fluorogold) are given elsewhere (2). Shortly, small amount of Fluorogold was injected unilaterally in the white matter of the ventral and ventrolateral columns of C6-C7 segments (n=3), Th8-Th9 segments (n=3) and L6-L7 segments (n=3), respectively. After surviving for 7 days the animals were anesthetized and perfused transcardially as needed for NOS immunoprocessing. Transverse sections (24 μm thick) from brainstem and selected spinal cord segments were processed for NOS immunocytochemistry and Fluorogold fluorescent microscopy. Double-labelled (FG, NOS-IR) neurons were detected in the interstitial nucleus of Cajal of the midbrain, in the fifth and sixth layers of the superior colliculus in the midbrain tectum and in the ponto-bulbar reticular formation (n. reticularis pontis caudalis and oralis and n. reticularis gigantocellularis) after injections of FG at C6-C7, Th8-Th9 and L6-L7 levels. Contrary to this, double-labelled neurons could be detected in the medial vestibular nucleus only after lower cervical injections of FG. In summary, our findings provide strong evidence that interstitiospinal, tectospinal and reticulospinal pathways contain a clearly detectable NOS-immunoreactive axonal components reaching distally to lower lumbar and sacral segments. In contrast to this, NOS-IR vestibulospinal pathway is traceable only to lower cervical and, to a lesser extent, into upper thoracic segments.

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ISCHEMIC PARAPLEGIA: IMPROVEMENT OF MOTOR FUNCTION AFTER SPINAL GRAFTING OF HUMAN hNT NEURONS OR NEURONAL PRECURSORS *M. Maršala*[1], O. *Kakinohana*[1], D. Čížková[2], [1]Department of Anesthesiology, University of California, San Diego, USA, [2]Institute of Neurobiology, Slovak Academy of Sciences, Slovak Republic.

Transient cessation of spinal cord blood flow (SCBF), the resulting spinal ischemia and subsequent loss of neurological function (spastic or flaccid paraplegia) represents serious complications associated with a transient aortic cross-clamp (used in repair of aortic aneurysm). In recent years, considerable interest has focused on the therapeutic potential of implanted neuronal progenitor cells after a variety of neurodegenerative insults including ischemia, trauma or neurotoxic lesions. In recent experiments we have employed a rat spinal ischemia model to evaluate the fate and therapeutical potency of spinally implanted neuronal progenitor cells (SNPs) or postmitotic human hNT neurons when implanted in rats with fully developed spastic paraplegia. In halothane anesthetized rats, spinal ischemia (10min) was induced by occluding the thoracic aorta. Seven days after ischemia, animals received grafts of hNT neurons or SNPs delivered in 30 separate injections targeting the gray matter of the L2-L5 segments. Animals were treated with FK-506 (1mg/kg/day) for the duration of the experiment. After grafting, the recovery of motor function (BBB scoring system) and motor evoked potentials (MEP) were assessed for 3 months. Animals were then perfused with 4% paraformaldehyde and the presence and phenotype of implanted cells was analyzed. Animals which received hNT or SNPs grafts showed a significant relief of spasticity and improved motor function (BBB: hNT:1→8; SNPs 1.5→8.5) when compared to controls. More rapid recovery was observed after grafting SNP (2 weeks) than was seen after grafting of hNT neurons (8 weeks). A positive correlation between the recovery of motor function and MEP was also seen. Confocal analysis revealed a rapid maturation of hNT and SNPs cells as evidenced by the expression of markers typical for mature neurons (NSE, NeuN, MAP2 or axonal HO14). A number of GABA positive neurons and their axons localized in the vicinity of persisting α-motoneurons were also identified. These data show that spinal grafting of hNT neurons or SNPs have a potential to ameliorate motor dysfunction after spinal ischemia and may represent an effective treatment modality in managing spasticity states after ischemic spinal cord injury.

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CHANGES IN THE ROSTRAL MIGRATORY STREAM AND SUBVENTRICULAR ZONE DURING POSTNATAL DEVELOPMENT IN RAT: ANATOMY AND IMMUNOHISTOCHEMISTRY *M. Martončíková, E. Račeková, J. Orendáčová, B. Poušová,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

For over thirty years it has been known that lateral walls of the forebrain lateral ventricles contain stem cells that give rise to neurons and glia throughout life (1). The cells that give rise to new olfactory neurons originate in the subventricular zone (SVZ) and migrate tangentially along the rostral migratory stream (RMS) to reach the olfactory bulb (OB). Within the OB these young neurons leave the RMS and migrate into the granule and periglomerular layers, where they differentiate into local interneurons. While neurogenesis has been extensively studied in the adult rodents no such data could be found about development of the SVZ and RMS in the developing brain during early postnatal period. The aim of this study was to analyze and compare anatomical, histochemical and immunohistochemical changes in the SVZ and RMS of P3, P7, P14, P21 and P28 rats. Brain sections of rat pups stained with Gill's hematoxylin were used to obtain general anatomy and morphology of the postnatal forebrain structures associated with neurogenesis. In order to reveal spatial-temporal changes in the distribution of the neuronal progenitor cells in the SVZ and RMS the pups received a single injection of cell proliferation marker - BrdU. Nitroergic neurons were identified by NADPH-diaphorase staining. Immunohistochemistry for BrdU showed that the number of neuronal progenitors are dependent on postnatal stages. NADPH-d-positive neurons were first observed in the SVZ and RMS at P14.

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MODIFICATION THE NUMBER OF IB4 AND NF200 - NEURONS AFTER NERVE INJURY *R.F. Masgutov, I.S. Raginov, Y.A. Chelyshev,* Kazan State medical University, Department of Histology, Russia.

An expression of NF200 (A-fiber marker) and binding with isolectin B_4 (IB_4) was examined immunohistochemically in the L_4–L_5 dorsal root ganglia (DRG) after ligation or transection of the rat's sciatic nerve. NF200 immunoreactivity was detected in 15% of all neurons in intact ganglia. Ligation of sciatic nerve caused decrease in a number of NF200 positive neurons upon 7.7% on the 90^{th} day after nerve injury. At the same term after ligation but under the influence of pyrimidine derivative xymedon the number of survival NF200 -positive neurons increase by 50.7%. In intact sensory ganglia 23.6% of neurons showed positive reaction with IB_4. Of the DRG neurons 2.6% were labeled by IB_4 at 30^{th} day after ligation of the nerve. At 90^{th} day after ligation IB_4-positive neurons do not revealed in L_4–L_5 (DRG). Xymedon increased the survival of IB_4-positive neurons up to 8-fold at 90^{th} day after ligation. Comparison of ability to survival neurons has shown two investigated populations, that IB_4 positive neurons with the greater probability enter in posttraumatic apoptosis. After nerve ligation survives less NF200 positive and IB_4 positive, than at transection a nerve that allows considering lengthening axon as the factor supporting a survival neuron. Xymedon promoted the survival in both subpopulation of neurons and predominantly prevents apoptosis of IB_4^+ - binding neurons.

DENSITOMETRIC AND MORPHOMETRIC VALUATION OF NADPH-d POSITIVE NEURONS IN THE DOG FOREBRAIN COMPARATIVE ONTOGENIC STUDY *P. Maslej, M. Pomfy, K. Kuchárová[1],* Department of Histology and Embryology, Faculty of Medicine, P. J. Šafárik University, Košice, [1]Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The use of nicotinamide adenine dinucleotide phosphate diaphorase histochemistry alone or combined with nitric oxide synthase immunoreactivity (NOS-IR) allowed a morphologically distinct and topographically precise localization of specific selective neuronal pools. Nitric oxide (NO) is a neurotransmitter and/or neuromodulator involved in a variety of physiological functions within the central nervous system (1). We evaluated the densitometric and morphometric parameters (the intensity of density of perikaryon and length of perikaryon diameter) of NADPH-d positive neurons in the cerebral cortex and adjacent subcortical white matter, nucleus caudatus and hippocampus. Puppies 1, 6, 21 and 60 days old as well as a group of adult dogs were used in our study. A computer UTHSCSA Image Tool program was used to investigate the nitrergic neuronal types differing by their density and cell body size. In the cerebral cortex, the density of neuronal perikarya increased continuously in 1, 6, 21 and 60 days old puppies. In the nucleus caudatus, we observed a significant increase of density in 1 and 6 days old puppies. In the hippocampus, we detected differences in nitrergic neuronal groups of 6 days old puppies and adult dogs. In the subcortical white matter, the highest density of neurons was found in adult dogs. Depending on the age, we also observed an increase of neuronal size. Our observation revealed changes of density and size of NADPHd-expressing neurons what demonstrate development of nitrergic neurons in postnatal period.

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THE PROLIFERATION OF PRECURSOR CELLS IN THE SUBEPENDYMAL LAYER OF ADULT ANIMALS IS AFFECTED BY PARTIAL NECROSIS RANGE *Y. Mazurová, V. Valoušková[1],* Department of Histology and Embryology, Faculty of Medicine, Charles University, Hradec Králové, [1]Institute of Physiology, Academy of Sciences, Prague, Czech Republic.

The ibotenic acid (IA) lesion is used as a model of neurodegenerative process (partial necrosis - PN) in Huntington's disease (HD), which is, like in humans, followed by reparative gliosis. With respect to long-term development of HD, we are interested in the reaction of the subependymal layer (SEL) during a longer time frame. In our previous studies we have proved that the reaction of the SEL is non-specific, i.e. it is a response to the neurotoxic lesion as well as sham-lesion, and only slowly decreases in time. Here we compared the level of proliferative activity of precursor cells in the SEL, and their possible capability to migrate into the degenerated striatum and to participate in reparative gliosis in relation to the extent of the IA lesion (i.e. of PN). Two groups of male rats (Long-Evans strain, n = 14), which were sacrificed at 5 and 13 weeks after the induction of unilateral IA lesion, were compared. Induced neurodegenerative process usually affected approx. 2/3 of the caudoputamen with preservation of tissue in the vicinity of the lateral wall of brain ventricle. In 3 animals, the PN and the following reparative gliosis spread up to the immediate neighbourhood of the SEL. This close contact between the SEL and PN of the striatum represented more intensive "irritation" of the SEL followed by increased proliferation and differentiation of precursor cells, as well as by their migration, in comparison with the stage after standard IA lesion. The enlarged SEL (particularly in the upper part of lateral ventricles) was entirely filled with GFAP-positive astrocytes, while CNPase-positive oligodendrocytes were present only in the outer layer. No beta-III-tubulin- or MAP2-positive cells, i.e. neurones, were detected in the SEL. Numerous BrdU- or Ki67-positive precursor cells were also present. Nevertheless, the decrease of cell proliferation was

found at 13-week surviving animals in comparison with 5-week survivors using the standard IA lesion, there were no conspicuous differences between these two groups in case the lesion expanded up to the SEL. On the other hand, number of migrating cells in our experiment was essentially time-independent but the migrating rate was obviously higher if the SEL was in close contact with the degenerated striatum. In this instance only, the participation of newly generated cells in reparative gliosis was observed.
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DISTRIBUTION OF UBIQUITIN IMMUNOREACTIVITY IN THE RABBIT SPINAL CORD AFTER ISCHEMIA/REPERFUSION INJURY AND THE EFFECTS OF PRETREATMENT WITH TANAKAN *E. Mechírová, I. Domoráková, P. Jalč*[1], Department of Histology and Embryology, Faculty of Medicine, Šafárik University, Košice, [1]Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The effects of transient spinal cord ischemia/reperfusion on ubiquitin antibody immunohistochemical staining (1) were investigated in the segment L_5 in the rabbit. 24 hours following 30 minutes occlusion of aorta below the left renal artery and 7 days Tanakan (Ginkgo biloba extract EGb 761) pretreatment, high ubiquitin immunoreactivity was present in the neurons situated in lamina IX of the ventral horns. The light cytoplasm of large neurons was filled up with dark-brown, ubiquitin positive granules that represent newly synthetized ubiquitin. The number of ubiquitin positive neurons in the intermediate zone was increased. The neurons in the dorsal horns did not show changes neither in number nor in colour. Our results indicate that there are changes in ubiquitin response to an ischemic/reperfusion insult, mainly in the most vulnerable neurons in the rabbit spinal cord. Tanakan, administered 7 days before ischemia/reperfusion is a scavenger of free radicals, improves the clinical signs – no paraplegia of hind limbs – and regulates the synthesis of stress protein ubiquitin (2).
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DIAZEPAM ADDS TO ANTINEOPLASTIC POTENTIAL OF HYPERICIN IN HUMAN GLIOMA CELLS *L. Mirossay, M. Šarišský, I. Šulla*[1]*, A. Mirossay, P. Miškovský*[2]*, J. Mojžiš,* Department of Pharmacology, [1]Department of Neurosurgery, P. J. Šafárik University, Faculty of Medicine, [2] Department of Biophysics, P. J. Šafárik University, Faculty of Science, Košice, Slovak Republic.

Hypericin (Hyp), a polycyclic aromatic naphtobianthrone displays antitumor and antiviral activities that are strongly enhanced by light. The physico-chemical mechanism of Hyp anticancer activity is still not fully understood. Diazepam is a non-selective ligand of peripheral benzodiazepine receptors (PBR). Higher PBR density in tumoral versus normal brain tissue has been reported and correlated with the grade of malignancy, cell proliferation and glioma patients prognosis. In U-87 MG glioma cells chemotherapy-potentiating and antiproliferative effects of diazepam have been described. In this report, the significance of diazepam potentiating effect for Hyp anticancer activity in the human primary glioma cell cultures, was investigated. Tumoral tissue specimens of 12 gliomas (8 patients with glioblastoma multiforme WHO gr. IV, 2 with anaplastic astrocytoma WHO gr. III, and 2 with oligodendroglioma) were obtained peroperatively. Cytotoxicity of Hyp and diazepam alone or in combination was tested by using the MTT assay. The results show that diazepam causes a marked potentiation of Hyp phototoxicity in primary human glioma cell cultures.

ANGIOGENESIS INDUCED BY NEURAL GRAFTING IS ASSOCIATED WITH NESTIN EXPRESSION IN ENDOTHELIAL CELLS *J. Mokrý, S. Němeček, D. Šubrtová, D. Duspivová, J. Karbanová,* Department of Histology and Embryology, Charles University Medical Faculty, Hradec Králové, Czech Republic.

Protein nestin originally identified in neuroepithelial stem cells is expressed by other cell types, e.g. developing cardiomyocytes and myotubes. Our previous findings (1) revealed that nestin expression occurred in vascular endothelial cells in the course of angiogenesis of most developing organs, intraembryonic connective tissue and extraembryonic structures. As the blood vessels mature, intermediate filament nestin is gradually replaced with vimentin. To find out whether vascular nestin expression reappears in blood vessels of adult mammals, we induced angiogenesis in the rat brain by the intracerebral grafting of i) solid pieces of E14 foetal forebrain, ii) solid neurospheres received from cultivation of foetal neural stem cells or iii) dissociated C6 glioma cells (2). At different survival periods, the animals were perfused with formalin, and the transplanted brains were processed for anti-nestin immunohistochemistry (using monoclonal antibody Rat-401, DSHB, Iowa or polyclonal antibody #4350 kindly provided by Prof. U. Lendahl from Karolinska Institute, Stockholm) in paraffin-embedded sections. As controls we processed the tissue obtained from intact rat brains, from biopsies of human brain tumours and peripheral organs for anti-nestin immunohistochemistry. In all experimental groups, we observed widespread immunoreactivity for nestin in capillaries that grew in the grafted tissue. Three to four weeks after transplantation of foetal grafts or neurospheres (when nestin expression ceased in differentiating neural elements), nestin was expressed in newly formed blood vessels nourishing the transplants. In distant and intact areas of the host brain, only sporadic nestin positive endothelial cells were observed (likely reflecting physiological turnover). In animals injected with glioma cells, nestin positivity was confined to neoplastic cells, reactive astrocytes and capillaries nourishing the tumour. In intact rodent brains, we could identify immunoreactivity in sporadic endothelial cells only. However in specimens derived from brain tumours, ingrowing blood vessels expressed a strong specific signal. In non-neural vascularized mature tissues, nestin expression was usually low and confined to few endothelial cells. Nestin-positive blood vessels were identified in the *corpus luteum* of the ovary. Our data indicate that nestin expression is associated with the process of angiogenesis. The work was supported by a grant No.6727-3/01 from IGA MZ.

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NEURAL STEM CELLS GROWN IN VITRO: A VERSATILE SOURCE OF MULTIPLE CELL TYPES *J. Mokrý, J. Karbanová, S. Filip*[1]*, J. Österreicher*[2]*, D. Šubrtová, D. Duspivová, L. Kotingová,* Department of Histology and Embryology, Charles University Medical Faculty, Hradec Králové, [1]Radiotherapeutic Clinic, Teaching Hospital, Hradec Králové, [2]Department of Radiobiology and Immunology, Purkinje Military Medical Academy, Hradec Králové, Czech Republic.

Differentiation potential of neural stem cells (NSCs) isolated from E14-15 mouse brains was assessed in the following experiments: 1) Spontaneous differentiation potential was evaluated in the differentiation assay or 2) by grafting neurospheres in the intact brains of histocompatible recipients. The full developmental potential was revealed by the following approaches: 3) NSCs were allowed to differentiate in the in vitro assay at densities 60000 cells per cm^2, 4) by cocultivation with ES-D3 cells in hanging drops or 5) by i.v. transplantation of dissociated NSCs in sublethally irradiated mice. The transplanted cells were tagged with *lacZ* or *eGFP* prior grafting to permit an unambiguous identification of donor cells. Their phenotypes were identified with the use of fluorescent immunocytochemistry or enzyme immunohistochemistry. Haematopoietic potential of cells

yielded from the bone marrow and spleen of irradiated animals was assessed using CFU GM colonies. In in vitro differentiation assays, NSCs produced β-III tubulin$^+$ neurons, GFAP$^+$ astrocytes, O4$^+$ oligodendrocytes and ciliated ependymocytes. Transplants derived from neural grafting of solid neurospheres contained neuronal cells with typical dendritic ramification, the neuropil was rich in synaptophysin immunoreactivity and astroglial cells established a complex three-dimensional network with endfeet contacting nourishing blood vessels. Ependymal cells were not observed likely due to the absence of signals required for initiation of ependymogenesis in the adult brain. Neurospheres that differentiated in vitro at low cell densities produced cells expressing muscle cell-specific markers desmin and SMA. In chimaeric embryoid bodies resulting from cocultivation experiments, NSCs participated in formation of endodermal, haematopoietic and other cellular elements (see our accompanying poster). Analysis and comparison of numbers of CFU GM colonies harvested from grafted irradiated animals with the group of non-treated irradiated animals confirmed that administration of NSCs supported haematopoiesis. Collectively our data confirm that NSCs under normal conditions behave as quadripotent stem cells and may be re-programmed to produce non-neural cell types.

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POSSIBLE MECHANISMS OF NEUROPROTECTIVE EFFECTS OF THE EXTRACT OF GINKGO BILOBA (EGB 761) *F. Monček, M. Kriška[1], M Laššanová[1], R. Dunčko, D. Ježová,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The standardized extract of Ginkgo biloba (EGb 761) is known to have antioxidant and neuroprotective properties, however the mechanisms underlying these effects have not been fully determined. Stress-induced hormonal and sympathetic activation can contribute to hippocampal neurodegeneration, which can result in dementia. The present study was aimed to evaluate the effects of EGb 761 on salivary cortisol and blood pressure responses during stress in healthy young volunteers (n=70) in a double blind placebo controlled design. A stress model involving a combination of static exercise (handgrip) and mental stimuli was used. Single treatment with EGb 761 (120 mg) reduced stress-induced rise in blood pressure without affecting the heart rate. Salivary cortisol responses showed differences with respect to the gender and the time of day of the stress exposure, with the activation only in male subjects in the afternoon. This activation was absent if they were treated with EGb 761. The performance in a short memory test with higher scores achieved by women remained unaffected by EGb 761 treatment. Thus, this study provides evidence that EGb 761 has an inhibitory action on blood pressure and it may influence cortisol release in response to some stress stimuli. These effects may play a role in neuroprotective action of EGb 761.

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POSTHYPOXIC EFFECTS OF HYPOTHERMIA ON BRAIN CORTEX METABOLISM IN EARLY ONTOGENY *J. Mourek, L. Šmídová, V. Šlapetová, J. Koudelová,* Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Many times in the past, the possible protective effect of hypothermia against any lack of oxygen was examined. The results were only partially successful and the methods were very often considered as doubtful. Several papers at present time indicate the positive effect of mild hypothermia, applied in the hypoxic newborns on their brain cortex only. Nevertheless the decrease of body temperature as a consequence of hypoxia in very young and immature animals could be interpreted as a very natural and physiological reaction which ensure their survival (1,2).

In our laboratory two series of experiments were performed in which the effect of hypothermic milieu on some metabolic parameters of brain cortex homogenates (14-day-old rats) was examined. The 14-day-old rats seem to be the most adequate to be compared with human newborn at term. Incubation for 30 minutes in three variously temperatured media (38°C – control, 30°C and 22°C – hypothermia) were realised in the first serie. In the second serie, the rats just before being killed, they were exposed to strong hypobaric hypoxia, corresponding 9000 m and lasting 30 minutes (pO_2 = 6.4 kPa). Results:

The effect of lower temperature in distinctly visible and mostly highly significant (lowered metabolic rate). As interesting finding we point out the massive decrease of lipoperoxidative activities and also the stability of pH. Both could be interpreted as a positive and perspective results.

In rats previously stressed by strong hypobaric hypoxia the obtained data with hypothermic conditions are giving a confused picture. The strong lack of oxygen is changing the brain cortex metabolism in such extent that the hypothemia remains without supposed effects.

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DOES DEAFFERENTATION OF BRAIN A11 DOPAMINERGIC CELL GROUP AFFECT STRESS-INDUCED INCREASE IN PLASMA CATECHOLAMINES? *B. Mravec, I. Bodnar, L. Kubovčáková, M. Palkovits[1], R. Kvetňanský,* Institute of Experimental Endocrinology, SAS, Bratislava, Slovak Republic, [1]Department of Anatomy, Semmelweis University, Budapest, Hungary.

Dopaminergic cells of the area A11 are situated in the dorso-caudal hypothalamus. Descending dopaminergic fibers project from the A11 cell group to the whole spinal cord. These dopaminergic fibers innervate dorsal horn (DH), the peri-ependymal region, ventral horn and the intermediolateral cell column (IML). IML cell column contains spinal preganglionic neurons, which innervate sympathetic ganglia and the adrenal medulla. The aim of this work was to investigate influence of the A11 dopaminergic cell group on plasma catecholamine levels during immobilization stress. Plasma epinephrine (EPI) and norepinephrine (NE) levels were measured in animals with deafferentation of A11 cell group and in sham operated animals at the rest conditions and during exposure to immobilization. We also determined dopamine, epinephrine and norepinephrine concentrations in A11 region, DH and IML cell column of the spinal cord. Blood was collected via chronically indwelling cannula in the tail artery. A11 cell group, DH and IML cell column were isolated using microdisection technique. A11 group deafferentation did not produce any significant changes in plasma catecholamine levels before and during immobilization compared to sham-operated animals. Deafferentation of A11 cell group increased dopamine concentration in A11 area of control and stressed animals. This finding is in accordance with the reduced DA transport from this area suggesting a success of the deafferentation procedure. DA concentration in the IML cell column and DH did not show the expected DA disappearance as a consequence of A11 deafferentation. In opposite, increase in DA concentration was found in these areas. Thus, the source of DA in IML cell column and DH of the spinal cord is not in the A11 cell group area, but of some other origin. Presented data suggest that dopamine from the A11 region does not significantly regulate sympatoadrenal system activity during immobilization stress.

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EXPRESSION OF SELECTED PROTEINS INVOLVED IN THE CA^{2+} HOMEOSTASIS AFTER ISCHEMIA-REPERFUSION INJURY *R. Murín[1,2], E. Babušíková[1], D. Dobrota[1], P. Kaplan[1], O. Križanová[3], J. Lehotský,* [1]Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic, [2]Physiologish-chemisches Institut, Universitat Tuebingen, Tuebingen, Germany, [3]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The lost of oxygen and glucose during brain ischemia initiates a great many intracellular ion and metabolite changes. These include decrease of ATP, decrease pH, and increase cytosolic Ca^{2+} and free radical overproduction. These changes have influence on gene expression; protein activity and finely they can lead to cell death. Some of these changes are recovering after upset of the blood recirculation, but others, including disturbance in Ca^{2+} are lasting during time of reperfusion. To evaluate possible influence of ischemic insult on expression of calcium homeostasis regulating proteins we measured relative levels of mRNA for alfa-1-A subunit of voltage-gated Ca^{2+} channel, Na^{+}-Ca^{2+} exchanger and inositol 1,4,5-triphosphate receptors of the type 1 and 2 (IPR–1, IPR–2). Wistar rats (5 mounts old, males) used for experiments were divided into five groups: control; with 15-minute global ischemia; and with 2 or 24 or 48 hour reperfusion after ischemia. Global ischemia was induced by 4-vessel occlusion model under complete anaesthesia. Body temperature of animals was measured and maintained at physiological level. From all forebrain mRNA was isolated and quantitative RT PCR was used to investigate possible changes in levels of mRNA for alfa-1-A subunit of voltage-gated Ca^{2+} channel, Na^{+}-Ca^{2+} exchanger and IPR–1, IPR–2. PCR products were analysed on 2% agarose gels, intensity of bands was measured and divided by the intensity of mRNA for GAPDH in each sample. Control group was sham operated. The intensity of mRNA signal for alfa-1-A subunit of voltage-gated Ca^{2+} channel decreased in all measured groups compared with control. mRNA for Na^{+}-Ca^{2+}- exchanger temporally decreased in groups with ischemia and 2 h reperfusion, and increased slightly over the control level after 24 and 48 hour reperfusion. Weak decrease in both values of the mRNA for IPR-1 and IPR-2 were observed in groups with ischemia and 2-hour reperfusion. Our results show, that ischemic insult induces the changes in calcium homeostasis regulating proteins in level of their mRNAs. Contribution of these changes to disturbance of calcium homeostasis after brain ischemia remains to be evaluated on the protein levels and their activity.
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ISCHEMIC PRECONDITIONING AND C-FOS IMMUNOREACTIVITY IN THE CA1 PYRAMIDAL LAYER OF THE POSTISCHEMIC RAT BRAIN HIPPOCAMPUS *M. Némethová, V. Danielisová, J. Burda,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

A large amount of injuries, including alterations in gene expression were caused by the cerebral ischemia, especially during reperfusion period. The expression of immediately early genes (like c-fos or c-jun) occurs in rodents following 1 - 90 min of cerebral ischemia (1) and their immunoreactive products are generally useful markers for mapping neural pathways or severity of damage. In this study we compared c-Fos immunoreactivity in the rat brain hippocampal CA1 region of pyramidal neurons after ischemia and following reperfusion using ischemic preconditioning. Rats were submitted to the standard 4-vessel-occlusion (2). The pretreatment was performed by inducing brief transient ischemia lasting 5 minutes. This short ischemic insult was followed by the recirculation 1 (IP1), 2 (IP2) or 3 (IP3) days, respectively. One group of rats was sham operated (SHC) as control animals. The more prolonged cerebral ischemia (30 min) was performed subsequently with following 2 hours of reperfusion. Brains of experimental animals were cut by the vibratome and 40 μm sections were used for immunohistochemistry floating processing with primary antibody anti-c-Fos (Calbiochem, PC 38) and ABC Vectastain complex. Positive labelled nuclei were dyed by diaminobenzidine, scanned by digital camera and analysed by ImageTool PC software equipment. Results were calculated in 1 mm^2 of hippocampal area and were expressed as percentual amount of dark colour in the black and white digital images. The very small differences among these experimental groups (IP1 = 2.31±0.27%, IP2 = 2.38±0.19%, IP3 = 2.05±0.19%, SHC = 2.77±0.21%) were reported. The statistical analysis and comparison did not show any important significancies. It is possible to suppose that the time interval of recirculation after ischemic preconditioning does not effect the number of c-Fos positive nuclei expressed after subsequent 30 min cerebral ischemia and following 2 hours of reperfusion. However, the decrease of c-Fos immunoreactivity in experimental animals compared with sham-operated animals indicates probable positive effect of the ischemic preconditioning.
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ISCHEMIC PRECONDITIONING DOES NOT IMPROVE THE OUTCOME AFTER SPINAL CORD CONTUSION INJURY IN THE RAT *T. Ondrejčák, I. Vanický, J. Gálik,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

It has been generally accepted that the pathophysiological mechanisms of traumatic spinal cord injury (SCI) involve both primary and secondary events. Secondary mechanisms seem to cause significant extension of the primary lesion into the white matter of the spinal cord and may be thus crucial for the final neurological outcome after SCI. Ischemic preconditioning (IPC) has been defined as the endogenous cellular protective mechanism evoked by brief ischemic periods. IPC renders a tissue of central nervous system more resistant to a subsequent lethal ischemic insult (1,2). In addition, similar protective effect of IPC has been observed after experimental traumatic brain injury (3). Spinal cord trauma differs from cerebral trauma in that the secondary processes act primarily on the white matter. In the present study we tested the hypothesis that a transient non-lethal ischemic insult would protect against subsequent traumatic spinal cord injury. 18 male Wistar rats were randomized into two groups. In the IPC group, 5 minutes spinal cord ischemia has been induced by aortic occlusion (4). 48 hours after IPC, moderate spinal cord injury has been induced by epidural balloon inflation at T8 level (5). In the control group, identical surgical procedures and SCI were performed except from IPC. During survival period, locomotor performance of all rats was regularly tested (BBB test), and after 4 weeks, they were perfusion-fixed for histopathology. Morphometric analyses were performed in order to quantify the extent of the spinal cord lesion. All animals were completely paraplegic after SCI, and showed partial neurological recovery during their survival period. No significant differences in BBB scoring were observed during the 4 weeks survival. Morphometry showed no differences in the spared grey matter, but there was significantly more spared white matter tissue in the control group ($p=0.01$). These data indicate that in contrary to cerebral trauma, IPC does not improve the outcome after SCI, and even worsens the damage to the white matter.
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EARLY SOCIAL ISOLATION INCREASES NITRIC OXIDE SYNTHASE ACTIVITY IN BRAIN REGIONS OF RODENTS *M.V. Onufriev[1], A.P. Bolshakov[1], N.M. Khonicheva[2], M.G. Ayrapetyanz[2], K. Braun[3], N.V. Gulyaeva[1]*, [1]Department of Functional Biochemistry of the Nervous System, [2]Group of Experimental Pathology and Therapeutics of Higher Nervous Activity, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia; [3]Magdeburg University, Magdeburg, Germany.

A direct measurement of nitric oxide synthase (NOS) activity as well as the evaluation of thiol levels were carried out in brain regions of rats subjected to the early social isolation. Isolation induced an increase of anxiety level in rats comparing with the rats reared in normal conditions. NOS activity was increased in hippocampus immediately after the isolation and in cerebral cortex and hippocampus 10 weeks after the isolation. Isolation did not affect the levels of non-protein thiols (glutathione) in brain regions. Thus, modulation of NOS activity was not related to changes of free radical status of the brain of isolated animals. In cerebral cortex and hippocampus of Octodon degu, early social isolation also induced an increase of NOS activity. NOS activation, revealed both immediately after the isolation stress and delayed, is suggested to mediate long lasting behavioral disturbances induced by social isolation in rodents.

EPENDYMAL REGION OF RAT SPINAL CORD AFTER ISCHEMIA AND ISCHEMIC PRECONDITIONING: AN IMMUNOHISTO-CHEMICAL STUDY *J. Orendáčová, E. Račeková, T. Ondrejčák, B. Poušová, M. Martončíková,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Our laboratory began to study regularities of rat ependymal cells transformation in health and pathology. Normal spinal cord ependyma showed limited proliferative activity and no immunoreactivity to nestin, a marker for neural precursor cells. In contrast, the spinal cord injured by clip compression demonstrated a dramatic increase in ependymal proliferation and strong nestin expression in the ependyma (1, 2). Re-expression of nestin has been observed also in astrocytes located at or close to the ischemic lesion (3). To induce spinal cord ischemia, the Fogarty 2F catheter was inserted into the descending thoracal aorta 10.5 cm retrograde from the left femoral artery. During the occlusion the catheter was inflated with saline solution for 12 min; continuous monitoring of mean distal aortic blood pressure and mean proximal aortic pressure were provided. In the preconditioned rats, 3-min ischemia and 30 min recirculation preceded the 12 min ischemia. The animals were subjected to neurologic testing before perfusion fixation. Proliferating and stem cells were immunohistochemically visualized on cryocut sections. The ischemic and preconditioned rats exhibited characteristic nestin immunoreactivity in vessel wall of lesioned and non-lesioned regions of spinal cord. Ischemia was characterized by strong nestin positivity of ependymal cells and astrocytes of lumbar and sacral spinal cord segments. The same ischemic injury in the preconditioned animals did not induce any specific immunohistochemical labelling. Our results are in accordance with finding that injured nervous tissue induces expression of developmental proteins. The results show beneficial effect of a very short ischemic preconditioning period on spinal cord ischemic injury.
Supported by VEGA Grants: 2/2082/22 and 2/7235/20.

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THE EFFECT OF ISCHEMIA AND SHORT- AND LONG-LASTING REPERFUSION ON THE CATALYTIC NOS ACTIVITY IN THE GRAY MATTER REGIONS OF THE RABBIT SPINAL CORD *J. Pavel, M. Silvašiová, N. Lukáčová, J. Maršala,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The latest research reveals that continually released NO may act as a mediator providing a basal vasodilator tone. In these experiments, the changes in catalytic activity of constitutive NOS isoforms were studied under threshold ischemia and short- and long-lasting intervals of reperfusion in the gray matter regions of the spinal cord. The results were compared with the neurological impairment. All experimental animals were divided into five experimental groups: (1) control (n=9), (2) animals subjected to 12.5 min ischemia and short (1h and 3h) intervals of survival (n=10), (3) animals subjected to 12.5 min ischemia and longer (24h and 4 days) intervals of survival (n=10). Temporary spinal cord ischemia was induced by 12.5 min of infrarenal balloon occlusion of the abdominal aorta. The catalytic NOS activity was determined by conversion of [^{14}C] - L - arginine to [^{14}C] - L - citrulline according to the method of Bredt and Snyder (1). Neurologic function was evaluated according to Tarlov´s criteria at the end of respective survivals. Threshold - ischemia with 1h of survival evoked a slight decrease in catalytic NOS activity under control values in all gray matter regions. More marked decrease of enzyme activity was detected after 12.5 min ischemia and 3h of survival, but statistical significance was found only in the intermediate zone (by 38%) and ventral horns (by 53%). On the other hand, 12.5 min ischemia and 24h of survival induced the upgrade of the catalytic activity in above mentioned gray matter regions, when the results were compared to ischemia and 3h of survival, but significant changes were detected only in the dorsal (by 32%) and ventral horns (by 23%). However, a small decrease of catalytic NOS activity was found again after 12.5 min ischemia and 4 days of survival. The results have shown that catalytic activity of constitutive NOS isoforms detected in gray matter spinal cord regions after 12.5 min ischemia with 1h, 3h and 4 days of survival has predominantly a downward character, but longer (24h) survival evoke the temporary rise of catalytic activity. The outcomes correlate with the neurological hindlimbs impairment of experimental animals.
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PENTYLENETETRAZOLE KINDLING INDUCES OXIDATIVE STRESS AND CASPASE-3 ACTIVATION IN RAT BRAIN *T.V. Pavlova, A.A. Yakovlev, M.Y. Stepanichev, I.I. Zarubenko, N.V. Gulyaeva,* Department of Functional Biochemistry of the Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia.

Acute and chronic (kindling) administration of pentylenetetrazole (PTZ) to rats (of 37.5 mg/kg i.p.) resulted in the oxidative stress in hippocampus and cerebellum. The degree of oxidative stress (accumulation of material reacting with 2-thiobarbituric acid, thiol content) depended on the mode of PTZ administration (acute, chronic), time after the last PTZ injection, and brain region studied. PTZ kindling (but not a single PTZ injection) induced caspase-3 activation in cerebral cortex, hippocampus, and cerebellum of rats as well as neurodegeneration in hippocampus. The number of neurons in CA3 subfield of hippocampus decreased significantly, while no apoptotic nuclei could be detected. The results suggest the involvement of oxidative stress in kindling-induced damage to brain and support possible non-apoptotic role of caspase-3 in brain plasticity.

HYPOTHALAMIC PARAVENTRICULAR NUCLEUS PLAYS ROLE IN THE REGULATION OF CEREBELLAR AND VESTIBULAR CELL ACTIVITIES DURING COLCHICINE STRESS *Z. Pirnik, A. Kiss,* Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic.

The hypothalamic paraventricular nucleus (PVN) serves as a primary site of stress response. Colchicine is known as a potent stressor eliciting massive Fos expression in many areas of the brain including PVN. Recently, we have shown Fos-activation in many cerebellar and vestibular nuclei (CVN) 48 h after centrally applied colchicine. Although direct anatomical interconnections between PVN and the cerebellum have not been established yet, there exists convincing neurophysiological evidence speaking about a possible functional interrelationships between these two structures during stress. To clarify this hypothesis effect of the unilateral PVN (uPVN) lesion was analyzed on the colchicine induced Fos expression in individual CVN nuclei in rats. Ten days after surgical lesion of the right PVN, colchicine (60 μg/10 ul) was injected into the lateral brain ventricle. Animals were sacrificed 48 h after colchicine administration. Coronal sections were Fos-immunostained by avidin-biotin peroxidase procedure. Light microscopical analysis of Fos-immunolabeled structures revealed that: 1) uPVN lesion reduced by colchicine elicited Fos expression in all CVN, 2) from cerebellar nuclei, the interpositus and dentate nuclei were ipsilaterally influenced by uPVN lesion, 3) from vestibular nuclei, greatest reduction was observed in the medial vestibular and lateral vestibular nuclei by contralateral manner, 4) lowest Fos-decrease was found bilaterally in the fastigii nuclei. The present study indicates that the PVN might have functional impact on the function of CVN in response to colchicine stress.
The study was supported by grants of EC ICA1- CT-2000-70008 and VEGA 2/2007.

DEVELOPMENT OF EFFECT OF KAINIC ACID ADMINISTRATION IN THE HIPPOCAMPUS OF RATS *J. Pokorný, M. Langmeier, R. Druga[1], S. Trojan,* Institute of Physiology, First Faculty of Medicine, [1]Department of Functional Anatomy, 2nd Medical Faculty, Charles University, Prague, Czech Republic.

Neurotoxic effect of kainic acid is related to the presence of specific receptors for this substance. It may also reflect the mutual relation of neurons in the neuronal circuits. Kainic acid was administered intraperitoneally to adult Wistar male rats in the dose of 10mg per kg, diluted in PS. Animals were fixed by intracardial perfusion 2, 4, or 6 days later. Cryostat slices were processed for Fluoro-Jade staining to visualise the dying cells. Material was evaluated and photographed under OLYMPUS Provis fluorescence microscope. Two days after the administration of kainic acid large numbers of neurons in CA1 and CA2-3 were stained. Few scattered neurons were stained in the hilus (interneurons of the hilar regions). Very few neurons were stained in the hilar and adjacent part of CA3. Neurons were well preserved and some of dendritic shafts were visible. Four days after the injection of kainic acid the predominant impairment was in the hilus and in the adjacent part of CA3. CA1 region was affected in its distal part only, the region CA2 had no dying neurons. In the six days interval, only few stained cells were present in CA1, CA3 and in the hilus. Most of the cells bore signs of a longer disintegration processes. Several days developing effects of kainic acid administration indicate, that the mechanism of cell death is related not only to the direct effect of the excitatory molecule, but also on the complexity and specific sensitivity of the neuronal circuits involved.
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NEUROGENESIS AND APOPTOSIS IN THE SVZ AND RMS OF THE ADULT RATS AFTER BILATERAL OLFACTORY BULBECTOMY *B. Poušová, E. Račeková, J. Orendáčová, M. Martončíková,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The subventricular zone (SVZ) is an important germinal layer that forms during development adjacent to the telencephalic ventricular zone and it persists into adulthood, where it retains the capacity to generate both neurons and glia. The SVZ precursors migrate through a network of tangential pathways in the lateral wall of the lateral ventricle and converge to rostral migratory stream (RMS), which leads into the core of the olfactory bulb (OB). Within OB, neurons leave the RMS and migrate into the granule and periglomerular layers, where they differentiate into local interneurons (1). Programmed cell death (apoptosis) is an important mechanism shaping the size of different cell populations within developing nervous system. Recent studies revealed that newly generated neurons and cells in the areas of adult neurogenesis also undergo apoptosis (2). The main objective of our study was to investigate proliferation and apoptosis in the SVZ and RMS in different survival times after bilateral bulbectomy.
Adult male Wistar albino rats were bilaterally bulbectomized under deep anesthesia. The trephine opening was made with a dental burr and the bulbs were removed by suction with a blunt pipette. After operation the animals were allowed to survive for 3 days, 3 weeks and 3 months. Two hours before perfusion the rats were injected with cell proliferation marker bromodeoxyuridine (BrdU). The brains were cut sagittally at the thickness of 24 μm on cryostat. Sections, which included the RMS, were alternatively processed for detection of proliferation (BrdU immunohistochemistry) and apoptosis (Fluoro-Jade B staining). Proliferating BrdU-positive cells were seen at all examined survival times (3 days, 3 weeks, 3 months). The RMS of bulbectomized rats has regular L-shape. Labelled cells were observed along the length of the migratory pathway and they were directed towards the place of the removed OB. On the other hand, apoptic cells were found in a high number only on the telencephalic margins close to the place of OB removal, i.e. in the most rostral part of the migratory stream. In the SVZ and main part of the RMS we did not notice any Fluoro-Jade positive cells. Our results put the evidence that the presence of OB is not essential for proliferation and migration of SVZ precursors and indicate that apoptosis is not directly controlled by the OB.
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NEONATAL OLFACTORY BULBECTOMY IN RATS: IPSILATERAL AND CONTRALATERAL ROSTRAL MIGRATORY STREAM *E. Račeková, M. Martončíková, B. Poušová, J. Orendáčová,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The rodent olfactory bulb (OB) retains the ability to acquire new neurons through life (1). These cells are born in the telencephalic subventricular zone (SVZ) persisting in the adult forebrain. The neuronal precursors migrate through the rostral migratory stream (RMS) to the OB where they differentiate into granule cells and periglomerular interneurons. The influence of the OB on neuronal cell migration through the RMS has been studied in adult rodents (2). In our experiment we intended to study the effect of olfactory bulbectomy performed during very early postnatal period, when the forebrain developmental processes are not finished. Neonatal (P3) Wistar albino rats were anaesthetized by hypothermia and the right OB was gently removed by suction. The bulbectomized animals were allowed to survive for 5 months. To label proliferative precursor cells, the rats received an intraperitonal injection of cell proliferation marker BrdU. We used camera lucida drawing to analyze the spatial distribution of the RMS on bulbectomized versus contralateral, non-lesioned hemisphere. Nitrergic neurons were identified by NADPH-diaphorase

histochemistry. Histological analysis of hematoxylin stained sections showed that the RMS persisted as a well-defined forebrain structure with regular L-shape on both bulbectomized and intact hemisphere. Although the RMS anatomical pattern of bulbectomized rats was similar to the control adult ones, an open olfactory ventricle was regularly present within it either in lesioned or intact side. Immunohistochemistry confirmed that proliferation of precursors along the RMS continued. Differentiated nitrergic neurons were observed in the SVZ and RMS in both hemispheres. We conclude that neonatal unilateral olfactory bulbectomy results in ipsi and contralateral morphological modification of the adult rat prosencephalic region - open olfactory ventricle.
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THE USE OF ISCHEMIC PRECONDITIONING AGAINST ISCHEMIC DAMAGE IN RATS *J. Radoňák[1], J. Vajó[1], E. Račeková[2], N. Lukáčová[2], J. Maršala[2],* [2]II. Surgical Clinic of Faculty of Medicine, P. J. Šafárik University, Faculty Hospital L. Pasteura, [2]Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Rat spinal cord ischemia and ischemic preconditioning were used as adjunct against ischemically damaged gray and white matter of the spinal cord. Spinal cord ischemic model combined with the obturation of the thoracic aorta was used inflating the balloon passing through femoral artery up to the branching of the left subclavian artery. In the first group of animals (n=12) the aortic occlusion during twelve minutes resulted in a clear neurological deficit affecting 81% of animals. In the second group (n=12) the application of ischemic preconditioning introduced by three min preischemia followed by 30 min recirculation and than 12 min ischemia applied 48 h later resulted in 27% animals suffering from paraplegia. The results were graded using the comparison of motor activity and histologic evaluation of transverse section cut through different spinal cord segments and impregnated according to Gallyas method. In the second experimental group an almost complete integrity of the gray and white matter without detectable necrotic loci could be detected. With regard to the first experimental group histopathologic changes could be seen mostly in the deep dorsal horn layers of lower lumbar and sacral spinal cord segments. Experimental approach applied above may be one of perspective methods permitting the application in surgery dealing with the surgical intervention on aneurysms of the thoracoabdominal aorta and having the potential leading to a decrease of neurological damage noted in patients with the above mentioned operations.
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REACTION OF PRIMARY SENSORY NEURONS FOLLOWING PERIPHERAL NERVE INJURY *M. Réthelyi, D. Zichó,* Department of Anatomy, Semmelweis University, Budapest, Hungary.

Peripheral nerves were either transected and the proximal stump ligated or squeezed in anesthetized adult rats. Three, five and seven weeks following the surgery the animals were sacrified with perfusion in deep anesthesia. The appropriate dorsal root ganglia (DRG) were collected and embedded in plastic. Toluidine blue stained 1 μm thick plastic sections and ultrathin sections were studied. Contralateral ganglia and DRG from intact animals were used as control. Large size (35-40 μm in diameter) neurons in the DRG on the operated side were found with cytoplasmic vacuolar changes following the transection of the peripheral nerves (sciatic nerve and nerves of the forelimb). The nucleus and nucleolus of the impaired neurons seemed intact even in the case when the vacuole filled almost the entire neuron. The cytoplasmic vacuoles were lined by membrane towards the cytoplasm and contained cytoplasmic debris. The initial portion of the axon of the neurons with vacuoles showed significant atrophy (1.5 to 2.5 μm in diameter) with respect to neurons in control animals as well as neurons in the contralateral ganglia in operated animals (3.5 to 5.5 μm in diameter). Neurons with vacuoles were found in the largest number five weeks following the surgery. No changes were found in experiments in which the sciatic nerve was squeezed but not transected. Repeated transection of the proximal stump (after five week survival) resulted in large number of neurons with vacuolar changes two weeks after the second operation. In another series of experiments 1% Cholera Toxin β subunit (CTb, List Labs.) was injected into the proximal stump of the sciatic nerve five weeks following the transection. Among the large number of CTb-labeled neurons also large neurons with vacuoles were found in the sections indicating the intact transport capacity of the morphological seriously impaired neurons. Loss of the distal portion of the axon causes severe changes in a population of large size primary sensory neurons such as cytoplasmic vacuoles and axon atrophy. Although the severe cytoplasmic alterations seem to indicate that the neurons are slowly deteriorating, the unimpaired transport capacity and the relatively long - 5 week - reaction time may suggest that the cytoplasmic vacuoles (*chromatolysis* in the truest sense) are signs of protracted regenerative processes in the sensory neurons.

NEONATAL ASPHYXIA AND DISTURBED SPATIAL LEARNING IN JUVENILE AND ADULT RATS: EFFECTS OF NEONATAL BODY TEMPERATURE AND CHELATION OF IRON *J. Rogalska, M. Caputa, K. Wentowska, A. Nowakowska, P. Waszak,* Institute of General and Molecular Biology, Department of Animal Physiology, N. Copernicus University, Toruń, Poland.

Newborn mammals, showing reduced normal body temperature, might be protected against iron-mediated, delayed neurotoxicity of perinatal asphyxia. Therefore, we decided to study the effects of (1) body temperature and (2) iron chelation with the deferoxamine in newborn rats, exposed to a critical anoxia, on spatial memory performance in adulthood. The performance was tested in holeboard. Neonatal anoxia at body temperature adjusted to a level typical of healthy (37°C) or febrile (39°C) adults resulted in impaired memory performance in adult rats. Both normal neonatal body temperature of 33°C and chelation of iron protected adult rats against the memory deficits. Surprisingly, 2 h exposure of the control (normoxic) newborns to body temperature of 37°C and 39°C itself led to substantial memory deficits. In conclusion, physiologically reduced neonatal body temperature can be recognized as a protection against development of iron-mediated postanoxic memory deficits in juvenile and adult mammals.
Supported by grant of 4.P05A.059.16 from the Polish State Committee for Scientific Research.

OPEN–FIELD–INDUCED STRESS RESPONSES IN JUVENILE AND ADULT RATS: EFFECTS OF NEONATAL ASPHYXIA, BODY TEMPERATURE AND CHELATION OF IRON *J. Rogalska, M. Caputa, K. Wentowska, A. Nowakowska,* Institute of General and Molecular Biology, Department of Animal Physiology, N. Copernicus University, Toruń, Poland.

Newborn mammals, showing reduced normal body temperature, might be protected against iron-mediated, delayed neurotoxicity of perinatal asphyxia. Therefore, we decided to study effects of (1) body temperature and (2) iron chelation with deferoxamine in newborn rats, exposed to a critical anoxia, on open field stress-induced behaviour in juvenile and adult rats. Neonatal anoxia at body temperature adjusted to a level typical of healthy (37°C) or febrile (39°C) adults led to the stress-induced hyperactivity in juvenile rats (5-45 days old) followed by hypoactivity in adult rats. Both normal neonatal body temperature of 33°C and chelation of iron prevented the behavioural disturbances. Neither neonatal body temperature nor anoxia affected spontaneous motor activity of rats, recorded in their home-cages with implantable transmitters. In conclusion, physiologically reduced neonatal body

temperature can provide a protection against iron-mediated postanoxic disturbances of stress responses in juvenile and adult mammals.
Supported by grant of 4.P05A.059.16 from the Polish State Committee for Scientific Research.

ALTERATION OF MICROGLIAL ACTIVATION IN A RAT MODEL OF SPINAL CORD ISCHEMIA *K. Saganová, I. Vanický, T. Ondrejčák, J. Gálik,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Transient cross-clamping of the thoracic aorta leads to spinal cord neuronal damage and loss of neurological functions. Microglial cells respond to neuronal damage by undergoing characteristic changes, the degree of microglial activation varies with the severity of neuronal injury. This study examined the early microglial response to transient spinal cord ischemia induced by intraluminal occlusion of the thoracic aorta for 8, 10 and 12 min accompanied by intra-ischemic reduction of mean proximal arterial pressure (MPAP) at 40 mm Hg (1). Evaluation of recovery of motor function as well as degree of microglial activation was assessed following intervals of postischemic reperfusion time ranging from 24 hours to 7 days. The microglial cells were detected on vibratome sections labeled by *Griffonia simplicifolia* B_4-isolectin (GSA I-B_4-HRP) (2) and their response to ischemic insult was studied in the affected lumbosacral spinal cord segments (L_3-S_2). Activated, lectin labeled cells were found as early as 1 day postischemia in the spinal cord gray matter of all spinal cords subjected to ischemia. Their response to transient ischemic insult was characterized by both increased lectin-binding and altered morphology. In the absence of proliferation and/or recruitment, this result indicates that the early microglial response consisted solely of the activation of resident microglia rather than to respond to the severity of insult. Depending on the degree of neuronal damage following 2 to 3 days postischemia, a progressive increase in the number of microglial cells, changes of cell morphology, recruitment of the activated microglia to the injured neurons, eventually to the side of ischemic lesions, were observed. Our data suggest that i) ischemia of different degrees gives rise to a uniform microglia response during the first 24 hours postischemia; ii) the activation of microglial cells occurs in a graded fashion in response to different degrees of neuronal injury within 2-3 days of postischemic reperfusion; iii) lectin labeling of microglia can serve for the study of the evolution of neuron-microglial interaction associated with spinal cord ischemia in the early postischemic period.
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EVALUATION OF MICROGLIAL RESPONSE TO EXPERIMENTAL SPINAL CORD INJURY IN THE RAT *K. Saganová, L. Urdzíková, I. Vanický,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

The activation of microglia which occurs predictably after acute spinal cord injury (SCI) is marked by a number of characteristic events affecting cellular morphology, cell size, cell number and a variety of changes on molecular level. These cellular changes form a graded response depending on the severity of the injury. This study sought to experimentally clarify time-dependent, differential microglial activation in the spinal cords of adult rats submitted to balloon-compression injury. SCI was produced by 2-French Fogarty catheter with volume of 15 μm inflated for 5 min at the dorsal epidural space at T8-9 spinal level (1). Evaluation of microglia response was performed 1 h and 1, 2, 3 and 7 days following SCI using *Griffonia simplicifolia* B_4-isolectin method (GSA I-B_4-HRP) (2). During the first 24 hours following SCI, lectin binding was most conspicuous in relation to red blood cells in hemorrhagic areas, while lectin labeling of microglial cells was found in a close vicinity of the lesion area. Increased number of lectin labeled macrophages and microglial cells with altered morphology occurred 2-3 days following injury; this pattern of cellular response persisted throughout the areas of compression at day 7 following spinal cord damage. Spinal cord injury of different origin leads to a time-dependent microglial activation (3). Our examination of the early response of microglia in the spinal cords subjected to SCI showed rapid microglial response in the proximity of lesion area. Activated microglia, macrophages and endogenous macrophages-like cells expanded proportionally to the irregularly-shaped lesions evolving with time in the rostrocaudal direction. This early microglial activation reflects the attempt of microglial cells to cope with drastically altered microenvironment.
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EFFECT OF CHRONIC ADMINISTRATION OF ONDANSETRON, A 5-HT_3 RECEPTOR ANTAGONIST, ON PENTYLENETETRAZOL-INDUCED SEIZURES *H. Sangraula, G.P. Rauniar, B.P. Das, K.K. Sharma,* Department of Pharmacology, B. P. Koirala Institute of Health Sciences, Dharan, Nepal.

The link between serotonergic (5-HT) and GABAergic system in brain is little known. In this study we concentrated on the interaction between 5-HT and GABA with the chronic administration of ondansetron (ODS, a 5-HT_3 receptor antagonist) on pentylenetetrazol (PTZ, a $GABA_A$ antagonist)-induced seizures.
Mice of either sex (25-35g) were treated with Ondansetron (0.5-2 mg/kg intraperitoneally, n=10) or saline (n=10) daily for 30 days. On day 31, a convulsive parameters were studied. Animals were treated as per the institute´s ethical guidelines.
PTZ produced frank tonic clonic convulsions in untreated mice. Ondansetron dose dependently protected against PTZ induced seizures. The number of mice developing convulsion was significantly less in ODS treated group compared to saline treated group (4 vs 8), the severity of convulsions was much less, and onset of convulsions was also significantly in ODS treated group compared to vehicle treated group (3.52 ± 0.21 min vs 2.42 ± 0.19 min, $p<0.01$). The results show that chronic blockade of 5-HT_3 receptor with ondansetron may protect against seizures even in conditions of reduced GABA activity as evidenced by administration of PTZ. Further studies are required to substantiate the findings.

THE ROLE OF ESPIN, AN ACTIN-BUNDLING PROTEIN, IN WILD-TYPE AND JERKER MUTANT MICE *G. Sekerková, L. Zheng, B. Changyaleket, R. Eytan, E. Mugnaini, J.R. Bartles,* Department of Cell and Molecular Biology, Institute for Neuroscience, Northwestern University Medical School, Chicago, USA.

The espins are a family of Ca^{2+}-insensitive actin-bundling proteins that are associated with the structures containing parallel actin fibers (1). So far 4 major isoforms were identified: a ~30 kD espin in the brush border microvilli of intestine and kidney, a ~50 kD espin in hair cell stereocilia in the auditory and vestibular system, a ~60-65 kD espin in Purkinje cells of the cerebellum and a ~110 kD espin in Sertoli cell junctions in the testis. These isoforms share a 116-amino acid C-terminal actin-bundling module, but contain different N-terminal peptides as a result of cell type-specific differences in transcriptional initiation and splicing. The espin gene maps to chromosome 4E1, the site to which jerker, a spontaneous autosomal recessive mutation, has been located. The most typical features of jerker homozygous mice are the Shaker-waltzer behavior (circling, head tossing, and hyperactivity) and deafness (2). While Sjöström and Anniko (3) found that the

deafness is caused by degeneration of hair cell stereocilia and cuticular plates resulting in total degeneration of the neuroepithelium, they could not pinpoint the molecular basis of this defect. When Western blot and immunohistochemical analyses were applied to various tissues from jerker mice, we found that the level of the espin protein decreased in the heterozygous animals, and that there was no espin in jerker homozygotes. Comparing data from DNA sequencing from wild-type mice and jerker homozygotes we found a deletion of a G nucleotide in the exon that encodes the C-terminal actin-bundling module. This frameshift mutation results in a protein that is 24 amino acids shorter protein that the wild-type and is responsible for the espin deficiency and phenotypical changes in jerker homozygotes (4). The most prominent effect of espin absence can be detected in the hair call stereocilia (3, 4); other organs show little or no change in histology. In hair cell stereocilia, the espin cross-links actin filaments in conjunction with Ca^{2+}-sensitive actin-bundling protein fimbrin. Lack of espin might cause collapse of the stereocilia during the Ca^{2+} influx associated with mechanosensory function of stereocilia.
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THERAPEUTIC EFFICACY OF PHOSPHABENZIDE AND PYRACETUM IN CORRECTION OF PATIENTS' ASTHENIC STATES *M.S. Schelkunova, R.K. Khafizyanova, K.K. Yakhin, A.M. Karpo,* Kazan State Medical Academy, Kazan State Medical University, Kazan, Russia.

Asthenic states in patients are characterized by a variety of clinical manifestations. Medications of polyvalent action in such states are the best. The objective was to study the spectrum of therapeutic activity of phosphabenzide compared with that of pyracetum in treatment of different structure asthenic states. Phosphabenzide belongs to new class of home psychotropic low toxic phosphororganic compounds without anticholinesterized action. The method used included: clinico - psychopathological, clinico - psychopharmaceutical, experimental - psychological and statistical. The study embraced 78 asthenic state patients (mean age - 44.5 years) of different psychopathological structure. Total of 42 phosphasbenzide monotherapy courses in a dose of 0.25 and 36 pyracetum courses in a dose of 0.4 were given. The medications chosen were taken orally 3 times a day for 30 consecutive days. Investigations showed that in the treatment of asthenic states of different structure both phosphabenzide and pyracetum cause positive ($p<0.01$) reduction of symptoms - targets determining psychoactivating, antiasthenic, nootropno - mnemotropic, antidepressive, tranquilizing, and vegetostabilizing effect of medications. Unlike pyracetum psychoactivating action of phosphabenzide is mild and acts at an earlier stages, demonstrating tropism to hypobulic symptomatics, its antiasthenic, antidepressive and tranquilizing effect being more potent; nootropic effects appear earlier promoting correction of memory and attention disorders among patients. Phosphabenzide contrast to pyracetum has been shown to produce prompt marked effect on obsesions and phobias. Thus, the spectrum of phosphabenzide therapeutic activity is shown to be wider than that of pyracetum. This testifies to preference of its use in correction of asthenic states of different structure.

THE EFFECT OF PHENYTOIN ON GLUTAMATERGIC NEURO-TRANSMISSION AND VOLUNTARY WHEEL RUNNING IN LEWIS RATS *M. Schwendt, R. Dunčko, F. Monček, A. Makatsori, D. Ježová,* Institute of Experimental Endocrinology SAS, Bratislava, Slovak Republic.

Receptors for glutamate are thought to be involved in a variety of higher brain functions including memory formation, stress response and addiction. Physical activities such as long-distance running can form a habit and might be related to drug-induced addictive behaviors. We have recently described that voluntary wheel-running, in addiction-prone Lewis rats is associated with altered gene expression of N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isooxazolepropionic acid (AMPA) glutamate receptor subunits in the ventral tegmental area (VTA) and the frontal cortex. In the present study, the functional role of glutamate receptors in the development of excessive wheel running was investigated. Rats were assessed to control and wheel running groups and treated with either saline or phenytoin (20 mg/kg i.p., 21 days). Phenytoin is an antiepileptic drug, which has been recently described to inhibit glutamate release. Lewis rats treated with saline progressively increased their amount of daily running reaching maximum levels of 4 – 6 km/day. Repeated administration of phenytoin significantly suppressed daily running, while it had no effect on locomotor and exploratory activity in the open field test. After 21 days of experiment, administration of phenytoin alone increased mRNA levels coding for NR1 and NR2A NMDA receptor subunits in the frontal cortex. Moreover, it potentiated the effect of running on AMPA receptor GluR1 and NMDA receptor NR2B subunit mRNA levels. Phenytoin also increased the binding of specific NMDA receptor antagonist [^{3}H]CGP39653 in the frontal cortex and the VTA. These findings are supporting the involvement of glutamate in the mechanism of phenytoin action. It may be suggested, that glutamate receptors are involved in the development of excessive voluntary wheel-running in Lewis rats.
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EFFECT OF ISCHEMIA/REPERFUSION ON NOS ACTIVITY IN THE WHITE MATTER COLUMNS OF THE SPINAL CORD *M. Silvašiová, J. Pavel, N. Lukáčová, J. Maršala,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

Ischemia of CNS causes damage in neuronal cell bodies/dendrites located in the gray matter and myelinated axons in the white matter. Numerous studies confirmed the role of nitric oxide as a neuromodulator in the CNS which affects synaptic transmission. There are only a few reports indicating the role of nitric oxide in the pathology of the white matter. In the present study calcium dependent NOS activity was investigated in course of reperfusions (1h, 3h, 1 day and 4 days) after 12.5 min of ischemia in rabbit spinal cord. Ischemia of lumbosacral segments was induced by 5 Fogarthy arterial embolectomy catheter engaged 12 cm into femoral artery (1). The catalytic NOS activity was determined by conversion of [^{14}C]-L-arginine to [^{14}C]-L-citrulline according to the method of Bredt and Snyder (2) and measured in the white matter devided into dorsal, lateral and ventral columns. The results have shown destructive effect of spinal cord ischemia on catalytic NOS activity, decreased during postischemic reperfusion periods in all white matter columns studied. Comparing to control values, short, 1h of reperfusion significantly decreased the enzyme activity in ventral columns. The most intensive damage of the lateral and ventral columns has been seen in the group of animals subjected to 12.5 min ischemia followed by 3h of reperfusion. We can conclude, that moderate ischemia followed by 1 and 3 hours of reperfusions is very damaging and capable of causing serious injury in above mentioned white matter columns. Longer times of reperfusion caused oscillatory continuance of changes in NOS activity. Renewal of enzyme activity has been seen during 1 day of reperfusion in the lateral and ventral columns, when the results were compared to 12.5 ischemia and 3h of reperfusion. A significant decrease of catalytic NOS activity

in all above mentioned white matter columns has been seen when reperfusion was prolonged to 4 days. Our results clearly show that the activity of catalytic NOS is affected during ischemia/reperfusion in white matter and that these changes are regional specific.
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CONTRIBUTION OF INNERVATION AND THYROID HORMONES TO THE DIFFERENTIATION OF MUSCLE FIBRE PHENOTYPE *T. Soukup, G. Zachařová, A. Vadaszová, I. Jirmanová[1], V. Smerdu[2], G. Asmussen[3], O. Križanová, M. Novotová[4],* [1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Institute of Anatomy, Medical Faculty, Ljubljana, Slovenia, [3]Carl-Ludwig-Institute of Physiology, University of Leipzig, Germany and [4]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic.

We have analyzed the effect of foreign innervation and altered levels of thyroid hormones on the expression of myosin heavy chain (MHC) isoforms using a model of s.c. heterochronous isotransplantation (1). The slow soleus (SOL) muscles from 2 to 4-week-old rats were grafted into the host fast extensor digitorum (EDL) muscles of adult eu-, hypo- or hyperthyroid female Lewis inbred rats. Two to 12 months after the transplantation, the fibre type composition of the SOL grafts, of the host EDL and of the control SOL and EDL muscles was determined according to the mATPase activity and immunocytochemical determination of MHC isoforms using the C.A.S.T. Grid system based on 2-D stereological methods (2). Furthermore, the MHC isoform composition was determined by the SDS-PAGE and their mRNA content was measured by the RT-PCR. In euthyroid rats, SOL grafts, reinnervated by the fast nerve of the host EDL muscle, developed as fast muscles containing 95.0% of fast fibres (2A, 2X/D and 2B). The transformation was even more pronounced in hyperthyroid rats, as regenerated SOL contained nearly 99% of fast, predominantly 2B fibres. Conversely, the transformation of the SOL graft was less pronounced in hypothyroid rats, as it contained about 65% of fast, mainly 2A fibres. The altered thyroid status led to similar, but less extensive changes in the host and control extrafusal fibres. This transformation was confirmed by SDS-PAGE, RT-PCR and electron-microscopical studies. We thus conclude that the reinnervation of the regenerating SOL graft by the foreign host EDL nerve triggers fibre type transformation from slow to fast muscle fibre types, but the extent of such transformation is further enhanced or suppressed by altered thyroid status.
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NITRIC OXIDE AND PARP IN MOLECULAR MECHANISM OF BRAIN AGING AND ISCHEMIA REPERFUSION INJURY *J.B. Strosznajder,* Department of Cellular Signalling, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

Poly(ADP-ribose)polymerase (PARP-1 EC 2.4.2.30) is a DNA associated enzyme that detects and converts DNA damage into intracellular signals and activates DNA repair programs or cell death pathways. The excessive production of nitric oxide (NO) and its interaction with superoxide radical is involved in activation of free radicals cascade responsible for DNA damage.

Up till now there is no consensus about the role of nitric oxide synthase (NOS) and PARP in brain aging and transient forebrain ischemia. The aim of this study was to determine the expression and activity of particular isoforms of NOS and PARP in rat brain (at the age of 4, 14, 24 months) and during reperfusion after short forebrain ischemia in gerbils. The radio-, immunochemical and RT-PCR methods were used. It was observed that: 1. neuronal NOS (nNOS E.C. 1.14.13.39) is mainly responsible for the higher NO release in aged and ischemic brain. There was no gene expression for iNOS in both conditions 2. alteration of phosphorylation state play important role in enhancement of nNOS activity 3. receptors induced activation of NO and PARP is decreased or abolished in aged brain 4. lower expression of genes for nNOS and PARP was detected in aged brain 5. the inhibition of PARP activity after ischemic episode protects the brain against oedema and neuronal death. Our data suggest that lower expression of gene for nNOS may protect the aged brain against it excessive activation. However down regulation of gene expression for PARP in aged brain may decrease the ability of brain for DNA repair. The inhibitor(s) of PARP may offer a new therapeutic strategy for global brain ischemia.

POLY(ADP-RIBOSE)POLYMERASE DURING REPERFUSION AFTER TRANSIENT FOREBRAIN ISCHEMIA - ITS ROLE IN BRAIN OEDEMA AND NEURONAL DEATH *R.P. Strosznajder, G.A. Czapski, H. Jesko, J.B. Strosznajder,* Department of Neurophysiology and Department of Cellular Signalling, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.
Poly(ADP-ribose)polymerase (PARP) can initiate an energy-consuming and inefficient repair cycle following cerebral ischemia – reperfusion by transferring ADP-ribose units to nuclear proteins leading to neuronal death. 3-minobenzamide (3-Aβ) a selective inhibitor of PARP significantly reduces brain damage after focal ischemia. However the role of PARP in global brain ischemia remains controversial till now. The activity of PARP in the reperfused brain after global ischemia has not been ever directly presented. The goals of our study were to determine PARP protein level and its activity and the effect of 3AB on brain damage after global ischemia. Our studies indicated different dynamic of PARP activity alteration in hippocampus during reperfusion after 3 and 10 min of transient forebrain ischemia in gerbils. The phasic stimulation of PARP activity was observed during reperfusion 15, 120 min and 4 days after 3 min ischemia with subsequent lowering of its activity close to control value on the 7th day of reperfusion. After 10 min of ischemic insult PARP activity significantly increased from 3rd up till 7th day of reperfusion period. Western blot analysis indicated slightly lower PARP protein level at 2nd and 3rd day after 3 and 10 min ischemia and similar level as in control at 7th day of reperfusion. 3-AB in a dose of 30mg/kg b.w. injected intravenously directly after 3 min ischemia protects over 60% of neuronal cells against death in CA_1 layer of hippocampus but has no effect after 10 min of ischemic episode. 3-AB decreased significantly forebrain oedema after 3 and 10 min of ischemia. Our data indicate that PARP inhibitors may offer a potent therapeutic strategy for the short global ischemia. The combination of PARP inhibitor with potent antioxidant may enhance its ameliorating effect.

MODULATION OF CHEMOSENSITIVITY OF HUMAN GLIOMA CELLS *M. Šarišský, J. Lavička, I. Šulla[1], L. Mirossay,* Department of Pharmacology, [1]Department of Neurosurgery, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic.

Malignant gliomas represent an extemely deadly neoplasm with one of the lowest survival rate of all malignant tumors. This is underlied by their typical resistance to all therapeutical modalities, especially chemotherapy. Various strategies might be employed to overcome chemoresistance of glioma cells. In this work, we sought to enhance chemotherapy effectiveness by combination with a ligand of peripheral benzodiazepine receptor (PBR). For the study, both human glioma cell lines and cells isolated from human glioma tissue specimens were used. Chemosensitivity of glioma cells to selected chemotherapeutic drugs

alone or in combination with diazepam, a PBR ligand, was tested by using MTT cytotoxicity test. Effects of diazepam on chemotherapy-induced apoptosis were also studied. In conclusion, viability of glioma cells was significantly reduced when chemotherapy was combined with PBR ligand. Thus, we anticipate that clinical effectiveness of chemotherapeutical regimens might be enhanced by including PBR ligands into anticancer treatment protocols.

PROTECTION OF NERVOUS TISSUE AGAINST OXIDATIVE STRESS: INDOLE DERIVATIVES AS MODEL PROTECTANTS

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Some of reactive oxygen species (ROS) play an important role in physiology of living tissues being involved especially in vascular tone regulation and cell signalization as well as in inflammatory defense mechanisms. However, if they are produced locally in excess and/or if they escape from an appropriate control they may represent a potential threat to particular tissue components. In nervous system they may impair neurons and glial cells resulting even in their death. This may occur under acute circumstances such as ischemia/reperfusion or trauma in brain or spinal cord as well as in long-lasting processes such neurodegeneration or aging. Undoubtedly, oxidative stress (OS), i.e. surplus of the uncontrolled ROS, is only one of numerous components in pathophysiology of the mentioned processes. Nevertheless, pharmacological intervention in the generation and/or control of ROS may be considered as an alternative approach to their prevention and treatment. Accordingly, it have been demonstrated in numerous preclinical studies made so far in different *in vitro* and *in vivo* models that antioxidants may indeed ameliorate the OS-induced impairment of nervous tissue. In spite of these positive observations, only a few drugs reached clinical practice, unfortunately, with frequently inconclusive and controversary outcome. Low efficacy and water solubility, inappropriate pharmacokinetics, slow blood/tissue barrier penetration, unsuitable organ distribution, high toxicity or even unacceptably high price of the drugs tested might be responsible for the controversy between the preclinical and clinical experiences. Consequently, the search for new compounds with suitable antiradical and other properties is still continuing. Although antioxidants may be found in different chemical groups, indole derivatives represent a distinct group involving both natural and synthetic compounds. Melatonin and pyridoindole stobadine are just two examples of the extensive group. We demonstrated that some well known indole derivatives as well as selected new tricyclic indole-derived compounds were able to increase survival of rat hippocampal slices in hypoxia/ reoxygenation and to ameliorate CNS impairment in mice head trauma model. Comparing to stobadine, a model substance, some of the new compounds prepared so far revealed up to two orders higher antioxidant efficacy in chemically-induced lipid–peroxidation and other models, they are devoid of undesired alpha-adrenolytic effect, etc. The new compounds may be considered as putative original neuroprotectants needing further and more detailed study.

NADPH - DIAPHORASE POSITIVE STRUCTURES AFTER COMPRE-SSIVE SPINAL CORD INJURY IN THE RAT

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Nitric oxide (NO) plays a role in secondary damage after traumatic spinal cord injury (SCI). NO is produced by the activity of nitric oxid synthase (NOS). Three isoforms of NOS, calcium-dependent (neuronal and endothelial) and calcium-independent (inducible) occur in the CNS. Recent in vitro studies indicate that NO and its metabolites are toxic to various structures in the white matter and may cause secondary degeneration of the long projection tracts in the spinal cord (1,2). Direct measurements of NO concentrations indicate rapid increase of NO concentrations immediately after injury (3) and both isoforms of NOS increase their activity at different time periods after SCI (4). To investigate the structures expressing NOS after spinal cord injury, NADPH-diaphorase histochemistry was performed in animals after SCI followed by different survival times: 15 minutes, 1 hour, 1 day, 3 days, 7 days and 28 days. In control animals, light-microscopic examination revealed selective NADPH-d positivity in different spinal cord structures including neurons, glia, endothelial cells, and macrophages. In general, the lesion site exhibited loss of stainability, and in the surrounding tissue, staining pattern and intensity remained unaltered. Increased staining was found only in two structures: 1, vessels surrounding the lesion, and 2, numerous disseminated NADPH-d positive cells present both in the necrotic center and surrounding intact tissue. Increased staining intensity was most prominent 3 days after SCI. At this period, the lesion is massively invaded by macrophages, and we assume that the NADPH-d positive cells are a subpopulation of macrophages expressing strong NADPH-d activity. Further studies are necessary to confirm the identity of these cells.

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PROTECTION AND RESTORATION OF CNS FUNCTION AFTER TRAUMATIC BRAIN LESION: LONG-TERM STUDY

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The transplantation of fetal brain tissue protects spatial memory impairment when done immediately after the cortical traumatic lesion and restricts ongoing degenerative processes following the lesion. The CNS grafts can influence the brain function 1) by formating synapses with host brain (restoration of neuronal circuits) 2) by production of trophic and growth factors supporting e.g. cell proliferation, neurites prolongation and neuronal viability (restriction of degenerative processes). Ameliorative effect of graft of pre-cultured astrocytes on spatial memory was comparable with the effect of homotopic grafts. Therefore, the process of memory amelioration was influenced by glial cells producing trophic and growth factors (e.g. glia derived growth factor II, known as basic fibroblast growth factor -bFGF, etc.). With aim to compare the effect of grafts with an influence of trophic factors on impaired spatial memory, bFGF and nerve growth factor (NGF) were continuosly infused in the damaged CNS during 14 or 28 days after the lesion surgery. Nootropic drug Cerebrolysin (EBEWE) containing aminoacids and small polypeptides was applied intraperitoneally. The animals were learned to find hidden platform in the water tank at 3 time points: before the lesion (pre-test), 14 days after the lesion (early test) and 6-8 months later (delayed test). An interaction of the treatment with a motor activity was also evaluated. These experiments revealed delayed restorative effect of spatial memory after b-FGF (0.2 μg/ml) treatment and partial short-term protective effect of NGF(11 μg/ml). Four-week administration of Cerebrolysin protects spatial memory declination due to the lesion (during minimally 6-8 months), while 2-week treatment has only temporal effect. Only b-FGF was found to protect long-lasting declination in motor activity that otherwise occurred in all the lesioned groups independently on the treatment. It is concluded that FGF-2 could support or initiate self-regenerative proceses in the CNS (compensatory synaptogenesis) in similar manner as fetal CNS graft, while NGF or Cerebrolysin restrict degenerative changes following traumatic brain lesion. Although growth factors are able to ameliorate CNS function their effect is not identical in time with the effect of CNS transplant.

STUDIES ON EXPERIMENTAL SPINAL CORD INJURY; WHITE MATTER - ORIENTED ANALYSES OF OUTCOME AND NEURO-PROTECTION *I. Vanický, L. Urdzíková, K. Saganová, J. Gálik,* Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic.

CNS white matter is sensitive to ischemia and anoxia. In the past decade, significant progress has been made in understanding the mechanisms of anoxic axonal injury, and new axonoprotective treatments have been described *in vitro* (1). Ischemic damage to spinal white matter is a part of secondary mechanisms after spinal cord injury (SCI), and axonal protectants should improve the outcome after experimental spinal cord injury. Here we describe a new rat model of SCI, induced by inflating a balloon in the epidural space at T8 level. The animals survive for 4 weeks and their neurological recovery is quantitated in an open field test and scored on a BBB scale discriminating between 22 grades of neurological injury (2). After 4 weeks, the animals are perfusion-fixed and the extent of the lesion quantitated by morphometry. The model produces reproducible and gradable intermediate stages of injury, and close correlations are found between neurological and morphometric outcomes. Spinal cord evoked potentials have been measured as an additional quantitative parameter of the outcome. Chronically implanted epidural electrodes enabled repeated and stable stimulation/recordings over the whole 4 weeks period. This technique allowed us to quantitate conductivity of the white matter between cervical and lumbar spinal cord levels. The model has been used in two treatment studies. First, we tested the effect of spinally administered methylprednisolone, a synthetic steroid approved for the treatment of SCI in humans (3). Spinal methylprednisolone elicited remarkable immediate reactions in paraplegic animals, but in long-term study, no significant changes were detected. In the second study, we have studied the effect of transient hyperthermia on SCI. Spinal injuries at higher thoracic or cervical levels cause serious dysbalances in thermoregulation, and in some patients, episodes of long-lasting and untreatable hyperthermia are observed (4). In our study, transient hyperthemia (40.5°C, 3hrs) significantly worsened neurological outcome as compared to normothermic animals. In conclusion, our rat model is a sensitive tool for studying the effects of various treatments after SCI.

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PRO-INFLAMMATORY CYTOKINE GENE EXPRESSION IN ASTROCYTES AFTER IMMUNOSUPPRESSANT FK506 TREATMENT AND IN RAT MODEL OF FOCAL CEREBRAL ISCHEMIA (MCAO) *M. Zawadzka, B. Kaminska,* Nencki Institute of Experimental Biology, Warsaw, Poland.

Brain injury and ischemia are often associated with proliferation and hypertrophic response of glial cells (reactive gliosis). Reactive astrocytes express growth factors that are critical factors regulating glial cell proliferation. However, ischemic brain astrocytes produce many cytokines and inflammatory mediators that may significantly influence the microenvironment of injured brain. We have found that tacrolimus (FK506), a widely used immunosuppressant, affects the survival of reactive astrocytes from striatal trauma and primary newborn astrocytes by inhibiting signalling pathways that regulate hypertrophic and/or proliferative responses. Exposure of astrocytes to immunosuppressant at cytotoxic doses evokes morphological changes, including cell body shrinkage and loss of extensions, followed by cell death. This death was accompanied by apoptotic changes in nuclear morphology and DNA fragmentation, as revealed by Hoechst 33258. Furthermore, FK506 treatment evokes changes in the expression of genes coding for growth factor and cytokine genes. In FK506-treated astrocyte cultures levels of mRNA encoding PDGF, bFGF, CNTF, TNF-alfa, and IL1-beta were decreased. We also characterised changes in the mRNA expression of cytokines in rat model of transient focal ischemia (middle cerebral artery occlusion – MCAO) by using RT-PCR. Down-regulation of growth factor expression by FK506 may play a role in the inhibition of astrocyte mitogenic potential and hypertrophic responses. Interestingly, in FK506-treated astrocytes the level of mRNA encoding BDNF increased in a dose-dependent manner. The up-regulation of BDNF mRNA and protein level in astrocytes exposed to FK506 may underlie the neuroprotective action of immunosuppressants.

CONVERSION OF MARROW AND CORD BLOOD MESENCHYMAL CELLS TO BRAIN CELLS *T. Zigova[1,2], S. Song[1,3,4], S. Kamath[1,3], A.E. Willing[1,2], S.N. Garbuzova-Davis[1,2], J.E. Hudson[1,2], N. Chen[1,2], P.R. Sanberg[1,2], J. Sanchez-Ramos[1,3,4],* Center for Aging and Brain Repair[1], Departments of [2]Neurosurgery, [3]Neurology, USF College of Medicine, and [4]James A. Haley VA Hospital, Tampa, USA.

Proliferation and differentiation of neural stem cells (NSC) derived from embryonic or adult central nervous system are highly dependent on the interplay between intrinsic and extrinsic factors that drive NSCs to differentiate into cell of a specific neural lineage. Recent studies, however, have demonstrated that cells outside the brain can be challenged through instructive environmental signals and may adopt neural fates. Our laboratory is focused on differentiation potential of two cell sources: bone marrow (BM)- and human umbilical cord blood (HUCB)-derived cells. In a series of *in vitro* studies we demonstrated that both cell types respond to exogenous soluble factors by acquiring new morphological and antigenic characteristics (1,2). Grafting experiments (3) utilizing pre-differentiated HUCB cells revealed that a small population of these cells survived in the microenvironment of the developing (non-immunosuppressed) rodent brain. Confocal microscopy confirmed that few glial and neuron-like cells within the subventricular zone originated from HUCB. Our current studies employing similar transplantation paradigm but utilizing murine GFP-tagged BM cells showed that these cells survived well within the neonatal rat brain and we found some of green-labeled, presumably BM-derived cells in relatively distant areas from the injection site. As controls we utilized GFP-labeled subventricular zone-derived precursors with well-described migratory behavior and phenotypic properties attributable to this transplantation paradigm. Immunocytochemical analyses to identify phenotype of BM-derived cells within various brain regions are in progress. In addition, a major objective is to identify and isolate the most primitive population of cells from both sources (BM and HUCB) that would rapidly expand *in vitro* and respond to instructive signals from the developing rodent brain.

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