SIXTH CONFERENCE OF THE CZECH NEUROSCIENCE SOCIETY

PRAGUE, NOVEMBER 19–20, 2007

The Sixth Conference of the Czech Neuroscience Society is organized by the Executive Committee of the Czech Neuroscience Society together with the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic and the Center of Neuroscience.

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Office Hours at the Registration Desk:

Monday, November 19	8:00–18:00
Tuesday, November 20	8:00–15:00

Posters
Posters should be set up between 8:30 and 9:00 on Monday (posters 1–44) or on Tuesday (posters 45–76). The author should be present at his /her poster between 13:30 and 14:30.

Programme

Monday, 19th November

08.30–09.00	Poster set-up
09.00–09.20	Opening of the Sixth Conference of the Czech Neuroscience Society with presentation of Society awards.
09.20–10.50	Session 1
10.50–11.10	Coffee break
11.10–12.40	Session 2
12.40–14.30	Lunch and Poster Session 1
14.30–16.00	Session 3
16.00–16.20	Coffee break
16.20–17.30	Session 4

19.30 Dinner at Best Western Hotel Kampa, Všehrdova 16, Praha 1

Tuesday, 20th November

08.30–09.00	Poster set-up
09.00–11.00	Session 5
11.00–11.20	Coffee break
11.20–12.20	Session 6
12.20–14.30	Lunch and Poster Session 2
14.30–15.30	Session 7

Monday, 19th November

9.00 **Opening of the Sixth Conference of the Czech Neuroscience Society with presentation of Society awards**
E. SYKOVÁ, President of the Czech Neuroscience Society

9.20 – 10.50 Session 1 Chairs: A. Saria, J. Syka

9.20 **First images: Activity patterns in the mouse visual cortex at eye opening**
A. KONNERTH, *Munich*

9.50 **Glutamate-mediated neuronal-glial transmission**
A. VERKHRATSKY, *Prague and Manchester*

10.20 **Stem cells, biomaterials, and their use for treatment of brain and spinal cord injury**
E. SYKOVÁ, *Prague*

10.50 **Coffee Break**

11.10 – 12.40 Session 2 Chairs: A. Konnerth, E. Syková

11.10 **Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement**
A. SARIA, *Innsbruck*

11.40 **Two signalling pathways from the metabotropic glutamate receptor of Purkinje neurons distinguished with flash photolysis and Ca^{2+} imaging**
D. OGDEN, *Paris and London*

12.10 **Temperature dependency of NMDA receptors**
L. VYKLICKÝ JR., O. CAIS, M. SEDLÁČEK, I. DITTERT, M. HORÁK, *Prague*

12.40 – 14.30 **Lunch and Poster Session 1**

14.30 – 16.00 Session 3 Chairs: H. Kettenmann, L. Vyklický Jr.

14.30 **What and Where in human audition: cortical organisation and plasticity**
S. CLARKE, *Lausanne*

15.00 **Aging of the auditory system**
J. SYKA, *Prague*

15.20 **Electrophysiological approach to evaluation of CNS ageing**
M. KUBA, J. KREMLÁČEK, Z. KUBOVÁ, J. LANGROVÁ, J. SZANYI, F. VÍT, *Hradec Králové*

15.40 **Laterality, cortical functions asymmetry, cerebellar dominance and hair whorl**
J. TICHÝ, J. BĚLÁČEK, *Prague*

16.00 **Coffee break**

16.20 – 17.30 Session 4 Chairs: S. Clarke, A. Verkhratsky

16.20 **Microglia in health and disease**
H. KETTENMANN, *Berlin*

16.50 **Bilateral changes of SDF-1 and its receptor CXCR4 in the dorsal root ganglia of chronic constriction injury and spinal nerve ligation models of neuropathic pain**
P. DUBOVÝ, I. KLUSÁKOVÁ, I. SVÍŽENSKÁ, R. JANČÁLEK, *Brno*

17.10 **The role of peripheral and central TRPV1 receptors in pain transmission and modulation**
J. PALEČEK, E. POSPÍŠILOVÁ, D. ŠPICAROVÁ, *Prague*

19.30 **Dinner at Best Western Hotel Kampa, Všehrdova 16, Praha 1**

Tuesday, 20th November

9.00 – 11.00 Session 5 Chairs: D. Ogden, J. Paleček

9.00 **Molecular Properties of P2X Receptor-Channels**
S. S. STOJILKOVIC, *Bethesda*

9.30 **Interactions between the 5-HT2a and mμ opioid receptors: implications for tolerance and responsivness to morphine**
G. MILLIGAN, *Edinburgh*

10.00 **Comparison of agonist-stimulated G-protein activity in brain cortex membranes isolated from control and morphine-treated rats**
P. SVOBODA, J. STOHR, L. BOUŘOVÁ, V. RUDAJEV, V. LISÝ, K. KLUČKOVÁ, J. NOVOTNÝ, *Prague*

10.20 **Opposite effects of rapacuronium on acetylcholine binding and signaling at muscarinic acetylcholine receptors**
J. JAKUBÍK, V. DOLEŽAL, PRAGUE

10.40 **Is nonquantal neurotransmitter release always nonvesicular?**
F. VYSKOČIL, *Prague*

11.00 **Coffee break**

11.20–12.20 Session 6 Chairs: G. Milligan, A. Chvátal

11.20 **Clinical application of BOLD fMRI**
J. VYMAZAL, R.JECH, PRAGUE

11.40 **In vivo metabolomics and genetics in patients with creatine deficiency**
M. HÁJEK, M. DEZORTOVÁ, V. MALINOVÁ, M. JIRSA, *Prague*

12.00 **New method for non-invasive measurement of head position**
R. ČERNÝ, M. BOJAR, J. HOZMAN, D. ŠTURM, *Prague*

12.20–14.30 **Lunch and Poster Session 2**

14.30–15.30 Session 7 Chairs: S. S. Stojilkovic, P. Dubový

14.30 **Effects of LiCl/PILOCARPINE-induced status epilepticus on rat brain benzodiazepine receptor binding: regional and ontogenetic study**
H. KUBOVÁ, L. SUCHOMELOVÁ, L. ROCHA, *Prague*

14.50 **Neuronal degeneration induced by status epilepticus in the nucleus accumbens and in the olfactory tubercle of immature rats**
R. DRUGA, H. KUBOVÁ, P. MAREŠ, *Prague*

15.10 **Effects of two GABA-B receptor agonists on cortical epileptic afterdischarges in immature rats are not identical**
P. MAREŠ, N. TABASHIDZE, *Prague*

POSTERS

Monday, 19th November, Poster Session 1

1. **Possible Role of the Ca^{2+}/Calmodulin Dependent Kinase II Phosphorylation Site T704 in Acute Desensitization of the Vanilloid Receptor TRPV1**
J. BENEDIKT, K. TOUŠOVÁ, K. SUŠÁNKOVÁ, A. SAMAD, L. VYKLICKÝ, J. TEISINGER, V. VLACHOVÁ, *Prague*

2. **PKA dependent effect of pregnenolone sulfate on NMDA receptors expressed in hippocampal neurons**
M. PETROVIC, H. CHODOUNSKÁ, M. SEDLÁČEK, L. VYKLICKÝ JR., *Prague*

3. **The changes in phosphatidyl inositol 4,5 bisphosphate (PIP2) levels related to the TRPA1 channel activity**
A. SAMAD, J. BENEDIKT, L. VYKLICKÝ, J. TEISINGER, V. VLACHOVA, *Prague*

4. **Fluorometry of thermosensitive TRP channels: more than complementary method for understanding structure-function relations**
F. TOUŠKA, J. KRŮŠEK, K. SUŠÁNKOVÁ, V. VLACHOVÁ, *Prague*

5. **TRPV1 endogenous agonist N-oleoyldopamine (OLDA) modulates nociceptive synaptic transmission in the spinal cord**
D. ŠPICAROVÁ, J. PALEČEK, *Prague*

6. **In vitro calcium imaging of spinal cord dorsal horn neurons**
D. SOJKA, J. PALEČEK, *Prague*

7. **Chronic constriction injury in weanling rats**
Š. VACULÍN, M. FRANĚK, R. ROKYTA, *Prague*

8. **Quantitative changes of IL-6 protein are presented not only in the lumbal, but also in the cervical DRG following unilateral sciatic nerve injury**
Z. VESELKOVÁ, P. DUBOVÝ, I. SVÍŽENSKÁ, I. KLUSÁKOVÁ, *Brno*

9. **Engraftment of donor cells in CNS depends on proper timing of host irradiation after preconditioning with cyclophosphamide**
P. JIROUTEK, L. ŠEFC, *Prague*

10. **Electrophysiological characterization of D6/GFP-neural stem/progenitor cells during in vitro differentiation and after transplantation into the injured rat brain**
M. ANDĚROVÁ, I. PRAJEROVÁ, P. HONSA, O. MACHOŇ, A. CHVÁTAL, *Prague*

11. **Differentiation of neural stem cells expressing Sonic Hedgehog and Wnt-7a**
I. PRAJEROVÁ, P. HONSA, M. ANDĚROVÁ, A. CHVÁTAL, *Prague*

12. **Ischemia-induced Volume Changes in Astrocytes: The Role of Chloride Movement**
J. BENEŠOVÁ, M. ANDĚROVÁ, M. HOCK, O. BUTENKO, I. PRAJEROVÁ, A. CHVÁTAL, *Prague*

13. **Changes in the expression of K+ and Na+ channels in astrocytes and NG2 glia after ischemia in vivo**
H. NEPRAŠOVÁ, M. ANDĚROVÁ, J. BENEŠOVÁ, O. BUTENKO, A. CHVÁTAL, *Prague*

14. **Human embryonic stem cells (CCTL14) differentiate into a neuronal phenotype**
N. KOZUBENKO, M. KAPCALOVÁ, M. ANDĚROVÁ, O. BUTENKO, A. HAMPL, P. JENDELOVÁ, E. SYKOVÁ, *Prague*

15. **Properties and growth of human bone marrow mesenchymal stem cells in different media**
K. TURNOVCOVÁ, K. RŮŽIČKOVÁ, P. JENDELOVÁ, E. SYKOVÁ, *Prague*

16. **Co-transplantation of olfactory ensheathing cells and mesenchymal stem cells improves hindlimb performance after spinal cord injury, but does not show synergistic benefits**
T. AMEMORI, K. RŮŽIČKOVÁ, G.D. ARBOLEDA TORO, P. JENDELOVÁ, E. SYKOVÁ, *Prague*

17. **Mobilization of bone marrow cells induced by G-CSF and FLT3 ligand**
K. LIKAVČANOVÁ, L. URDZIKOVÁ, J. ŠEDÝ, P. JENDELOVÁ, E. SYKOVÁ, *Prague*

18. **Experimental spinal cord injury reconstruction using hydrolytically degradable hydrogels**
A. HEJČL, P. LESNÝ, J. ŠEDÝ, M. PŘÁDNÝ, J. MICHÁLEK, P. JENDELOVÁ, E. SYKOVÁ, *Prague*

19. **Growth of rat mesenchymal stem cells on layers of nonwoven nanofibers from different biocompatible polymers**
M. KAPCALOVÁ, P. LESNÝ, P. JENDELOVÁ, L. MARTINOVÁ, E. SYKOVÁ, *Prague*

20. **Nonwoven nanofiber materials as three-dimensional tissue constructs in spinal cord injury**
P. LESNÝ, O. JIRSÁK, P. JENDELOVÁ, J. MICHÁLEK, M. PŘÁDNÝ, L. MARTINOVÁ, E. SYKOVÁ, *Prague*

21. **Development of neurogenic pulmonary edema in spinal cord injured rats – the role of isoflurane anesthesia**
J. ŠEDÝ, L. URDZIKOVÁ, K. LIKAVČANOVÁ, A. HEJČL, M. BURIAN, P. JENDELOVÁ, J. ZICHA, J. KUNEŠ, E. SYKOVÁ, *Prague*

22. **Extracellular space diffusion parameters in focal cortical dysplasia**
A. HOMOLA, L. VARGOVÁ, J. ZÁMEČNÍK, P. KRŠEK, P. MARUSIČ, E. SYKOVÁ, *Prague*

23. **Diffusion parameters in the rat cerebral cortex during pilocarpine-induced status epilepticus**
L. DMYTRENKO, K. ŠLAIS, I. VOŘÍŠEK, E. SYKOVÁ, *Prague*

24. **Time course of water diffusivity in the rat brain after global ischemia**
I. VOŘÍŠEK, H. NEPRAŠOVÁ, M. ANDĚROVÁ, D. JIRÁK, M. HÁJEK, E. SYKOVÁ, *Prague*

25. **Calbindin and S100 protein expression in the developing inner ear of mice**
D. BUCKIOVÁ, J. SYKA, *Prague*

26. **Changes in GAD levels in the central auditory system of two rat strains with aging**
J. BURIANOVÁ, L. OUDA, O. PROFANT, J. SYKA, *Prague*

27. **Aging influences parvalbumin expression in the central auditory system of two rat strains**
L. OUDA, R. DRUGA, J. SYKA, *Prague*

28. **Age-specific impairment of hearing function in the rat**
N. RYBALKO, D. ŠUTA, J. PELÁNOVÁ, S. HAMSOVÁ, J. SYKA, *Prague*

29. **Mapping the auditory cortex in the rat by the expression of cytoskeletal neurofilament protein**
R. DRUGA, J. SYKA, *Prague*

30. **Functional and electrical membrane properties of neurons in the auditory cortex in rats**
O. PROFANT, K. LOMAKINA, R. TUREČEK, J. SYKA, *Prague*

31. **Effect of noise exposure on the amplitudes of auditory evoked responses in rats**
J. GRÉCOVÁ, J. POPELÁŘ, J. SYKA, *Prague*

32. **Spontaneous otoacoustic emissions in children and adolescents – effect of cisplatin treatment**
J. PELÁNOVÁ, D. SUMERAUER, M. ZÁPOTOCKÝ, M. JILEK, D. GROH, Z. KABELKA, J. STARÝ, J. SYKA, *Prague*

33. **Hearing thresholds in the extended frequency range as a function of age and sex**
M. JILEK, J. SYKA, *Prague*

34. **Auditory cochlea-threedimensional MRI of the left and right cochleo-vestibular organ**
J. TICHÝ, T. VITÁK, O. DLOUHÁ, *Prague*

35. **Adaptation of the olfactory receptor neuron of a moth to the natural pheromone signal**
L. KOŠŤÁL, P. LÁNSKÝ, J.-P. ROSPARS, *Prague*

36. **Optimal odor intensity in simple olfactory neuronal models**
O. POKORA, P. LÁNSKÝ, *Prague*

37. **Rats do not learn to avoid a cued moving region in a stationary environment but they learn to avoid the cued region if it is stationary in a moving environment**
K. BLAHNA, B. OSECKÁ, V. MAŇÁSKOVÁ, D. KLEMENT, *Prague*

38. **Are inertial stimuli and/or motor skills sufficient for successful navigation of rats in the AAPA task (moving world)?**
I. FAJNEROVÁ, J. KENNEY, D. KLEMENT, PRAGUE

39. **Strategies used by rats during navigation toward a visible moving target**
D. KLEMENT, K. BLAHNA, *Prague*

40. **Role of posterior parietal cortex of the rat in two tasks involving dynamic environment**
J. SVOBODA, P. TELENSKÝ, J. BUREŠ, *Prague*

41. **Study of animal cognitive functions using an active allothetic place avoidance task: assets, problems, questions**
A. STUCHLÍK, K. VALEŠ, *Prague*

42. **All subtypes of mild cognitive impairment are impaired in episodic-like memory**
K. VLČEK, J. LACZO, O. VAJNEROVÁ, M. ORT, M. VYHNÁLEK, J. HORT, *Prague*

43. **The effect of enforced physical activity and cerebellar transplantation on spatial learning in Lurcher mutant mice**
J. CENDELÍN, I. KORELUSOVÁ, F. VOŽEH, *Brno*

44. **Retrospective longitudinal study of titres of specific antibodies to ß tubulin class III in patients with MS and depression treated by glatimeracetate and SSRI**
Š. CIHELKOVÁ, M. MINÁRIKOVÁ, M. HLADÍKOVÁ, D. ŠKODA, P. JINOCH, M. BOJAR, *Prague*

Tuesday, 20th November, Poster Session 2

45. **Regulation of RGS3 function by 14-3-3 protein**
E. BOUŘA, T. OBŠIL, *Prague*

46. **Does 14-3-3 Protein Affect Conformation of FoxO4 DNA-Binding Domain?**
J. ŠILHÁN, E. BOUŘA, P. VÁCHA, P. HERMAN, J. VEČER, T. OBŠIL, *Prague*

47. **Determinants of calmodulin binding site on the C-tail of TRPC6 channel**
E. FRIEDLOVÁ, L. GRYCOVÁ, Z. LÁNSKÝ, M. ŠULC, J. TEISINGER, *Prague*

48. **ATP binding studies on the C - termimus TRPV1**
L. GRYČOVÁ, Z. LÁNSKÝ, E. FRIEDLOVÁ, M. KUBALA, J. TEISINGER, *Prague*

49. **Possible involvement of ryanodine receptor-controlled calcium release in sensitization of GnRH-stimulated IP3 receptor in melatonin-sensitive neonatal gonadotrophs of the rat**
A. BALÍK, I. SVOBODOVÁ, H. ZEMKOVÁ, *Prague*

50. **Identification of Transmembrane Residues Contributing to Channel Gating and Interaction with Ivermectin at Rat Purinergic P2X4 Receptor**
H. ZEMKOVÁ, I. JELÍNKOVÁ, V. VÁVRA, M. JINDŘICHOVÁ, T. OBŠIL, H.W. ZEMKOVÁ, S.S. STOJILKOVIC, *Prague and Bethesda*

51. **Disruption of the plasma membrane structure by depletion of cholesterol impairs effectiveness of TRH receptor-mediated signal transduction via Gq/11-alpha protein**
P. OSTAŠOV, J. NOVOTNÝ, L. BOUŘOVÁ, L. HEJNOVÁ, P. SVOBODA, *Prague*

52. **Calcium responses to thyrotropin-releasing hormone and angiotensin II. The role of plasma membrane integrity and effect of G11-alpha protein overexpression on homologous and heterologous desensitization**
J. NOVOTNÝ, P. OSTAŠOV, J. KRUŠEK, D. DURCHÁNKOVÁ, P. SVOBODA, *Prague*

53. **Delayed effects of xanomeline on evoked ACh release from rat brain slices**
E. MACHOVÁ, J. JAKUBÍK, E.E. EL-FAKAHANY, V. DOLEŽAL, *Prague*

54. **Cholesterol differentially influences G-protein signalling activated by the muscarinic M2 receptor**
P. MICHAL, E.E. EL-FAKAHANY, V. DOLEŽAL, *Prague*

55. **Looking for fluorescent probes for allosteric binding sites at muscarinic receptores: Unusual, but not exclusive, mechanism of interactions of tacrine at muscarinic receptors**
J. PROŠKA, M. DVOŘÁK, *Prague*

56. **M1 receptors participate in the nonquantal acetylcholine regulation via feedback NO action**
AI MALOMOUZH, MR MUKHTAROV, EE NIKOLSKY, F. VYSKOČIL, *Prague*

57. **Photoperiodic entrainment of the circadian molecular clock in the mice SCN**
S. SOSNIYENKO, R. HUT, K. MATĚJU, M. SLÁDEK, H. ILLNEROVÁ, A. SUMOVÁ, *Prague*

58. **Subunit composition of hippocampal NMDA receptor in heuristic (animal) model of schizophrenia**
F. ŠŤASTNÝ, J. KLASCHKA, I. KOZMÍKOVÁ, H. TEJKALOVÁ, M. VRAJOVÁ, *Praha*

59. **Acute and subchronic administration of quinolinic acid and psychotic-like behaviour in neurodevelopmental model of schizophrenia**
H. TEJKALOVÁ, J. KLASCHKA, F. ŠŤASTNÝ, *Prague*

60. **Protein expression of NMDA-NR1 subunit and acoustic startle in genetic (animal) model of schizophrenia**
M. VRAJOVÁ, H. TEJKALOVÁ, J. KLASCHKA, F. ŠŤASTNÝ, *Prague*

61. **Lateralization of 17beta-hydroxysteroid dehydrogenase type 10 in hippocampi of demented and psychotic patients**
Z. KRIŠTOFIKOVÁ, D. ŘÍPOVÁ, P. HOVORKOVÁ, A. HOŘÍNEK, E. MAJER, J. ŘÍČNÝ, *Prague*

62. **Absorption of high-frequency electromagnetic radiation by the mouse brain; theoretical model and real experiment**
J. BARCAL, V. ŽALUD, F. VOŽEH, J. VRBA, *Prague*

63. **Calretinin-containing neurons in normal and epileptic human temporal neocortex**
F. BAŘINKA, R. DRUGA, J. ZÁMEČNÍK, *Prague*

64. **Changes in the permeability of blood brain barrier: Cortical photothrombosis and epileptic seizure induced by flurothyl inhalation**
D. KRÝSL, L. TŮMA, J. POKORNÝ, J. MAREŠ, *Prague*

65. **Preconditioning effect of normobaric intermittent hypoxia on behavioral consequences of chemically induced seizures in rats**
K. DEYKUN, M. POMETLOVÁ, J. MAREŠ, *Prague*

66. **The Effect of ET-1 on the Excitability of the Rat Cortical and Hippocampal Slices in Vitro**
R. KONOPKOVÁ, I. VILAGI, S. BORBELY, H. KUBOVÁ, J. OTÁHAL, *Prague*

67. **Alteration of regulation of the regional cerebral blood flow in chronic epileptic rats**
J. OTÁHAL, G. TSENOV, P. MRÁZOVÁ, H. KUBOVÁ, *Prague*

68. **Effects of metabotropic glutamate receptor 5 antagonist MPEP on learning in developing rats**
A. MIKULECKÁ, P. MAREŠ, *Prague*

69. **Does paraldehyde treatment participate in short-term effects of status epilepticus in immature rats?**
G. TSENOV, H. KUBOVÁ, P. MAREŠ, *Prague*

70. **Software for synchronous electrophysiologic and image recordings and high level mathematical analysis**
E. KRAJČOVIČOVÁ, M. MARTÍNKOVÁ, R. KONOPKOVÁ, M. FEJTOVÁ, J. OTÁHAL, *Prague*

71. **Effect of methamphetamine exposure and postnatal care on sensorimotor development of rat pups**
L. HRUBÁ, B. SCHUTOVÁ, M. POMETLOVÁ, R. ŠLAMBEROVÁ, *Prague*

72. **Effect of methamphetamine on social and locomotion behaviors of adult male rats**
B. SCHUTOVÁ, L. HRUBÁ, M. POMETLOVÁ, K. DEYKUN, R. ŠLAMBEROVÁ, *Prague*

73. **Effect of acute methamphetamine administration on seizures in adult male and female rats prenatally exposed to the same drug**
R. ŠLAMBEROVÁ, B. SCHUTOVÁ, L. HRUBÁ, K. BERNÁŠKOVÁ, I. MATĚJOVSKÁ, R. ROKYTA, *Prague*

74. **Analgesic effect of acute methamphetamine in prenatally stressed and methamphetamine-treated rats**
A. YAMAMOTOVÁ, R. ŠLAMBEROVÁ, R. ROKYTA, *Prague*

75. **Grooming induced by intraperitoneal application of malanotan II, a dirivative of α-MSH**
S. HYNIE, M. FLEGEL, V. KLENEROVÁ, *Prague*

76. **Carbetocin improves deterioration of stress-induced behavior in the open-field device**
V. KLENEROVÁ, M. FLEGEL, P. ŠÍDA, S. HYNIE, *Prague*

ABSTRACTS

LECTURES

Stem cells and biomaterials in treatment of brain and spinal cord injury

E. SYKOVÁ

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In the last decade, neurotransplantation research has focused on the potential of embryonic and adult stem cells to replace damaged cell populations and to produce missing transmitters, neuroactive substances and growth factors. Neural as well as non-neural stem cell therapy might overcome the low regenerative capacity of the human CNS. Embryonic stem cells (ESCs) and bone marrow stromal cells (MSCs) are pluripotent progenitor cells that have the capacity to migrate towards lesions and induce or facilitate site-dependent differentiation in response to environmental signals. The behavior of rat fetal cells, mouse ESCs, rat and human MSCs and olfactory glia (OEGs) labelled with superparamagnetic iron-oxide nanoparticles and grafted to injured rat brain or spinal cord is studied to elucidate whether these cells are capable of survival, do not produce tumors, differentiate into neurons and astrocytes, prevent scar formation, promote neurogenesis and enhance regeneration. Magnetic resonance imaging (MRI) provides a noninvasive method to study the fate of transplanted cells in vivo. Rat or human ESCs, MSCs or OEGs can be labeled with various types of iron-oxide nanoparticles (Neurodegen. Dis. 3:62-67, 2006) and human CD34+ cells can also be labeled with magnetic MicroBeads (Miltenyi). In animals with a cortical or spinal cord lesion, cells were grafted intracerebrally, contralaterally to a cortical photochemical lesion, or injected intravenously. During the first two weeks post-transplantation, transplanted cells migrated to the lesion. Labeled MSCs, ESCs and CD34+ cells were visible in the lesion on MR images as a hypointensive signal, persisting for more than 2 months. Various biocompatible hydrogels (degradable and nondegradable), including those based on non-woven nanofibres, have been developed for bridging tissue defects and for use as 3D stem cell carriers (J. Mat. Sci. Mat. Med. 17:829-833, 2006). We also used nanofibre constructs seeded with MSCs or OEGs labeled with iron-oxide nanoparticles to bridge a spinal cord lesion. Our studies demonstrate that scaffolds seeded with adult as well as with embryonic stem cells can bridge a lesion site in the brain as well as in the spinal cord. Autologous BMC implantation has been used in a Phase I/II clinical trial in patients with SCI (n=30). The implantation was safe, and partial improvement was seen in patients who received BMC in the first month after injury (Cell Transplantation, 15:675-687, 2006). We conclude that adult stem cells derived from bone marrow are promising candidates for spinal cord lesion repair.

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Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement

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Neurotransmitter release in the nucleus accumbens core (NACore) during the acquisition of remifentanil or cocaine reinforcement was determined in an operant runway procedure by simultaneous tandem mass spectrometric analysis of dopamine, acetylcholine, and remifentanil or cocaine itself. Run times for remifentanil or cocaine continually decreased over the five consecutive runs of the experiment. Intra-NACore dopamine, acetylcholine, and drug peaked with each intravenous remifentanil or cocaine self-administration and decreased to pre-run baseline with half-lives of_10 min. As expected, remifentanil or cocaine peaks did not vary between the five runs. Surprisingly, however, drug-contingent dopamine peaks also did not change over the five runs, whereas acetylcholine peaks did. Thus, the acquisition of drug reinforcement was paralleled by a continuous increase in acetylcholine overflow in the NACore, whereas the overflow of dopamine, the expected prime neurotransmitter candidate for conditioning in drug reinforcement, did not increase. Local intra-accumbens administration by reverse microdialysis of either atropine or mecamylamine completely and reversibly blocked the acquisition of remifentanil reinforcement. Our findings suggest that activation of muscarinic and nicotinic acetylcholine receptors in the NACore by acetylcholine volume transmission is necessary during the acquisition phase of drug reinforcement conditioning.

Two signalling pathways from the metabotropic glutamate receptor of Purkinje neurons distinguished with flash photolysis and Ca^{2+}imaging

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The type 1 metabotropic receptors at parallel fibre synapses of Purkinje neurons are coupled by Gq protein to two mechanisms of Ca^{2+}elevation in the dendrites. Following burst stimulation of parallel fibres or photorelease of L-glutamate an early Ca^{2+} release from stores mediated by phospholipase C is precisely timed at 100 ms following stimulation, generates a brief outward current, and requires prior spiking in the Purkinje neuron. This is followed by a slower Ca^{2+} influx during the PF slow excitatory PSC which is mediated by Ca^{2+} permeable cation channels independently of Ca^{2+}release, PLC and Purkinje neuron activity. The kinetics, pharmacological characteristics and distribution of the two signalling paths will be described.

Temperature dependency of NMDA receptors

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NMDA receptors are highly expressed in the CNS, mediate the slow component of excitatory transmission and play key roles in synaptic plasticity and excitotoxicity. We have used patch clamp technique to examine temperature sensitivity of recombinant and native NMDA receptors. Temperature dependency of the amplitude of NR1/NR2B receptor responses was bell-shaped with maximum at ~35°C. Responses normalized with respect to the amplitude of the single channel currents were only little temperature sensitive at 25–35°C, however, were reversibly diminished at 45°C. Rate constants were assessed by fitting 6-state kinetic scheme to the time courses of macroscopic currents induced by rapid glutamate (1 mM) application at 25–45°C. Arrhenius transformation of the rate constants characterizing NMDA receptor activity indicate that the most temperature sensitive were the rate constants of desensitization (Q10 = 10.3), resensitization (Q10 = 4.6) and unbinding (Q10 = 3.6). Other rate constants and the amplitude of single channel currents were less temperature sensitive. Deactivation of responses mediated by NR1/NR2B receptors after a brief application of glutamate was best fit by a double exponential function with mean time constants: temperature coefficients (τ fast: Q10 = 3.7) and (τ slow: Q10 = 2.7). In contrast, time constants characterizing the deactivation of NMDA receptor mediated EPSCs recorded from pyramidal neurons of the rat cortex were only weakly temperature sensitive (τ fast: Q10 = 1.7) and (τ slow: Q10 = 1.8). The amplitude of NMDA EPSCs was diminished in the presence of ifenprodil. Both the ifenprodil-sensitive and ifenprodil-resistant decay time constants exhibited low temperature sensitivity. These data suggest that NMDA receptor desensitization may affect the time course of receptor deactivation as well as the amplitude of responses. In addition, the data indicate an existence of endogenous factors affecting temperature sensitivity of native NMDA receptors.

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What and Where in human audition: cortical organisation and plasticity

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Several lines of evidence indicate that sound recognition and sound localisation depend on two distinct, anatomically partially segregated processing streams, often referred to as the auditory What and Where streams:
The supratemporal plane contains several histologically identified non-primary auditory areas, some of which are specialised in sound recognition and others in sound localisation (1, 2).
The functionally defined What and Where streams are also present on the convexity of either hemisphere (3, 4). Hemispheric lesions centred on one or the other stream are associated with the corresponding deficits (5). Auditory processing within these streams is highly plastic, as demonstrated at several levels:
The representation of auditory objects differs between object categories (living vs man-made) as soon as 100 ms post-stimulus onset. Plasticity in auditory object representations, as indexed by repetition suppression, takes place within the next 100 ms and modulates distinct brain areas from the initial categorization of sounds (6, 7).

Cortical auditory representations of space are dynamic and subject to rapid reorganisation. Plasticity induced by a 40 minute auditory spatial discrimination training lasts for ca 6 h, is specific for trained locations and goes beyond strengthening the representation of the trained locations (8).

Unilateral hemispheric lesions cause major changes in the processing within the What and Where streams in the contralateral, intact hemisphere, involving the loss of parallel processing within the streams (9).

Thus, the auditory What and Where networks adapt at short- and long-term to functional requirements as different as the recognition of a previously heard stimulus, the increase in discrimination ability and postlesional reorganisation.

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Aging of the auditory system

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All mammalian species, including man, suffer from age-related hearing loss or presbycusis. Presbycusis affects 40% of the human population over 75 years of age. Typically, hearing in high frequencies deteriorates especially in a noisy background conditions. The most pronounced aging-associated pathological changes appear in the peripheral part of the auditory system; they are, however, accompanied by pathological processes occurring in the central auditory system. The first signs of deterioration occur in presbycusis in the cochlear outer hair cells, as detected by the recording of oto-acoustic emissions. Hearing thresholds evaluated either by subjective audiometry or objectively by evoked potentials show a combination of peripheral and central defects in auditory function. Deteriorated temporal resolution is demonstrated by increased gap detection thresholds.

A question arises to what extent are in presbycusis involved genetic factors. A suitable model for the study of presbycusis is rodents, such as the mouse or rat, particularly because of their short life-span. Among mouse strains, the most frequently used is the C57BL/6J; rapid progress of presbycusis is also evident in Fischer 344 (F 344) rats. The fast deterioration of hearing thresholds is, in F 344 rats, accompanied by the fast disappearance of otoacoustic emissions. The reasons for this process are pathological changes in the function of fibrocytes in the spiral ligament and in cells of the stria vascularis. Also, changes in the neurochemistry of the central auditory system accompany aging in F 344 rats. In the auditory cortex, the number of parvalbumin-positive neurons significantly decreases and calretinin immuno-positive neurons become more densely stained and the cross-sectional area of their somas increases. A possible means of preventing the start of presbycusis was recently demonstrated in C 57BL/6J mice by treating young animals with atorvastatin or by using caloric restriction.

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Electrophysiological approach to evaluation of CNS ageing

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Methods for an objective evaluation of CNS ageing are very limited and boundaries between non-pathological (biological) brain ageing and age-associated pathologies (e. g. dementias) are unclear. Considering the relationship between biological ageing of the brain and cognitive ageing, we tried to compare the ageing effect on three different levels of visual information processing with the use of three types of visual evoked potentials (VEPs).

In 133 healthy volunteers at age of 19 - 83 years (38 ± 17 years) we examined reactions of the primary visual cortex (to pattern-reversal stimuli), responses of extrastriate areas to motion-onset and P300 wave in visual cognitive task (recognition of digits and letters).

The most distinct change of visual evoked potentials parameters (VEPs) was a prolongation of P300 latency representing highly significant delay of cognitive processes toward elderly (2 ms/1 year of age, r = 0.71), which is more expressed in men. Decreased cognitive functions due to ageing are signalized also by reduction of P300 amplitude (r = -.48). Motion processing (activity of the magnocellular system/ dorsal stream) seems to be more influenced by ageing (irrespective of gender) compared to the parvocellular system/ventral stream function according to age related changes in latencies of the motion-onset VEPs (increase of 0.5 ms/1 year) and of the pattern-reversal VEPs (0.3 ms/1year increase. More detailed info can be found on our Web page http://www.lfhk.cuni.cz/elf.

We believe that VEPs might contribute as an objective tool for recognition of physiological and pathological functional changes of the ageing brain.

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Laterality, cortical functions asymmetry, cerebellar dominance and hair whorl

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Body asymmetry has been studied not selectively in human beengs. Julius Verne has mentioned captains dysphoria over a lost of shell ulit with curious contralateral whorl. Handedness has been claimed to be a privilidge of man. Righthandness seems to be present in 90 per cent of people on this globe. Lefthanded people have the cortical symbolic functions localized in more than in 70% in the left hemisphere. About 4% of „pure“ righthanded people have speech centers localized in the right hemisphere. Variability of handedness has been found in twins. Cerebellar dominance seems to be in accordance with handedness. Clockwise hair whorl has been found in about 85–90% of righthanded persons. About 50% lefthanded people have counterclockwise hair whorl.

221 healthy school children 9–11 years of age have been studied by means of Edingurgh´s questionnaire and its modification. Five tests for increased passivity (decreased muscle tonus) on nondominant hands and legs were recorded. Cerebellar dominance is in our study strongly related to handedness. Hair whorl has been found as impropriate in about 15% of girls due to hair frizzle and care. Only one child from a group of eleven lefthanded has a counterclockwise hair whorl. Crossed footness and handedness is present in a high percentage. The puzzle of laterality is still a provoking event. Hair whorl, namely in boys, may be used as a complementary test for the laterality.

Bilateral changes of SDF-1 and its receptor CXCR4 in the dorsal root ganglia of chronic constriction injury and spinal nerve ligation models of neuropathic pain

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There is compelling evidence indicating that hyperalgesia, allodynia and ongoing pain associated with peripheral nerve injury are due to changes in the DRG. Chronic constriction injury (CCI) and spinal nerve ligation (SNL) are most frequently used experimental models of neuropathic pain based on nerve injury.

Chemokines are small chemotactic cytokines which produce their effects by activating a family of G-protein coupled receptors. Some recent studies have shown that regulation of chemokines is one of the mechanisms underlying the development and maintenance of neuropathic pain. The biological activity of stromal cell-derived factor (SDF1) is signaled through the chemokine receptor CXCR4 which is unique among chemokine receptors, having only one known ligand. The role of SDF1/CXCR4 in the peripheral nervous system is implied by their early expression in neural crest cells/derivatives as well as in the dorsal root ganglia (DRG). The goal of our experiments was to compare an immunohistochemical staining for SDF-1 and CXCR4 proteins in the L4-L5 DRG of naive rats and those operated for unilateral L4-L5 SNL and CCI of sciatic nerve.

The naive DRG displayed a sharp immunofluorescence for SDF1 (SDF1-IF) at the surface of neuron/satellite glial cell units. A significant decrease of SDF1-IF was induced bilaterally in the L4-L5 DRG 3 days after both CCI and SNL. A diffuse immunofluorescence for SDF1 was found in SGC after both types of nerve injuries for 1 and 2 weeks with a higher intensity following CCI.

CXCR4-IF was present in the small- and medium sized neurons and the satellite glial cells (SGC) enveloping the large-sized neurons of naive DRG. CXCR4-IF was unchanged in the small- and medium sized neurons in the DRG from operated rats for all periods of survival. A significant reduced CXCR4-IF was observed in the SGC of contralateral DRG after both types of nerve injuries, but was elevated at the surface of large-sized neurons of ipsilateral DRG when CCI was applied. Latter pattern of staining was not significantly developed following SNL.

Our results suggest different regulation of SDF1/CXCR4 expression in the DRG during various periods of survival and by the type of nerve injury used in neuropathic pain models.

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The role of peripheral and central TRPV1 receptors in pain transmission and modulation

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Transient receptor potential vanilloid (TRPV1) receptors are expressed on both the peripheral and central branches of dorsal root ganglion neurons. The aim of this study was to examine the role of the TRPV1 receptors in cutaneous hypersensitivity, present after surgical tissue injury and to evaluate the effect of intradermal and intrathecal TRPV1 antagonist and endogenous agonists treatment. Paw withdrawal responses to mechanical stimuli of plantar skin with von Frey filaments and to thermal stimuli with radiant heat were tested before and at several time points after the surgery in rat plantar incision model. Magnitude of central sensitization changes was also judged by the number of spinothalamic (STT) and postsynaptic dorsal column (PSDC) neurons expressing c-Fos. In the control group of animals treated with vehicle, mechanical and thermal sensitivity increased significantly following the incision. TRPV1 antagonist (SB 366791) applied intradermally slightly attenuated the development of thermal hyperalgesia and mechanical allodynia. Intrathecal application of this TRPV1 antagonist was much more effective, it greatly reduced postoperative thermal hyperalgesia and also attenuated mechanical allodynia. High concentration intradermal capsaicin application reduced the number of STT and PSDC neurons expressing c-Fos after the incision. Intrathecal application of an endogenous TRPV1 agonist N-oleoyldopamine (OLDA) did not change sensitivity to peripheral thermal stimuli. However, it potentiated the effect of intrathecal bradykinin application. These results were further studied using patch clamp recordings from superficial dorsal horn neurones in spinal cord slices. Our results show that intrathecal application of TRPV1 antagonist can reduce postoperative thermal and mechanical hypersensitivity and that both peripheral and central TRPV1 receptors play an important role in pain transmission and modulation.

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Molecular Properties of P2X Receptor-Channels

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The P2X receptors (P2XRs) are a family of ATP-gated ion channels expressed in numerous excitable and nonexcitable cells that play important roles in the control of cellular functions such as neurotransmission, hormone secretion, transcriptional regulation, and protein synthesis. P2XRs are homomeric or heteromeric proteins, formed by assembly of at least three subunits, termed P2X1-P2X7. All subunits possess intracellular N- and C-termini, two transmembrane domains and a large extracellular ligand-binding loop. ATP binds to ectodomain, leading to a sequence of conformational transitions between closed, open and desensitized states of channel. No crystal structures are available for P2XRs and receptors have no obvious similarity to other ion channels or ATP binding proteins. These factors limit progress in the understanding of relationships between molecular structure and receptor function. To identify regions important for binding and gating, we used homology modeling of P2XR based on secondary structure similarities between the Lys-180-Lys-326 ectodomain region of P2X4R and the class II aminoacyl-tRNA synthetases. The interplay between homology modeling and site-directed mutagenesis identified several residues that could contribute to ATP binding, including Asp-280, Lys-190, and Arg-278. Using the same approach, we also found that Tyr-315 is the last ectodomain residue that could contribute to the agonist binding module, and Ile-317–Ile-333 sequence could operate as a signal transduction module, with Gly-316 serving as a flexible residue linking the binding and transduction modules. Using ivermectin, a positive allosteric modulator of P2X4R, we were also able to identify the critical roles of Lys-67, Lys-313, and Arg-295 residues in forming the proper three-dimensional structure of receptor. Furthermore, alanine and cysteine scanning mutagenesis of the two transmembrane domains combined with ivermectin treatment helped us to identify amino acid residues facing the pore site and playing important role in gating, including Tyr-42, Gly-340 and Asp-354, as well as residues facing lipids in the open conformation state. We also studied the relevance of the N- and C-terminal structures on the channel activity. Experiments with P2X2R and P2X7R revealed that both termini contribute to the control of gating. The efficiency of bioluminescent resonance energy transfer between luciferase and fluorescent proteins attached to the N- or C-termini of P2X2R subunits also suggested that the extent of subunit interactions prior to ATP application could contribute to conformational changes associated with agonist-dependent desensitization.

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Comparison of agonist-stimulated G-protein activity in brain cortex membranes isolated from control and morphine-treated rats

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Rat brain cortex was fractionated by two-step density gradient centrifugation. Plasma membrane fraction isolated in PercollR-gradient was sub-fractionated into the bulk of plasma membranes (BPM) containing the major part of PM markers Na, K-ATPase, G-protein coupled receptors, trimeric G protein α and ß subunits, caveolin(s) and adenylyl cyclases I/II and the low-density membrane compartment, LPM. Functional activity of trimeric G protein(s) was measured in PM compartments of different density as agonist-stimulated [32P]GTPase. All PM fractions were characterised by high level of basal high-affinity GTPase. Baclofen (GABAB-receptor) was the only GPCR agonist significantly increasing this activity.

Considering the high basal GTPase in PM isolated from the brain tissue, we tried to distinguish among different [35S]GTPγS binding sites and improve the assay conditions for detection of agonist-stimulated G-protein activity. Saturation binding curves were measured in wide range of [35S]GTPγS concentrations in the presence or absence of increasing concentrations of GDP. Under optimum conditions, baclofen (GABAB-R agonist), DAMGO (DOR agonist) and DADLE (MOR agonist)-stimulated binding represented 193±33%, 158±23% and 135±16% of the basal level, respectively. Net-increment of agonist-stimulation was 17 ± 4, 11 ± 3 and 7 ± 3 pmol.mg-1.

These methodological improvements were used in the last step of our work for comparison of OR-stimulated G-protein activity in PM isolated from morphine-treated and control animals. The net-increment of DADLE (DOR)-, DAMGO (MOR)- and U-23554 (KOR) stimulated [35S]GTPS binding was measured at 30 μM GDP. Our data indicated that there was not significant difference between the two groups. This conclusion applied to the absolute level of agonist-stimulated component (net-increment) as well as relative proportion between agonist-stimulated and the basal level (expressed as % of basal level of binding, 100%). The order of potency among different agonists was: baclofen > DADLE > DAMGO > U-695 93 and was the same in PM isolated from control and morphine-treated rats. It may be therefore concluded that the prolonged exposure of experimental animals (Wistar rats) to morphine does not influence the OR-mediated increase of overall G protein activity in rat cerebral cortex when performed under experimental conditions employed in this study. This occurs together with the marked effect of morphine on behaviour of these animals characterised by typical drug dependence.

Opposite effects of rapacuronium on acetylcholine binding and signaling at muscarinic acetylcholine receptors

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One of a promising approaches to alleviate symptoms in neurodegenerative diseases (e.g. Alzheimer disease) is to support synaptic transmission of degenerating or damaged neurons with allosteric drugs which potentiate binding of their natural neurotransmitter to postsynaptic receptors in a subtype-specific manner that would enable efficient targeting of distinct functions. Our previous finding of the positive cooperativity between allosteric modulator thiochrome and natural transmitter acetylcholine (ACh) at muscarinic M4 receptor that displayed absolute subtype specificity attested viability of this concept (1). In our present study of interactions between ACh and allosteric modulator rapacuronium at muscarinic receptors we found that rapacuronium binds to all five subtypes of muscarinic receptors (M1-M5) and strongly diminishes both high and low affinity binding of ACh at all subtypes. In contrast to these findings we demonstrate in functional assays that rapacuronium enhances receptor activation, particularly at M3 receptor, during a brief period after ACh application. We show that the binding of GTPγS to membranes and accumulation of inositol phosphates in intact CHO cells expressing M3 receptor subtype induced by 10 μM acetylcholine are accelerated by 1 μM rapacuronium. In accordance with these findings we show that calcium signal after 5 second stimulation by 300 nM ACh relaxes more slowly in the presence of 1 μM rapacuronium. These results are in line with the view that allosteric modulators not only change occupancy of agonist but also induce conformation different from that of receptor-agonist complex. The implication of these findings is the necessity to employ fast functional assays which much better resemble physiological conditions than the long lasting equilibrium binding experiments, in screening for potential allosteric enhancers of neurotransmition.

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Is nonquantal neurotransmitter release always nonvesicular?

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There is considerable evidence for a continuous release of ACh from motor nerve terminals associated with the activity of vesicle ACh transporter (VAChT) inserted into the nerve membrane during exocytosis. Early experiments implied the existence of NQR from the hyperpolarization of the muscle (the ĹH-effectÍ) at the NMJ in response to block of nAChRs in muscles in which the AChE activity had also been blocked. Normal AChE activity would prevent this effect but appears to allow enough ACh to reach the muscle to activate nAChRs. This in turn appears to trigger a postsynaptic phosphorylation cascade that involves both Ca++ and NO as second messengers. This cascade suppresses the activity of an inward Cl- transporter and this supports the local hyperpolarization of the muscle in the region of the NMJ, thus helping to maintain muscle excitability. A number of factors including AChE activity and purines released from the nerve appear to influence the magnitude of NQR and the effects resulting from it. VAChT hypothesis indicates that NQR is a residue of the quantal release. During synapse formation and reinervation, NQR appears earlier, by about 3 days, indicating the nonvesicular nonquantal release. This important fact is furher discussed. While the true physiological significance of NQR is slowly emerging, it seems that a „byproduct“ of the exocytosis causing ACh release may play a significant role in regulating postsynaptic excitability at the NMJ. A number of factors including AChE activity and ATP released from the nerve also influence the magnitude of NQR and the effects resulting from it are quite substantial.

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Clinical application of BOLD fMRI

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Blood oxygen level dependence (BOLD) contrast is a powerful tool in functional magnetic resonance imaging (fMRI). BOLD is based on different magnetic properties of oxyhemoglobin and deoxyhemoglobin. While oxyhemoglobin is diamagnetic i.e. has no specific magnetic properties, deoxyhemoglobin is paramagnetic and thus causes inhomogeneities of the local magnetic field that can be detected with a sensitive imaging sequence. Thus, BOLD does not show directly the synaptic neuronal activity but rather the increase in the regional cerebral blood flow (rCBF) with a relative increase of oxyhemoglobin in activated areas. The deoxyhemoglobin effect (on T2 and T2* relaxation), responsible for BOLD, is roughly quadratically dependent on the external magnetic field strength.

Using various paradigms BOLD can detect different brain centers and also relate them to various pathologies. BOLD has been widely used in neurosurgery, neurology, psychiatry and neuroscientific research.

The resulting activated areas are displayed as statistical maps that are displayed over the morphological images.

We present our experience with BOLD fMRI in the routine intraoperative neurosurgical navigation especially for brain gliomas where the functional blobs are incorporated into the 3D brain imaging. Furthermore, we present the BOLD data from deep brain stimulation (DBS) for Parkinson´s disease, in lateralization of speech center in epileptology and in the combination with transcranial magnetic stimulation in some extrapyramidal disorders.

New method for non-invasive measurement of head position

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Background
Quantitative assessment of head alignment in space has potential importance in many diseases of nervous system – movement disorders, neuro ophthalmology and vestibular disorders. Surprisingly little is known about limits of natural head position in healthy human, particularly in torsional (yaw) plane. Today, head position is evaluated by simple assessment, by deviation from plumb line or by sophisticated or invasive techniques (eg. magnetic search coil technique for eye-head co registration in vestibulo-ocular reflex studies, X-ray evaluation of head posture in orthodoncy). In view of these shortcomings, we have proposed a simple, non-invasive, but precise method of undisturbed head position in all 3 dimensions of space.

Methods
Authors developed a simple, non-invasive method of head position measurement. Pictures of the head with marks placed on tragus and eye outer canthus on each side are taken simultaneously by two digital cameras aligned in space by laser beam. Angle deviation of orbito-meatal and interorbital lines from horizontal plane and head rotation are automatically evaluated by digital picture analysis.

As evidenced by experiments done with mechanical head model, head position is measured with precision of 0.5 degree in all three dimensions (rotation, flexion and inclination).

Results
We present details of measurement methodology and results from a control group consisting of 100 healthy volunteers.
Mean values of head position in control subjects: retroflexion 21 deg, inclination to the right 0.2 deg, and head rotation to the left 1.7 deg.
Illustrative cases of acute vestibular failure, torticollis and compensatory head posturing in ocular muscle palsy are shown.

Conclusions
New method of non-invasive, high precision head posture measurement using digital picture analysis is presented.
Results from a control group and clinical examples are demonstrated.

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Effects of LiCl/PILOCARPINE-induced status epilepticus on rat brain benzodiazepine receptor binding: regional and ontogenetic study

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Convulsive status epilepticus (SE) represent highly destructive and epileptogenic brain insult. Severity of SE-induced damage as well as incidence of epilepsy increases with age at SE onset. There is however no clear correlation between severity of morphological damage and epileptogenesis or seizure pattern. Recent data demonstrate that changes in receptor structures play an important role in changes of brain excitability and development of epilepsy. The involvement of GABA receptors in seizure development and also their role during epileptogenesis was well documented by neurochemical, pharmacological as well as electrophysiological studies. Some studies suggest that the role of GABA receptors in epileptogenesis can be age-related, data are however limited. Thus, present study was designed to extend present knowledge about SE-induced changes of GABA receptors at different levels of brain maturation.

In present study, LiCl/pilocarpine-induced status epilepticus (SE), which leads to development of epilepsy in all adult andin a subpopulation of immature rats (Kubova et al, EJN, 2004), was induced in P12, P25 and/or adult rats. Using in vitro autoradiography, benzodiazepine (BDZ) receptor binding was evaluated one week (early phase of epileptogenesis) and 3 months (chronic phase) after SE in 27 brain structures, involved in seizure generation and spread (amygdala complex, hippocampus, basal ganglia, thalamic nuclei and several cortical regions). The pattern of receptor binding changes was highly related to the age at SE, interval after SE and to the brain structure evaluated. One week after SE, enhanced BDZ binding was found in P12 group in most of cortical areas and in both P12 and P25 groups also in the amygdala complex and dentate gyrus. No changes of BDZ binding occurred in adults at that time point, but three months after SE a decrease of binding was found in both adult and P25 rats in all evaluated areas. In P12 group, a significant decrease of BZS binding occurred only in the entorhinal cortex. The decrease of binding did not reflect neuronal loss. Our data support hypothesis that age-related pattern of changes of receptor properties may participate in different functional consequences of SE including epileptogenesis and altered behavior.

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Neuronal degeneration induced by status epilepticus in the nucleus accumbens and in the olfactory tubercle of immature rats

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The basal forebrain consists of a number of closely related nuclei and regions with complicated architecture. Prominent among these are the nucleus accumbens septi (NAS) and the olfactory tubercle (OT) which share morphological and chemical characteristics with striatal regions and are considered the major component of the ventral striatum. Nucleus accumbens is divided into ventromedial shell and dorsolateral core compartments. Olfactory tubercle exhibits in macrosmatics laboratory animals laminated structure. Both compartments of the nucleus accumbens and the olfactory tubercle differ in structure and functions. In an effort to better understand the development of neuronal damage in the striatopallidal complex after status epilepticus (SE) neuronal degeneration was analyzed within both compartments of the NAS and in the olfactory tubercle.

Experiments were carried out in Wistar pups 25 days old. Lithium-pilocarpine model of SE was used. The rats survived for 4, 8, 12, 24, 48 hours and 1 week after SE. Degenerated neurons were detected with a fluorescent stain Fluoro - Jade B (FJB). The basal forebrain was partitioned into different nuclear groups according to Paxinos and Watson (1997).

A small to moderate number of FJB – positive (degenerated) neurons was found 4–12 hours after SE in the NAS. These neurons were distributed in the shell compartment of the NAS. The core region contained only isolated degenerated neurons in short intervals after SE. At survival interval 24 hours the number of degenerated neurons in the shell subdivision of the NAS significantly increased and FJB – positive neurons were evident in the whole extend of the nucleus. At longer survival intervals (48 h, 1 week) degenerated neurons persisted in the shell subdivision of the NAS but their number was significantly reduced. In the ventral pallidum was negative finding. In the OT degenerated neurons were discernible for the first time 12 h after SE and prevailed in deeper layers.

In summary, data show that the lithium – pilocarpine model of SE resulted in degeneration of neurons not only in the dorsal striatum but in the same survival intervals after SE also in the nucleus accumbens and in the olfactory tubercle. In all survival intervals degenerated neurons significantly prevailed in the shell subdivision of the NAS. In the core subdivision of the NAS were degenerated neurons rare. Olfactory tubercle was less damaged than shell compartment of the NAS. In the ventral pallidum was in all survival intervals negative finding.

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Effects of two GABA-B receptor agonists on cortical epileptic afterdischarges in immature rats are not identical

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RATIONALE: Baclofen, a classical GABA-B receptor agonist, was found to elicit both anticonvulsant and proconvulsant effects. In addition, these effects change during postnatal development. Therefore we compared the effects of baclofen with another agonist SKF97541 to know if these actions are a general feature of GABA-B receptor agonsts or if they are specific for baclofen.

METHODS: Epileptic afterdischarges (ADs) elicited by stimulation of sensorimotor cortex were used as a model. To study developmental changes 12-, 18- and 25-day-old rats were used. Animals with implanted electrodes were repeatedly stimulated with 15-s series of 1-ms pulses at 8-Hz frequency. Intensity of stimulation current was stepwise increased with repeated stimulations from 0.2 to 15 mA. Thresholds for four phenomena (movements directly elicited by stimulation, epileptic ADs characterized by spike-and-wave EEG rhythm, clonic seizures bound to this type of ADs and a transition into the second, mixed type of ADs with features characteristic for ADs generated in limbic structures) were established, intensity of motor phenomena was scored and duration of ADs was measured.

RESULTS: Decreased intensity of motor phenomena was observed after either agonist, i.e. they exhibited an anticonvulsant action. At the same time proconvulsant effects were registered - decreased threshold intensities necessary for elicitation of mixed ADs, for transition of epileptic activity into limbic structures and significant prolongation of ADs. SKF97541 is much more potent than baclofen. Differences between actions of the two drugs were also found. SKF97541 increased threshold intensities of stimulation current necessary for elicitation of stimulation-bound movements, spike-and-wave type of ADs and accompanying clonic seizures in 12- and 18-day-old rats, baclofen did not exhibit this effect. Quantitative differences were found with all effects. They were clearly seen in suppression of intensity of both motor phenomena (movements and clonic seizures) was more marked with SKF97541 in 12- and 18-day-old rats than with baclofen where this effect was only marginal.

CONCLUSIONS: Differences between the action of the two GABA-B receptor agonists may be due to their action on different subsets of receptors but further studies are necessary.

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POSTERS

Possible role of the Ca^{2+}/calmodulin dependent kinase II phosphorylation site T704 in acute desensitization of the vanilloid receptor TRPV1

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Capsaicin-induced desensitization of the Transient Receptor Potential Vanilloid receptor-1 (TRPV1) is one of the key strategies for the treatment of neuropathic and inflammatory pain. This process is initiated by TRPV1 receptor activation and the subsequent entry of extracellular Ca^{2+} through the channel into primary afferent neurons. It is generally assumed that TRPV1 desensitization is caused by dephosphorylation by the Ca^{2+}/calmodulin-dependent enzyme, phosphatase 2B (calcineurin). Of several consensus phosphorylation sites identified so far, the most notable are two sites for Ca^{2+}/calmodulin dependent kinase II (CaMKII) at which the dynamic equilibrium between the phosphorylated and dephosphorylated states presumably regulates the binding of the agonist. Using whole-cell patch-clamp technique in HEK293T cells expressing the wild type or CaMKII phosphorylation site mutants of TRPV1, we investigated the mechanisms of acute Ca^{2+}-dependent desensitization. The nonphosphorylatable mutant S502A/T704I was capsaicin-insensitive but the S502A/T704A construct retained its full function, indicating a requirement for a specific residue at position 704. Alanine mutation at the nearby conserved residue R701 strongly affected the heat, capsaicin and pH-evoked currents. As this residue constitutes a stringent CaMKII consensus site but is also predicted to be involved in the interaction with membrane phosphatidylinositol 4,5-bisphosphate (PIP2), this data suggests that in addition to dephosphorylation, or as its result, a short C-terminal juxtamembrane segment neighbouring to the TRP box comprised of R701 and T704 might be involved in the decelerated gating kinetics of the desensitized vanilloid TRPV1 channel.

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PKA dependent effect of pregnenolone sulfate on NMDA receptors expressed in hippocampal neurons

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Activity of NMDA receptors can be modulated by neurosteroids, the substances of steroid origin, having a direct nongenomic effect on neuron excitability.

We examined mechanism of action of endogenous neurosteroid pregnenolone sulfate (PS), a positive modulator of NMDA receptor activity. Our surprising finding was that, when PS was pre-applied to outside-out patches pulled from hippocampal neurons grown in the culture, strong potentiating effect of PS rapidly decayed in repetitive applications. After the first PS pre-application (300 μM), NMDA currents were potentiated 2.11±0.11 times relative to control, but after fifth cycle, there was practically no potentiation, 1.01±0.07, n=6. In whole-cell mode, degree of PS potentiation was unchanged after first (3.02±0.17) and fifth application (2.94±0.06), n=6. We also tested effect of endogenous neurosteroid pregnanolone sulfate (3α5ßS), but there was no difference in time-course of its effect on patches and whole cells.

The activity of NMDA receptors is influenced by their phosphorylation status. We tested the effect of substances that influence the activity of neuronal kinases or phosphatases. Addition of cyclosporine, okadaic acid, PKA catalytic subunit or BAPTA to intracellular solution (or combination of these) produced significant changes both in degree and the time-course of PS potentiation on NMDA receptors patches, slowing its decay.

In whole cells, bath application of staurosporine (1h) reduced the degree of PS pre-application to 2.02±0.12 times (n=6). Exposing the cells to NMDA (100 μM) for 30 s at the half of staurosporine application further decreased the PS potentiation to 1.68±0.05 times (n=6), but subsequent application of 8-Br-cAMP (500 μM, stimulator of PKA) partially recovered the PS potentiation to 2.33±0.09 (n=6). The subsequent application of phorbol 12-myristate 13-acetate (0.5 μM, stimulator of PKC) produced no recovery 1.72±0.16 (n=6).

To specifically inhibit PKA, we bathed the cells in H-89 (50 μM) for 1 h, which reduced PS potentiation to 2.18 ± 0.23 times (n=6). Interposing NMDA application (30 s) further reduced the PS effect to 1.83±0.13 times (n=6). As expected, the PS potentiation was recovered by subsequent application of 8-Br-cAMP (500 μM) to 2.58±0.13, n=6.

Our conclusion is that the potentiating effect of PS on NMDA receptors strongly depends on their phosphorylation status and is primarily related to activity of protein kinase A.

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The changes in phosphatidyl inositol 4.5 bisphosphate (PIP2) levels related to the TRPA1 channel activity

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The Transient Receptor Potential Ankyrin receptor-1 (TRPA1) is a nonselective cation channel that is gated in response to pungent chemicals found, e.g. in mustard oil, wasabi, horse radish and garlic. In primary nociceptors, this channel is also activated by an inflammatory peptide bradykinin known to stimulate the activity of phospholipase C which, in turn, may influence the concentration of phosphatidyl inositol bisphosphate (PIP2). Although this membrane lipid is supposed to be required for TRPA1 function, it is not clear to what extent it could alter the channel activity. We examined the dynamics of PIP2 in the plasma membrane during TRPA1 activation by using the PIP2-sensitive potassium inward rectifier ion channel (Kir2.1) coexpressed with TRPA1 in HEK293T cells, and by measuring whole-cell inward currents elicited by allyl isothiocyanate (AITC; 100-200 μM) in the presence of 2 mM Ca^{2+}. We found that intracellular dialysis with anti-PIP2 antibody or with the substances lowering PIP2 (e.g. PIP2 scavenger poly-L-lysine, activation of endogenous PLC, and inhibitors of PI-kinases required for PIP2 synthesis) significantly reduced the Kir 2.1 channel activity. The activation of TRPA1 by AITC caused a substantial suppression of Kir2.1 currents suggesting that the activation of TRPA1 results in potent depletion of PIP2, presumably due to the Ca^{2+} influx. These results form a starting point for future studies aimed at the unraveling of the exact molecular mechanisms underlying PIP2 modulation of TRPA1.

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Fluorometry of thermosensitive TRP channels: more than complementary method for understanding structure-function relations

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Thermosensitive transient receptor potential (TRP) receptors are cation-selective ion channels that are gated in response to changes in ambient temperature. In primary nociceptors, these channels are also activated by pungent irritant plant-derived chemicals found, e.g. in red pepper, peppermint oil, mustard oil, horse radish or garlic. Recent electrophysiological, pharmacological and biochemical studies have provided considerable insights on how these channels contribute to thermosensation, but many aspects of their regulation remain elusive.

In the present study, we set out to develop an approach for exploring agonist- and temperature-induced activity of thermosensitive TRP channels by using fluorescence imaging techniques, which in combination with electrophysiological studies, can provide a sensitive and specific read-out of channelĺs gating and ion selectivity functions. By using fura-2AM ratiometric Ca^{2+} imaging, we examined the changes in $[Ca^{2+}]i$ induced by applications of heat or chemical stimuli in HEK293T cells transiently transfected with either TRPV1, TRPV4, or TRPA1. We also attempted to characterize the factors that could generally influence the multimerization of thermosensitive channels by using the fluorescence resonance energy transfer technique on TRPV4/TRPV6 labeled with CFP and YFP (1). Our initial results prove the feasibility of microfluorometry to be used for future studies aimed at the unraveling of the unique molecular mechanisms underlying thermal and chemical activation and structure-function relationships among thermosensitive TRP channels.

(1) Hellwig et al, J Cell Sci. 2005; 118:917-28.

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TRPV1 endogenous agonist N-oleoyldopamine (OLDA) modulates nociceptive synaptic transmission in the spinal cord

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Transient receptor potential vanilloid (TRPV1) receptors are expressed predominantly in a subpopulation of primary sensory neurons, that convey nociceptive information from the periphery to the spinal cord dorsal horn (DH). The TRPV1 receptors are expressed on both the peripheral and central branches of these dorsal root ganglion neurons and can be activated, beside capsaicin, by heat, low pH and also by recently described endogenous lipids derived from dopamine. Using patch-clamp recordings from superficial DH neurons in acute spinal cord slices prepared from 21 days old rats, the effect of N-oleoyldopamine (OLDA) application on the frequency of miniature excitatory postsynaptic currents (mEPSCs) under different temperature conditions and after protein kinase C (PKC) activation was evaluated. At room temperature (24°C) high concentration of OLDA (10μM) was needed to increase the frequency of mEPSCs, while low concentration of OLDA (0.2μM) did not evoke any change. However, after activation of PKC by phorbol 12-myristate 13-acetate (PMA, 1μM), application of the low concentration of OLDA increased significantly the frequency of mEPSCs. The control frequency of mEPSCs was increased significantly when the bath temperature was raised to 34°C. Changes in mEPSCs frequency induced by OLDA application were similar to those obtained at 24°C. Our results suggest that central TRPV1 receptors could play an important role in modulation of nociceptive signalling at the spinal cord level. The presumed endogenous agonists of these receptors, like OLDA, could have a considerable impact on synaptic transmission at DH especially under conditions when TRPV1 receptors are phosphorylated.

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In vitro calcium imaging of spinal cord dorsal horn neurons

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The aim of our study was to develop a technique to study mechanisms of central sensitization in vitro, on population of dorsal horn neurons. Acute spinal cord slices (300-400 μm) with spinal roots attached were prepared from rats 12-15 days old. The slices were incubated with Fluo-4 AM dye. Intracellular calcium concentration changes were recorded with Leica LSM confocal microscope. Activity of the neurons evaluated as relative fluorescence changes was evoked by electrical stimulation of the dorsal rootlet by control stimulation (10 Hz, 1s duration, 0.5ms pulse length). Control stimulation resulted in increased calcium concentration in number of dorsal horn neurons. The calcium changes were confirmed as neuron specific by using an in vivo glial marker sulforhodamine 101 and KCl solution of low and high concentration. Repeated control stimulations led to consistent changes of calcium concentration in the neurons recorded. Rate of calcium peak onset and sensitivity to AMPA and NMDA receptor antagonists CNQX and MK801 and to Ca^{2+} store depletor caffeine allowed to distinguish two types of neuronal responses to the stimulation. First type was potentiated by caffeine, suppressed by CNQX and MK801 coapplication and its calcium peak onset followed immediately after the stimulation. The second type of calcium response was highly reduced by caffeine application and the calcium peak onset was delayed 3 to 8 s after the stimulation. Application of NK1 receptor antagonist decreased the delayed response to stimulation in number of neurons suggesting involvement of neurotransmitters of peptidic origin and activation of metabotropic receptors. Both calcium concentration changes were totally suppressed by application of sodium channel blocker TTX. Our data suggest possible important role of internal calcium stores activated by metabotropic receptor pathway in pain transmission and possible participation of internal stores in induction of the central sensitization.

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Chronic constriction injury in weanling rats

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Neuropathic pain is known to appear in infants and younger children, however, the incidence is much lower than in school-age children and adults. Development of neuropathic pain was studied in modified peripheral neuropathic pain model in weanling rats. In order to evoke pain, two ligations (chronic constriction injury – CCI) were placed on right sciatic nerve at postnatal day 22. CCI evoked significant difference in paw withdrawal latency between the hindlimbs in response to thermal stimulation ten days later. Both non-significant increase of pain threshold in contralateral limb and non-significant decrease of pain threshold in ligated limb contributed to the difference. Regarding mechanical stimulation, non-significant difference in paw withdrawal latency between the hindlimbs was observed. In sham operated rats, significant difference between the hindlimbs appeared neither in mechanical nor in thermal stimulation. Our results show that thermal hyperalgesia, but not mechanical allodynia, develops following modified CCI in weanling rats.

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Quantitative changes of IL-6 protein are presented not only in the lumbal, but also in the cervical DRG following unilateral sciatic nerve injury

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Several lines of evidence suggest that pro- and anti-inflammatory cytokines contribute to both the induction and maintenance of neuropathic pain derived from cellular and molecular changes in the DRG including the activity of the primary sensory neurons.
Interleukin-6 (IL-6) is a pleiotropic cytokine of the family containing IL-11, ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), leukemia inhibitory factor (LIF), oncostatin M. A significant involvement of IL-6 in neuropathic pain induction and modulation of nerve regeneration is suggested from several experiments. Chronic constriction injury (CCI) of sciatic nerve is well-characterized experimental model of neuropathic pain. We used this model to study quantitative changes of IL-6 protein in the rat cervical and lumbal DRG. ELISA was employed to quantify IL6 protein in both ipsi- and contralateral cervical (C-DRG) as well as lumbal (L-DRG) dorsal root ganglia from naive rats and those operated for unilateral CCI of sciatic nerve with periods of survival for 1, 3, 7, and 14 days.

One day after CCI, an increase of IL-6 protein was measured in the ipsilateral cervical dorsal root ganglia (C-DRGi) with a peak on day 3. On day 14 from operation a level of IL-6 protein was close to the control baseline level. Contralateral cervical ganglia (C-DRGc) displayed only a mild decrease of IL-6 protein on day 7. No significant changes were found in other periods of survival, and the IL-6 protein level reached to control baseline levels on day 14. In comparison with the naive DRG, the IL-6 protein of ipsilateral lumbal ganglia (L-DRGi) elevated from postoperation day 1, and the higher level of IL-6 remained up to 14 days from operation. The IL-6 protein level in the contralateral lumbal ganglia (L-DRGc) peaked on day 3, and enhanced level was measured till 14 days.

These data provide evidence for changes of IL-6 protein levels not only in the DRG associated with damaged nerve, but also in the DRG non-associated with nerve injury in the rat experimental neuropathic pain model.

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Engraftment of donor cells in CNS depends on proper timing of host irradiation after preconditioning with cyclophosphamide

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INTRODUCTION
The presence of donor bown marrow (BM) derived cells in CNS seems to have neuroprotective effect in animal models of various neurological disorders. The positive effect of BM transplantation or human umbilical cord blood transplantation was observed in mice model of ALS, ischemic brain lesion and traumatic spinal cord lesion. We believe that the crutial factor in the process of neuroprotection madiated by BM derived cells is the long term survival of the donor cells in CNS.

AIM
We studied the factors that affect the long term survival of BM derived donor cells in host CNS after BM transplantation. We have tested the hypothesis that the successful BM engraftment is one of the most important features that increses the pool of donor cells in host CNS.

METHODS
Three gopups of C57BL/6 mice were irradiated (4Gy, whole body irradiation) and transplanted with GFP+ BM (4*10exp6 nucleated cells). Group CY2 was preconditioned with cyclophosphamide (CY) (135mg/kg bw) two days before the BM transplantation (BMT) and group CY5 five days before the BMT. No CY was administrated in the control group. We evaluated the time course of BM chimerism and the number of GFP+ cells in the host CNS twelve month after BMT. The changes of microenvironment in BM after CY administration were analysed properly.

RESULTS
The number of CFU-S in BM after CY administration fell rapidly to its minimum of 10% on day 1 and raised again to the local maximum of 70% on day 3 and than decreased slowly. Percentage of CFU-S in S phase increased to its maximum (52%) on day 2 and reached the minimum on day 5, where almost none (1%) CFU-S in S phase were detected. The BM chimerism reached 87% in CY5 group, 25% in CY2 and 29% in the control group.
In the CNS the density of GFP+ cells (10exp-8/μm2) was 8.98 ($p<0.001$) in CY5 group, 1.44 in CY2 group and 1.60 in the control group.

CONCLUSION
The timing of BMT after preconditioning with CY strongly influence the BM engraftment. The microenvironment of high proliferating CFU-S on day 2 after CY administration probably interferes with the ability of the donor cells to keep in the BM in contrast to the BMT performed on day 5. The degree of BM chimerism strongly correlates with the long time presence of donor cells in CNS. Successful BM engaftment seems to be an important factor for maintaining the donor cells pool in CNS after BMT.

Electrophysiological characterization of D6/GFP-neural stem/progenitor cells during in vitro differentiation and after transplantation into the injured rat brain

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D6 is a promotor/enhancer of the mDach1 gene, which is involved in the development of the neocortex, including the ventricular zone and hippocampus, and is expressed in the proliferating neural stem/progenitor cells of the cortex (Machon et al., 2002, Neuroscience 4, 951-966). The differentiation potential of embryonic neural stem/progenitor cells isolated from the cortex of E12 mouse embryos, in which the expression of GFP is driven by the D6 promoter was, studied in vitro and after transplantation into non-injured adult rat brain as well as into the site of a cortical photochemical lesion. The electrophysiological properties of D6/GFP cells were studied using the whole-cell patch-clamp technique, and immunohistochemical analyses were carried out. Six days after the onset of in vitro differentiation, two cell populations were identified. Large flat cells forming an underlying layer expressed GFAP and/or nestin. Their average membrane potential (Vm) was -78.3 mV, membrane resistance (IR) 89.9 MOhms and membrane capacitance (Cm) 37.3 pF, and they displayed large passive K+ currents. Smaller cells with multiple long processes expressed neuronal markers such as ßIII tubulin, MAP-2 or DCX. These cells, with Vm of -61.2 mV, IR of 909.5 MOhms and Cm of 11.2 pF, expressed fast activating A-type K+- channels, delayed outwardly rectifying K+ channels and TTX -sensitive Na+-channels. One week after transplantation into intact tissue and 4 weeks after transplantation into the site of a photochemical lesion, the D6/GFP-cells survived and expressed markers characteristic of mature neurons, such as NeuN, NF68, ßIII tubulin and MAP2 and they displayed typical neuronal curent pattern. Based on these results, D6/GFP-cells could provide a suitable tool for studying neurogenesis in vivo as well as neural stem/progenitor cell survival, migration and differentiation under pathological conditions.

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Differentiation of neural stem cells expressing Sonic Hedgehog and Wnt-7a

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Sonic hedgehog (Shh) and Wnt-7a are secreted morphogens involved in neurogenesis of the adult brain. GFP-labeled P0 mouse neural stem cells expressing either Shh (Shh/GFP) or Wnt-7a (Wnt-7a/GFP) were used to study their effect on neural stem cell proliferation and differentiation in vitro. Electrophysiological characterization using the patch-clamp technique and immunohistochemical analysis were carried out 8 days after the induction of differentiation by retinoic acid; wild-type cells (WT/GFP) were used as a control. In WT/GFP cells three distinct cell populations were identified. Large flat cells with a cell-body diameter of 40μm formed an underlying layer (19%), expressed GFAP, and displayed passive, time- and voltage-independent K+ currents, with an average membrane potential (Vm) of -87 mV and input resistance (IR) of 61MOhms. The second population of cells (16%) with a triangular cell-body (diameter 25 μm) expressed GFAP or NG2 and predominantly displayed passive, time- and voltage-independent K+ currents together with an inwardly rectifying current activated by hyperpolarization. Their mean Vm and IR were -90 mV and 72 MOhms, respectively. The third group of cells (65%) with a cell-body diameter of 15μm (termed neuron-like cells) were MAP-2, DCX or ß-III tubulin positive with a mean Vm of -83 mV and IR of 357 MOhms and mostly displayed voltage-dependent KA, KDR and TTX-sensitive Na+ currents (INa). Shh expression led to increased numbers of both flat and triangular cells, but their passive membrane properties were not significantly different from those in control cells. The number of neuron-like cells decreased by 25%, and moreover, INa currents were not detected. In Wnt-7a/GFP cells the number of large flat cells was decreased by 16% and the number of triangular cells increased by 23%, while the number of neuron-like cells was not significantly different when compared to controls. All three cell populations showed increased IR and decreased Vm; further, in neuron-like cells the INa amplitude was significantly increased. Based on electrophysiological data we can conclude that Wnt-7a/GFP cells showed marked differences compared to Shh/GFP and WT/GFP cells, thus these morphogens might play an important role in postnatal neural stem cell differentiation.

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Ischemia-induced Volume Changes in Astrocytes: The Role of Chloride Movement

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Volume changes of eGFP-labeled astrocytes during ischemia/reperfusion in situ were studied in the cortex of GFAP/EGFP transgenic mice using confocal microscopy combined with 3D reconstruction. Inhibitors of chloride channels (NPPB, DIDS, Cd^{2+}, tamoxifen), the K-Cl cotransporter (DIOA) and the Na-K-Cl cotransporter (bumetanide) were used to study the contribution of chloride movement to cell volume changes and regulatory volume processes. A 20-minute application of a solution modelling oxygen-glucose deprivation (OGD) revealed two populations of astrocytes: low response (LR) astrocytes and high response (HR) astrocytes. In LR-astrocytes, the application of OGD led to a small volume increase of 5%; subsequent 40-minute reperfusion led to complete volume recovery. In HR-astrocytes, the application of OGD evoked a marked volume increase of 41%; reperfusion led within the first 20 minutes to an additional volume increase with no apparent volume recovery. OGD application together with NPPB, DIDS or tamoxifen led to a smaller volume increase compared to OGD alone, while bumetanide and DIOA had no effect on the volume changes evoked by OGD. The application of DIDS, NPPB, Cd^{2+}, tamoxifen or bumetanide during reperfusion evoked a significant volume decrease. A pH shift from 7.4 to 6.8, modeled by ACSF (artificial cerebrospinal fluid) saturated with 85% O_2 / 15% CO_2 (ACSFpH) or by the addition of lactic acid, caused a volume decrease of 15% or 25%, respectively. Two populations of astrocytes were not detected during the shift of pH, as all measured cells displayed consistent volume changes. NPPB, DIDS, Cd^{2+}, or bumetanide applied together with ACSFpH reduced the volume decrease induced by pH 6.8, while tamoxifen evoked a volume increase. When applied during reperfusion, DIDS and Cd^{2+} caused cell swelling, NPPB and bumetanide did not affect cell volume and tamoxifen significantly inhibited volume recovery. Our data suggest the presence of two populations of astrocytes in the cortex of GFAP/EGFP mice that respond differently to ischemia and also imply an essential role for Cl^- movement, carried by the Na-K-Cl transporter and Cl^- channels, in volume regulation.

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Changes in the expression of K+ and Na+ channels in astrocytes and NG2 glia after ischemia in vivo

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Time-dependent changes of astrocyte and NG2 glia membrane properties were studied together with tissue damage and glial scar formation in hippocampus (CA1 region) of 8-week-old rats after global cerebral ischemia followed by reperfusion for 2, 6, 24 hours, 3, 7 and 35 days. Glia membrane properties were determined by the whole-cell patch-clamp technique and tissue damage in the CA1 region was evaluated using markers of neurons, glia, newly derived cells, apoptosis and proliferation. In both sham-operated rats and those after ischemia, astrocytes from the CA1 region displayed passive symmetrical non-decaying K+ currents with the additional expression of delayed outward K+ rectifier (KDR) and/or inward K+ rectifier (KIR) currents. NG2 glia displayed a complex current pattern, i.e. KDR, KIR, A-type K+ current and voltage-dependent Na+ currents (INa). Astrocyte depolarization was observed starting 2 hours after ischemia and their membrane capacitance was decreased from the first day after ischemia. The KIR current density in astrocytes was increased 6 hours after ischemia, when apoptotic markers appeared in neurons. After 5 weeks of reperfusion, apoptotic markers in CA1 astrocytes were detected, coinciding with an increase in the KDR current density. In NG2 glia, an increase in KDR, KIR and INa was observed 6 hours to 1 day after ischemia. Proliferation of NG2 glia was observed during the first week after ischemia. Moreover, NG2/nestin double positive cells were found in CA1 region 3D and 7D after ischemia. Our data show that global cerebral ischemia results in changes typical of neuronal damage and astrogliosis in the CA1 regio. Transient changes in membrane properties and expression of voltage-dependent K+ and Na+ channels occur both in astrocytes and NG2 glia and correlate with proliferation and nestin expression in NG2 glial cells.

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Human embryonic stem cells (CCTL14) differentiate into a neuronal phenotype

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Human embryonic stem cells (hESCs) demonstrate remarkable proliferative and developmental capacity. Clinical interest arises from their ability to provide an apparently unlimited cell supply for transplantation and from the hope that they can be directed to desirable phenotypes in high purity. The CCTL 14 ES cell line (derived by the Department of Molecular Embryology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic) has been cultivated in vitro for extended periods while maintaining the expression of markers characteristic of pluripotent primate cells (Nanog, Oct-3/4, SSEA-4). The karyotype was stable during the entire time of the experiments. We induced embryoid body (EB) formation using a Noggin (500 ng/ml) –4/+4 classical protocol and then a cocktail of basic fibroblast growth factor (bFGF) (20 ng/ml) and epidermal fibroblast growth factor (EGF) (20 ng/ml) during the next 8 days. Subsequently, the extent of neural differentiation in the EBs was assessed by analyzing the percentage of cells expressing markers of neural precursors, including neural cell adhesion molecule (NCAM) and Nestin. The percentage of NCAM- and Nestin-postive cells was 95.5±2.3% and 96.1±2.8%, respectively. Neuronal differentiation was induced by culturing the dissociated EBs on poly-D-lysine/laminin in growth medium supplemented with bFGF (10 ng/ml), NT-3 (10 ng/ml), brain-derived neurotrofic factor (10 ng/ml), insulin-like growth factor (1 ng/ml) and ascorbic acid (160 μM) for 4 weeks. During 14 days, cells displayed the morphology and also expressed the structural markers of immature neurons such as β-III-tubulin and PSA-NCAM. The finally differentiated cells expressed mature neuronal markers – NF160, MAP-2 and synaptophysin. Some cells were positive for glutamate and γ-aminobutyric acid (GABA). Electrophysiological recording of cell membrane currents indicated the presence of mature functional neurons in differentiated culture. These cells had sodium channels, responded to GABA and fired action potentials. We have developed a basic platform for the efficient and directed differentiation of hESCs and a potential source of cells for use in transplantation studies.

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Properties and growth of human bone marrow mesenchymal stem cells in different media

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Human mesenchymal stem cells (hMSCs) are mutipotent cells with the capacity to differentiate into various cell types such as osteoblasts, chondroblasts or myoblasts. Moreover, they have the ability to differentiate into non-mesenchymal cell phenotypes such as astrocytes or neurons. Therefore, hMSC can be an important tool for cell therapy and tissue engineering. The application of hMSCs in clinical medicine requires rapid cell expansion in media suitable for clinical use. For this reason, we tested the influence of several culture media on cell expansion and cell properties. In our study we examined the colony-forming properties (CFU - colony forming unit test), the doubling time (PD - population doublings) and cell cycle and the expression of surface markers. We isolated hMSCs from bone marrow obtained from healthy donors; the mononuclear fraction was collected using a Ficoll-gradient. Cells were seeded and expanded in different culture media: α-MEM (Gibco) containing penicilin/streptomycin with 2.5%, 5%, 10% or 20% fetal bovine serum (FBS), 5% or 10% human cord blood serum (hCBS), 5% or 10% human blood serum from AB adult donors (hABS) or hMSC-medium (Lonza).

We assesed the colony-forming properties by counting colonies with a diameter > 2 mm and calculating the total area of the colonies. The colony forming efficiency was best in α-MEM/hCBS and α-MEM/hABS, good in hMSC-medium and worse in α-MEM/FBS. Among FBS-containing media, a 10% concentration was the best and 2.5% and 20% the worst. The shortest time for PD was achieved in media enriched with human sera - 10% hABS followed by 5% hABS and hMSC-medium - and it gradually increased from 10% hCBS to α-MEM/FBS. The cell cycle was examined using BD Pharmingen PI/Rnase Buffer and assesed by FACS analysis. In all samples the proliferating fraction (S+G2/M) was < 10%, independently of the media used. Phenotyping of 16 surface markers was performed with a FACSAria: CD10, CD 29, CD34, CD44, CD45, CD49a, CD61/51, CD71, CD73, CD90, CD105, CD235a, CD271, MHC-I, MHC-II and fibroblast surface marker. CD34, CD45, CD235a, CD271 and MHC-II were negative and CD 90, CD105 and MHC-I were strongly positive with all tested media. CD10, CD29, CD44, CD49a, CD51/61, CD71 and fibroblast surface marker showed broad variability in α-MEM/hABS and hMSC-media. The expression of CD29, CD44 and CD73 decreased with lower concentrations of serum. The choice of serum influences hMSC expansion and cell properties; hABS seems to be a promising candidate for hMSC cell expansion in clinical use.

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Co-transplantation of olfactory ensheathing cells and mesenchymal stem cells improves hindlimb performance after spinal cord injury but does not show synergistic benefits

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Olfactory ensheathing cells (OECs) and mesenchymal stem cells (MSCs) have been used as promising candidates for transplantation therapy in the central nervous system. OECs are known as glial cells residing in the lamina propria of the olfactory mucosa and in the outermost layer of the olfactory bulb and the surrounding olfactory receptor neurons, which have a continuous turnover throughout life. MSCs have the pluripotency to differentiate into cells of mesenchymal origin and neurons. OECs express several growth factors and extracellular matrix molecules that provide a hospitable environment for both injured and remaining intact axons in the spinal cord and that promote axonal regeneration. MSCs produce growth factors and cytokines and excert their neuroprotective effects on neurons and oligodendrocytes by downregulating Caspase-3-mediated apoptosis after spinal cord injury (SCI). The transplantation of other cell types along with OECs benefits SCI treatment by inducing more effective neurotrophic support. In our study, the effects of OEC and/or MSC transplantation were examined. Both cell types are appropriate for autologous transplantation therapy. OECs were isolated from the lamina propria and MSCs from the bone marrow of one-month-old male Wistar rats. SCI was made 1 cm anterior to T10 by balloon inflation in adult male Wistar rats. One week after SCI, a total of 3x105 OECs and/or MSCs were transplanted into the injured spinal cord through a glass pipette connected to an automatic microinjector. Control rats received saline instead of cells. Hindlimb locomotor activity was assessed weekly by the Baso, Beattie, and Bresnahan (BBB) test. Motor evoked potentials (MEPs) were tested every week by transcranial electric stimulation. BBB scores improved in transplanted rats, but the results following co-transplantation of OECs and MSCs did not differ significantly from those following transplantation of one cell type alone. MEPs drastically decreased their amplitude from about 6mV to about 20μV following contusion and recovered partially, but never regained normal values throughout the experiments. OECs and MSCs obtained from green fluorescent protein transgenic rats showed that both types of cells survived in the spinal cord lesions, but did not migrate far from the injection sites during two months observation. Although OECs and MSCs had the ability to foster hindlimb recovery, the co-transplantation of both types of cells did not reveal any additional benefit.

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Mobilization of bone marrow cells induced by G-CSF and FLT3 ligand

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Our previous studies showed that mobilization of bone marrow cells with granulocyte colony stimulating factor (G-CSF) or FLT3 ligand or a combination of G-CSF and FLT3 ligand improved the motor performance and sensitivity of rats with spinal cord injury in comparison with saline-treated animals. The sensitivity improvement caused by combined therapy (at the same total dose) was significantly greater than that resulting from treatment with either G-CSF or FLT3 ligand alone. Morphometric measurements showed that treatment with G-CSF, FLT3 ligand or a combination of G-CSF and FLT3 ligand also increased the spared white and gray matter, evaluating the cross-sectional area as well as the volume of the spinal cord lesion. We studied the increase in peripheral blood monocytes during and after the injection of G-CSF, FLT3 ligand or a combination of G-CSF and FLT3 ligand. Wistar male rats were divided into four groups. The first group received FLT3-ligand (50 μg/kg, i.v., n = 6) for five days, the second group was injected with G-CSF (50 μg/kg, i.v., n = 6), the third group received a mixture of G-CSF and FLT3-ligand (G-CSF 25 μg/kg + FLT3 25 μg/kg, i.v., n = 6) and the fourth group was injected with saline (1ml/kg, i.v., n = 5). Blood samples (0.5–1.0 ml) were taken from the eye orbital sinus of each animal under isofluorane anesthesia one day before bone marrow stimulation, on the 1st, 2nd and 4th day of stimulation, and on the 2nd and 4th day post-stimulation. For cell counting we used a standard hematology analyzer. G-CSF and a combination of G-CSF and FLT3 ligand increased the absolute number of leukocytes (Lkc) during the time course of bone marrow stimulation. Lkc subpopulation ratios changed according to the injected drugs. The relative number of lymphocytes (Ly) was decreased and the number of neutrophils (N) increased in animals with G-CSF injection during the bone marrow stimulation period and on the 4th day post-stimulation. However, FLT3 ligand injection decreased Ly and increased N subpopulations only after bone marrow stimulation was completed. The combination of both factors lowered Ly and increased N subpopulations during bone marrow stimulation and post-stimulation. The observed improvement in behavioral and morphological parameters in animals with SCI treated with a combination of both drugs could be explained by the prolonged time course of the mobilization of bone marrow cells.

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Experimental spinal cord injury reconstruction using hydrolytically degradable hydrogels

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Spinal cord injury leads to axonal damage, necrosis, demyelinization and extracellular matrix deposition, which ultimately lead to the formation of a glial scar and post-traumatic pseudocystic cavities. Three-dimensional biocompatible macroporous polymer hydrogels can be utilized in experimental models of spinal cord injury repair. They bridge the pseudocystic cavity and provide a scaffold for the ingrowth of regenerating axons. The advantage of using degradable hydrogels is that they may bridge the lesion and provide a scaffold for axonal regeneration without leaving any remnants of artificial material several months after implantation. Three types of hydrolytically degradable hydrogels based on copolymers of 2-hydroxypropyl-methacr ylamide (HPMA) with degradation times of 18, 23 or 32 days, with decreasing hydrophility in the same order, were prepared and implanted into a spinal cord hemisection in rats. The animals were sacrificed one or two months after implantation, and their spinal cords were histologically evaluated. One month after implantation, all of the hydrogels integrated well into the spinal cord tissue, with only minimal signs of foreign body reaction. We observed loose mesenchymal tissue with ingrowing connective tissue elements, blood vessels and neural processes in all of the hydrogels. The ratio between neural processes and connective tissue was highest in the hydrogel with the highest hydrophility. Two months after implantation, the ingrowth of the neural processes continued only in the hydrogels degradable within 23 or 32 days, in which pseudocystic cavities were minimal. The hydrogel with the shortest degradation time (18 days) was resorbed, and large pseudocystic cavities had developed. Hydrophilic degradable hydrogels may be utilized for spinal cord tissue reconstruction. However, the stability of newly formed tissue is insufficient when the degradation time is less than 3 weeks.

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Growth of rat mesenchymal stem cells on layers of nonwoven nanofibers from different biocompatible polymers

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The importance of biomaterials has steadily increased in recent years, while the number of polymer applications in tissue engineering continues to grow. Polymeric scaffolds created from nonwoven electrospun nanofibers have the potential to be used in the reconstruction of skin, bones, muscles, veins and nerves, supporting the growth of cell cultures utilized in cell therapies. Multipotent mesenchymal stem cells play an important role in stromal support for hematopoietic stem cells, immune modulation, and tissue regeneration.

We studied the effect of nanofiber surface chemistry on the growth of rat bone marrow mesenchymal stem cells (rMSCs). We compared nanofiber layers with similar morphological structure, created from different biocompatible polymers (polyamide 6, methacrylate copolymer, poly(lactic-co-glycolic) acid and polyurethane). The nanofiber layers were prepared by electrospinning on a Nanospider™ device (ELMARCO and Technical University of Liberec). As a negative control, we utilized polystyrene tubes; the positive control was polystyrene modified for tissue culture (cell culture wells; both from Techno Plastic Products). We studied the growth of rMSCs, isolated from rats expressing green fluorescent protein (GFP), in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and primocin (2 μl/ml) on the nanofiber layers and control surfaces. The rMSCs were scanned on the first and third days after seeding, and the cellular density was determined using the Cavalieri principle within MATLAB software (The MathWorks, Inc.). The GFP-rMSCs grew on all of the nanofiber surfaces significantly better than on the unmodified polystyrene; however, their growth was less than on the tissue culture polystyrene. The results show that although rMSCs adhered to all of the nanofiber layers, the chemical composition of the fibers plays a role in their adhesion. The highest cellular density was measured on the polyamide 6 nanofiber layers, the lowest on the polyurethane nanofiber layers. Biocompatible polymer nanofibers can be utilized as cell carriers for tissue reconstruction; however, further studies are necessary in order to determine the best polymer structure.

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Nonwoven nanofiber materials as three-dimensional tissue constructs in spinal cord injury

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The preparation of three-dimensional tissue replacements for bridging a spinal cord lesion is at the center of attention of tissue engineering. Polymeric scaffolds created from nonwoven electrospun nanofibers support the growth of various supportive cell types utilized in the treatment of spinal cord injury, such as bone marrow stromal cells (MSC) or olfactory ensheathing glial cells (OEGs). It is therefore possible to create three-dimensional implants by folding the cell-containing layers. Using a Nanospider device (International patent WO 2005/024101, Technical University of Liberec), we prepared layers from the copolymer of 2-hydroxyethylmethacrylate and ethoxyethylmethacrylate with mesenchymal stem cells (MSCs) or olfactory glial cells (OEGs) growing on these layers. The growth of the cells was evaluated using confocal microscopy. After reaching confluence, the individual layers were folded, gently compressed and implanted into a spinal cord lesion. In culture, MSCs grew to confluence on all of the nanofiber layers, while OEGs remained in a subconfluent state. The cell-containing folded scaffolds were implanted into hemisected rat spinal cords. After two weeks, the nanofiber scaffold containing the cells had adhered to the host tissue; the MSCs were able to leave the implant and migrate into the host tissue. There were neural cell processes stained for neurofilaments present in the implanted scaffold; however, astrocytes did not enter the implants. Our results show that nonwoven polymeric nanofiber scaffolds can be utilized in vitro to cultivate MSCs and OEGs; these scaffolds seeded with cells have the ability to induce tissue formation when implanted into injured spinal cord tissue and therefore support the regeneration of the nervous tissue. The implantation of nonwoven polymeric nanofiber scaffolds may serve as an alternative to conventional grafting technologies.

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Development of neurogenic pulmonary edema in spinal cord injured rats – the role of isoflurane anesthesia

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Anesthetics can either promote or inhibit the development of neurogenic pulmonary edema (NPE) after central nervous system injury. The influence of isoflurane was examined in male Wistar rats using 1.5%, 2%, 2.5%, 3%, 4% or 5% isoflurane in air. Epidural balloon compression of the thoracic spinal cord was performed. The development of NPE was examined in vivo and on histologic sections of lung tissue. Animals anesthetized with 1.5% or 3% isoflurane were behaviorally monitored using the BBB and plantar tests for 7 weeks post-injury. The spinal cord was examined using MRI and morphometry of the spared white and gray matter. All animals from the 1.5% and 2% groups developed NPE. Almost 42% of the animals in the 1.5% group died of severe pulmonary hemorrhage and suffocation; X-rays, the pulmonary index and the histological picture revealed a massive NPE. More than 71% of the animals from the 2.5% and 3% groups did not develop any signs of NPE. Blood pressure after spinal cord compression rose more in the 1.5% group than in the 3% one. In the 1.5% group, sympathetic ganglionic blockade prevented neurogenic pulmonary edema development. Animals from the 3% group recovered behaviorally more rapidly than did the animals from the 1.5% group; morphometry and MRI of the lesions showed no differences. Thus, low levels of isoflurane anesthesia promote NPE in rats with a compressed spinal cord and significantly complicate their recovery. The optimal concentration of anesthesia for performing a spinal cord compression lesion is between 2.5 and 3% isoflurane in air. A concentration of 1.5-2% isoflurane anesthesia can be used as a model of severe NPE in rats with balloon-induced spinal cord injury.

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Extracellular space diffusion parameters in focal cortical dysplasia

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Focal cortical dysplasia is a common cause of pharmacoresistant epilepsy, but the mechanisms of enhanced excitability in the dysplastic neuronal network are poorly understood. In this study we determined the extracellular space (ECS) diffusion parameters in human neocortical tissue, obtained from 16 surgically treated patients with temporal lobe epilepsy. The ECS diffusion parameters – volume fraction α (α = ECS volume/total tissue volume) and tortuosity λ (λ^2 = free/apparent diffusion coefficient) – were determined by the real-time iontophoretic method using ion-selective microelectrodes. Diffusion measurements were performed in 400 μm thick slices, which were subsequently histologically processed; three categories of cortical malformations were determined by microscopic analysis: 1) cortex with normal cytoarchitecture, 2) focal cortical dysplasia (FCD) type I and 3) FCD type II. In tissue with normal cytoarchitecture, α = 0.24 ± 0.01 and λ = 1.48 ± 0.01 (mean ± SE, n = 10 patients, N = 44 slices), which are not significantly different from those values found previously in healthy human cortex. In both FCD I and FCD II slices, tortuosity was significantly increased (1.57 ± 0.01, n = 4, N = 11 and 1.67 ± 0.02, n = 3, N = 10, respectively). Extracellular volume fraction α was not significantly changed in FCD I but was increased in FCD II (0.27 ± 0.04), reaching in some locations values of 0.35–0.45. Histological evaluation revealed the disordered structural organization of neuronal and astrocytic processes, typical for dysplastic cortex, and increased staining for tenascin C. We conclude that diffusion in dysplastic cortical areas is compromised due to increased diffusion barriers manifested by an increase of tortuosity. Together with structural disorganization, the impaired diffusion of neuroactive substances may contribute to the development of epileptic seizures.

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Diffusion parameters in the rat cerebral cortex during pilocarpine-induced status epilepticus

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Extrasynaptic transmission between nerve cells, mediated by the diffusion of neuroactive substances in the extracellular space (ECS), is an alternative mode of signal transmission that may modulate synaptic transmission itself and thus affect tissue excitability. The aim of this study was to determine the diffusion parameters in vivo in the brain cortex of 3-month-old male Wistar rats after pilocarpine-induced seizures. Animals were pretreated with lithium chloride 14-18 hours before the experiment, anesthetized by urethane and artificially ventilated. The epileptiform seizures were evoked by pilocarpine administration (300 mg/kg, i.p.). Volume fraction α (α = ECS volume/total tissue volume) and tortuosity λ (λ^2 = D/ADC, where D is the free and ADC is the apparent diffusion coefficient) were determined from concentration-time profiles of tetramethylammonium (TMA) applied by iontophoresis, and the apparent diffusion coefficient of water (ADC_w) was measured by diffusion-weighted magnetic resonance imaging (DW-MRI). ADC_w maps were evaluated in the primary somatosensory cortical region, corresponding to the site of the TMA measurements. In addition, the extracellular potassium concentration [K+]e was recorded using ion-selective microelectrodes. Before pilocarpine application, the ECS parameters were: α = 0.19 ± 0.01 and λ = 1.58 ± 0.01 (n = 7, mean ± SEM). The volume fraction started to decrease several minutes after the application of pilocarpine and reached a minimum value of 0.13 ± 0.01 in 80–100 minutes. Approximately at 120 minutes α started to increase, reaching 0.18 ±0.01 four hours after pilocarpine application. There were no significant pilocarpine-evoked changes in tortuosity observed. However, ADC_w was significantly decreased 80 minutes after pilocarpine application (563 ± 18 $\mu m^2 s^{-1}$, n = 5) compared to controls (655 ± 11 $\mu m^2 s^{-1}$, n = 5). By the end of the experiments, ADC_w had returned to control values. Decreases in α and ADC_w were accompanied by an increase in extracellular potassium concentration, indicating increased neuronal activity. Our results show that pilocarpine-induced seizures result in long-lasting cell swelling. Compensatory ECS shrinkage may contribute to an increase in the concentrations of extracellular metabolites and neuroactive substances that can further aggravate seizures. Recovery of ECS volume corresponds to the progressive reduction of epileptiform activity 120 min after the administration of pilocarpine, probably attributable to the depletion of cell energy reserves.

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Time course of water diffusivity in the rat brain after global ischemia

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Global cerebral ischemia is characterized by delayed neuronal loss and reactive gliosis, especially in the CA1 region of the hippocampus. The aim of this study was to evaluate the time course of changes in the apparent diffusion coefficient of water (ADC_w), measured by diffusion-weighted magnetic resonance, in the CA1 region after ischemia/reperfusion injury. We correlated the ADC_w results with immunohistochemical analysis of the damaged tissue, focusing on neuronal loss, reactive gliosis and the expression of apoptotic markers. Global cerebral ischemia was induced in 2-month-old male Wistar rats by a bilateral 15 min occlusion of the common carotid arteries combined with hypoxic conditions (6%O_2 / 94%N_2). ADC_w was measured in control rats, after 1 hour, 6 hours, 1 day, 3 days, 7 days and 5 weeks of reperfusion. Sham operated animals were measured 3 days after surgery. In the CA1 region global cerebral ischemia led to an increase in ADC_w (762 ± 5 $\mu m^2 s^{-1}$, n = 6) during the first hour of reperfusion. No significant differences from control values (714 ± 7 $\mu m^2 s^{-1}$, n = 7) were found at 6 or 24 hours after ischemia or in sham operated animals. Subsequently, ADC_w significantly increased to 756 ± 9 $\mu m^2 s^{-1}$ and 859 ± 12 $\mu m^2 s^{-1}$ (n = 6) after three days and five weeks of reperfusion, respectively. Immunostaining revealed reactive gliosis and neuronal loss in the CA1 region. The apoptotic markers casp-3 and PARP-1 were mostly activated in neurons in the CA1 region 6 hours after ischemia; after five weeks they became detectable in astrocytes. The increase of ADC_w at the begining of reperfusion is probably caused by an increase in extracellular space volume after ischemia, while the second peak in ADC_w, three days after ischemia, coincides with neuronal death in the CA1 region. The marked rise in ADC_w five week after global ischemia might reflect changes in astrocyte morphology and membrane properties. Caspase-3 and PARP-1 immunoreactivity in this period implies either ischemic preconditioning in astrocytes or ongoing programmed cell death in astrocytes (apoptosis).

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Calbindin and S100 protein expression in the developing inner ear of mice

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Calbindin D28K (CB) and S100 are calcium binding proteins known to be expressed in the inner ear of adult mice. Limited information exists about their occurrence during the inner ear development. This study demonstrates that the sites of CB and S100 expression in the mouse inner ear during embryonic and early postnatal development do not overlap and signal independent developmental patterns. The expression patterns of CB and S100 were investigated in C3H mice by immunohistochemistry, from embryonic day 11 (E11) to postnatal day 10 (P10). CB was expressed in the otocyst and vestibulocochlear ganglion (VCG) from E11. In the developing cochlea at E17, CB immunoreactivity clearly labeled the auditory ganglion, the precursors of the outer and inner hair cells, and the stria vascularis. CB staining was also present in the VCG and vestibular hair cells, including their nerve fibres. Two days later, to this CB expression pattern was added the labeling of Kölliker´s organ. Early postnatal CB expression encompassed spiral ganglion neurons, auditory hair cells, their afferent nerve fibres and cells of the cochlear lateral wall. Cup-like calyceal terminals at vestibular cells (type I) were CB immunostained as well.The first signs of S100 immunostaining of cochlear epithelial cells appeared at E14. At E17 S100 protein showed a spatially restricted expression pattern in the cochlea. The stained area was separated by a morphological furrow corresponding to the future site of the tunnel of Corti. A few spiral ganglion nerve fibres labeled by S100 indicated that the developing inner hair cells contain S100 as well. This finding and evidence that the pillar and Deiters cells were immunostained at E19 supports the idea that S100 protein is involved in pillar cell development. Immunostaining was also present in the vestibular cells and their afferent fibres. Postnatally, S100 staining appeared in the inner hair cells, their afferent nerve fibres, the outer hair and Deiters cells. Vestibular I and II hair cells in the macula and crista ampularis and some vestibular neurons were labeled. In addition, S100 immunostaining was present in the spiral limbus, the spiral prominence and the intermediate cells of the stria vascularis. This study demonstrates Ca^{2+} involvement in several important steps of inner ear development in the mouse. Ca^{2+} ions participate in cell migration and neuroblast differentiation. Also, the development of the organ of Corti and the differentiation of auditory and vestibular sensory cells, including their inervation, is influenced by Ca^{2+}.

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Changes in GAD levels in the central auditory system of two rat strains with aging

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian neocortex and is critically involved in shaping neuronal responses in the central auditory system. In the current study we evaluated age-related changes in the levels of glutamate decarboxylase (GAD), the key enzyme in the formation of GABA, in the inferior colliculus (IC) and auditory cortex (AC) of young and old animals of two rat strains - Long Evans (LE), a strain with normal aging and preserved hearing function up to late senescence, and Fischer 344 (F344), a fast aging strain with an early onset of hearing impairment. The comparison was made between rats aged 3-5 months in both strains and LE animals aged 27-33 months and F344 animals aged 20-24 months. GAD is present in two isoforms, GAD65 and 67; immunohistochemistry was used to investigate changes in GAD67 content and western blot technique to investigate changes in GAD 65 and 67 content. GAD67-immunoreactive(-ir) neurons were present in all subdivisions of the IC and in all layers of the auditory cortex in all examined animals. Age-related changes in GAD67 immunoreactivity comprised a significant decrease in the optical density of GAD67-ir neurons in the IC and AC of both old LE and F344 rats. A decrease in the optical density was accompanied by a significant decrease in the number of GAD67-ir neurons in the AC of both strains, especially in the superficial layers (I-IV). In western blots, pronounced age-related decreases in the levels of GAD65 and 67 in the IC and AC of both strains were found as well. In contrast, in the visual cortices of both strains only a tendency towards a decrease in the levels of the examined proteins was observed in old rats. In addition, western blots revealed a striking difference in the amount of GAD65 and 67 between the IC and the cortical regions. The densities of both isoforms were almost three times higher in the IC compared to the auditory and visual cortices in young animals of both strains. With either technique, no strain-specific changes were found with aging.

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Aging influences parvalbumin expression in the central auditory system of two rat strains

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Changes in the levels of calcium binding proteins are known to occur in different parts of the brain during aging. In our study we attempted to find out the effect that aging has on the parvalbumin-expressing system of neurons in the higher parts of the central auditory system. Age-related changes in parvalbumin immunoreactivity were investigated in the inferior colliculus (IC), medial geniculate body (MGB) and auditory cortex (AC) in two strains of rat, Long Evans (LE), a strain with normal aging and preserved hearing function up to late senescence, and Fischer 344 (F344), a fast aging strain with an early onset of hearing impairment. The comparison was made between rats aged 3-5 months in both strains and LE animals aged 27-30 months and F344 animals aged 20-22 months. In young animals of both strains, parvalbumin-immu noreactive(-ir) neurons were present in all subdivisions of the IC and in layers II-VI of the auditory cortex. The number of PV-ir neurons in the MGB in all examined animals was very low independently of the strain and age. In old LE rats the number of PV-ir neurons was greater in the central nucleus of the IC; the optical density of their somas was increased in comparison with young LE animals. In addition, the optical density of PV-immunoreactive somas was also enhanced in the AC in this strain. In contrast, old F344 rats showed a pronounced decline in the number of PV-ir neurons in the AC in comparison with young F344 rats. In some parts of the AC of old F344 animals, almost no PV-ir neurons were present at all. Changes in the AC in old Fischer 344 rats were accompanied by a reduction in the cross-sectional areas of PV-ir neurons in the central nucleus of the IC. The results clearly demonstrate that aging is associated with significant changes in PV-immunoreactivity in the central auditory system of the rat and that the changes are strain-dependent.

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Age-specific impairment of hearing function in the rat

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The aim of our study was to assess age-related changes in the auditory system of pigmented rats (Long Evans strain). These animals, with a relatively short life-span in which age-specific cochlear impairment occurs later in life (Popelar et al., Neurobiology of Aging, 2006), are useful subjects for the investigation of presbycusis. Auditory sensitivity (hearing threshold and gap detection threshold) and auditory discrimination ability (frequency difference limen and gap duration difference limen) were examined in young-adult (2-4 months) and old (28-34 months) rats using behavioral and electrophysiological methods. The results show a small difference in hearing thresholds between young-adult and old rats: the hearing thresholds increased by 5-25 dB over the frequency range of 2-32 kHz during aging. Hearing thresholds assessed by behavioral and electrophysiological methods in both age groups were similar; the difference in thresholds did not exceed 5-10 dB. Frequency discrimination slightly deteriorated during aging; age-related changes were revealed in some but not all old rats. Old rats exhibited a considerable deficit in temporal resolution and in their ability to discriminate the time parameters of acoustic stimuli. Gap detection thresholds significantly increased from 1.8 ± 01 ms to 3.4 ± 0.5 ms during ageing. In all old rats the values of gap duration different limens were larger than in young rats; Weber ratios for the discrimination of gap duration increased two-fold with age. The results suggest that aging in Long Evans rats is accompanied by deteriorated hearing function. The deficit in the processing of temporal sound features present in old rats indicates an age-related impairment, which may be localized in the central part of the auditory system.

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Expression of cytoskeletal neurofilament protein in the auditory cortex of rat

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Although according to the prevailing opinion the rat's auditory cortex conform to the basic mammalian plan. The growing body of data indicate significant differencies between parcellation of the auditory cortex derived from the principal morphological approach (packing density of neurons) on one side and from the electrophysiological and functional metabolic techniques and data about the connections on the other side. According the morphological criteria the auditory cortex is divided to three fields (Te 1, Te 2, Te 3 ; Zilles and Wree, 1995) while the functional data and connectional pattern offer parcellation to 4 – 6 cortical fields. We designed this study to analyze the distributions of non-phosphorylated neurofilaments within rat temporal neocortex in order to obtain additional data enabling the comparison with maps of the auditory cortex based on connectivity and functional data. Selective neurofilament expression was formerly used as a sensitive and specific method for detection of boundaries between individual cortical fields and for the more detailed parcellation of the cortical areas.

Five young adult rats (strain Long Evans, age 4 – 5 months, weight 250 – 300 g) were used for this study. Animals were deeply anesthetized and perfused with 4 % paraformaldehyde. Brains were postfixed, cryoprotected and 40 μm thick frozen frontal sections were treated in a solution of SMI – 32 antibody (antibody against nonphosphorylated epitopes in neurofilament subunits NF – M and NF – L; Sternberger Monoclonals Inc, MD, USA) and further processed with ABC peroxidase method and DAB as a chromogen.

Analysis of SMI – 32 immunoreactivity patterns revealed differencies in the distribution of neurofilament protein-containing neurons across the temporal neocortex. Striking differencies were evident between the primary auditory area (Te 1, Au 1) and ventrally and dorsally situated associative auditory areas (Te A, Au V, and Au D; Paxinos and Watson 2007). In the primary auditory area are evident two distinct layers of strong immunopositivity in neurons and neuropil localized in the 3rd and 6th layer. Additional less intensive immunostaining was evident in the 5th layer. Between the primary auditory area and the ectorhinal cortex were discernible two areas where SMI – 32 immunostaining was weaker in all cortical layers and the positivity was evident only in the 5th and 6th layers. The dorsal field AuD exhibited stronger immunostaining for neurofilament protein only in the 3rd layer.

In summary the regional distribution of SMI – 32 immunoreactivity in the rat temporal neocortex corresponds more with cortical areas as defined by functional analysis than with the classical subdivision by Zilles and Wree (1995).

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Functional and electrical membrane properties of neurons in the auditory cortex in rats

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The study focused on the functional and electrical membrane properties of neurons in the auditory cortex (AC). In our previous study, five individual fields were distinguished based on the neuronal response to simple acoustic stimuli such as broad band noise (BBN) and pure tone bursts: primary auditory field (AI), anterior auditory field (AAF), suprarhinal auditory field (SRAF), posterior auditory field (PAF) and belt area. Neurons in the AI, AAF, SRAF and PAF typically responded to both pure tones and also to BBN stimulation. In contrast, neurons located in the belt reacted only to BBN stimulation. Individual fields were also characterized by latency, pattern, duration and strength of the response. Auditory fields which differed most in the studied features were the AI and belt area. The AI was the field with the shortest latency, the highest response strength to BBN stimulation and the highest occurence of an onset response pattern. On the other hand, the belt area had the longest latency and the lowest response strength. Based on these findings, the AI and belt area were chosen for further examination of the electrical membrane characteristics of pyramidal neurons in layer V. A glass electrode was inserted into the AC in ketamine-xylazine anaesthetised, 30-35-day-old Wistar rats, and the approximate borders of the AI, AAF and belt area were distingusihed on the basis of the neuronal response to BBN and pure tone bursts. The neurons were then studied in supravital brain cortex slices. The current-clamp mode of the patch-clamp technique was used to record the voltage responses of neurons in brain cortex slices (300 – 400 μm thick) to both hyperpolarizing and depolarizing current injections of variable amplitude. Two distinct classes of neurons, formerly named as regularly spiking (RS) and fast spiking (FS), were found in both areas of the auditory cortex (A1 and belt). The two populations clearly differed in their steady state firing frequencies (34.3 $\pm$ 8.5 Hz evoked by a 0.3 nA current step in FS and 25.0 $\pm$ 6.2 Hz for RS) and in the length of the after hyperpolarization phase (AHP) of postsynaptic action potentials (AHP peaked at 25.1 $\pm$ 6.1 ms after a spike in FS neurons and 58.9 $\pm$ 14.4 ms in RS neurons). By comparing the passive membrane properties of the A1 and belt neurons, we found that RS neurons from the belt showed a markedly higher time membrane constant than their A1 counterparts (27.9 $\pm$ 6.5 ms vs. 17.5 $\pm$ 4.6 ms). This suggests a significantly lower resting membrane resistance of the A1 neurons. Consistently, RS neurons from the belt showed a shift of the spike activation threshold to more hyperpolarized potentials by 3.4 mV compared to the RS neurons from the A1. Based on these findings, both extra- and intracellular features of neurons significantly differ between the AI and belt area.

Effect of noise exposure on the amplitudes of auditory evoked responses in rats

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Noise exposure affects not only the peripheral, but also the central part of the auditory system. Previously, we found enhanced cortical middle latency response (MLR) amplitudes after noise exposure, but with large inter-individual variability. The aim of this work was to record MLRs and auditory brainstem responses (ABRs) and to analyze the relationship between hearing threshold shifts, changes of MLR and ABR amplitudes and the slope of MLR and ABR amplitude-intensity functions (AIFs) after noise exposure. MLRs and ABRs were recorded with electrodes implanted on the surface of the rat brain; hearing thresholds were assessed on the basis of ABR recordings. To produce various hearing treshold shifts, rats were exposed twice for one hour to broad-band noise of 118 dB SPL (first exposure) and 122 dB SPL (second exposure); the interval between the exposures was three weeks. One day after the first exposure, hearing threshold shifts ranged between 5-45 dB at frequencies of 8–32 kHz. Whereas maximal MLR amplitude enhancement reached up to 250% and the average slope of MLR AIFs increased from the pre-exposure value of 2.75 ± 1.2 to 7.4 ± 3.7 postexposure, ABR AIFs after noise exposure were shifted in parallel to higher stimulus intensities and ABR amplitudes were reduced. The almost full recovery of thresholds and evoked response amplitudes to pre-exposure values was observed during two weeks. The second noise exposure resulted in a more pronounced hearing threshold shift of about 80 dB, but the maximal MLR amplitude enhancement was similar to that measured after the first exposure, and the average slope of the MLR AIFs increased to 14.1 ± 3.7.

The second noise exposure produced a greater suppression of ABR amplitudes than did the first exposure, and ABR AIFs were more shifted to higher stimulus intensities while keeping their slope more or less unchanged. The results demonstrate that noise exposure differentially affects ABR and MLR amplitudes: whereas ABR AIFs after noise exposure were shifted in parallel to higher stimulus intensities and ABR amplitudes were reduced and negatively correlated with threshold shifts, MLR amplitudes were enhanced after noise exposure and MLR AIFs were significantly steeper, correlating their slope with the threshold shift. Thus, changes in the slope of MLR AIFs characterize more the effect of noise exposure on the central part of the auditory system, whereas ABR suppression reflects mainly noise-induced changes at the periphery.

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Spontaneous otoacoustic emissions in children and adolescents – effect of cisplatin treatment

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Spontaneous otoacoustic emissions (SOAEs) are spontaneous oscillatory products of the outer hair cells recordable in the external acoustical meatus. They are considered to represent a manifestation of the normal active feedback mechanism in the cochlea. Previous studies have shown that typical SOAEs are characterized by one or more peaks in the frequency spectrum with great stability in individual ears over time. The aim of the study was to analyze the appearance and characteristics of SOAEs in a group of 124 normal hearing children and adolescents aged from 6 to 25 years, to correlate the findings with other audiological parameters measured in this group and to compare them with findings in a group of 32 children treated with cisplatin as a cure for several types of cancer.

In the normally hearing group, SOAEs were present in 70.1% of the individuals in either one ear (55.4%) – with a higher occurrence in the right ear (55.4%) – or in both ears (44.6%). They were present more frequently in girls (81.6%) than in boys (58.1%). The frequency of the individual peaks of the SOAEs ranged between 0.5 and 6 kHz in all measured spectra, with a maximum in the speech-related range, especially around 2 kHz. The presence of SOAEs was significantly correlated with larger amplitudes of transiently evoked otoacoustic emissions (TEOAEs) and distortion-product otoacoustic emissions (DPOAEs). The audiometric parameters were compared in four age groups, each spanning five years of age. In the group of 21–25-year-olds, individual hearing thresholds at 16 kHz were significantly better in individuals with SOAEs than in those without SOAEs.

The results obtained in the normally hearing group were compared with the results from the recording of SOAEs in children and adolescents treated with cisplatin in a cumulative dose ranging between 200 – 1000 mg/m^2 (469,6 ± 158,71 mg/m^2) for the presence of osteosarcoma, chondrosarcoma, neuroblastoma or meduloblastoma. In the cisplatin-treated group, SOAEs occurred in approximately 12% of ears only, with the majority of the SOAE peaks found at frequencies ranging from 1 to 3 kHz. Their incidence was very low even in children with relatively well preserved audiograms. The mean number of SOAE peaks in the normally hearing group was 3.3 in one ear whereas in the cisplatin treated group the number decreased to 1.4 in one ear.

In summary, the appearance of SOAEs may serve as an indicator of a healthy ear with normally functioning outer hair cells whereas most pathological states results in a decrease in the occurrence of SOAEs, up to their complete disappearance.

Hearing Thresholds in the Extended Frequency Range as a Function of Age and Sex

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If we want to compare the audiograms of persons of different age, we have to normalize them. The normal thresholds of hearing by air conduction as a function of age and sex are given by the ISO 7029 standard (ČSN EN ISO 7029). The threshold shift depending on age is, in this standard, expressed as $\mathbf{H} = \alpha\ \mathbf{(Y-18)^2}$, where **H** is the hearing threshold level in dB, **Y** is age in years and **α** is a coefficient that, in this document, is given separately for men and women for each frequency used. However, even the last version of this standard (2002) does not contain **α** for frequencies higher than 8 kHz. Our aim was to determine **α** for frequencies of 10 kHz, 12.5 kHz and 16 kHz.

We measured the hearing thresholds of 102 (49 male and 53 female) otologically normal persons over a frequency range of 125 Hz – 16 kHz with an Madsen Orbiter 922 audiometer equipped with Sennheiser HDA 200 high frequency headphones. The results of these measurements enabled us to plot a graph of hearing levels vs. age. For frequencies of 10 kHz, 12.5 kHz and 16 kHz, we used curve fitting in order to find the optimal values of coefficient **α**. However, the fitting was not precise for high frequencies. With the linear approximation

H = a (Y–18) the fitting was more exact. The values of the coefficients **α** and **a** were calculated separately for men and women.

Because the ISO 7029 standard does not cover frequencies higher than 8 kHz, our results can be used in high frequency audiometry. Our data show, that for the highest audible frequencies, the square-law is not valid and better fitting may be obtained with a linear approximation.

Auditory cochlea-threedimensional MRI of the left and right cochleo-vestibular organ

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The human body is asymmetrical and/or symmetrical. Some of body parts or organs are in a mirror shape. The membraneous and osseous labyriths may be examples of a such mirror shape arrangement.

The data about the left-right asymmetry could be found even in biology of plants, in biochemistry as alpha- or beta- helices. About 90% of humans have clock-wise hair whorl. The simillar percentage has been found in spiral shape in shell ulits.

Hypothesis

We were curious if such asymmetry of auditory cochlea could be found different in left and right-handed human individuals.
A small group of left-handed individuals (-100%) has been compared with right-handed persons (+100%) according The Edingurgh Inventory in this preliminary study.

Adaptation of the olfactory receptor neuron of a moth to the natural pheromone signal

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Studies on stimulus-response relationships were carried out on a model of olfactory transduction in the pheromone receptor neuron of the male moth Antheraea polyphemus. These neurons are capable of detecting rapid changes in stimulus intensity encountered in natural pheromone odor plumes. Knowing the densities and reaction rate constants of the perireceptor and receptor processes we predicted several characteristics the pheromone stimulus should possess under the hypothesis that the receptor neuron performs optimally, i.e. transfer as much information on the stimulus as possible. Statistical characteristics of the stimulus were obtained and compared with the experimental observations. The öoptimalö probability density function of the fluctuations in the pheromone concentration was found to be exponential in good agreement with experimental data. The predicted intermittency (12%) falls within the lower range of experimentally observed values (10-40% depending on experimental conditions).

Optimal odor intensity in simple olfactory neuronal models

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Signal processing in olfactory systems is initiated by binding of odorant molecules to receptor molecules embedded in the membranes of sensory neurons. Three models of olfactory sensory neurons have been investigated. Their behavior is described by stochastic processes of binding (and activation). The models assume that the response, concentration of bound (activated) receptors, is determined by the signal, which is fixed log-concentration of odorant in a perireceptor space. Dependency of the mean response on the signal is realized through the input-output transfer function (usually the logistic curve). How the concentration of bound (activated) receptors can code the intensity of odorant is analyzed using the statistical properties of the steady-state responses.

Classical, deterministic, approach to the problem of finding a suitable signal is based on the steepness of the input-output transfer function. For the logistic curve, the best detectable signal is located at the inflexion point of the input-output function.

We use approach, which is based on stochastic variant of the law of mass action as a neuronal model. We consider a model experiment in which a fixed odorant concentration is applied several times and realizations of steady-state characteristics are observed. The response is assumed to be a random variable with some probability density function belonging to a parametric family with the signal as a parameter. Different types of distributions of the response are considered. As a measure how well the signal can be estimated from the response, the Fisher information and its lower bound are used.

Results are compared with the classical approach to determine the coding range via steepness of the input-output transfer function. The point in which the first derivative of the input-output function is maximal coincides with the point of maxima of the Fisher information in the simple models. The obtained results differ in the models including the activation step.

Rats do not learn to avoid a cued moving region in a stationary environment but they learn to avoid the cued region if it is stationary in a moving environment

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Rats easily learn to avoid place in their enviroment. Several aversive behavioral tasks take advantage of it in order to test spatial memory and cognition. However to-be-avoided place might not be only defined with respect to stationary objects but also with respect to moving ones. In order to test whether rats can learn to avoid a region bounded to a moving object we modified the behavioral task called AAPA (Bureš et al, 1997; Fenton et al., 1998). Rats (n = 9) were trained on a stable arena to avoided a region which rotated together with a distant object around the arena. Performance of the rats did not improve during 7 days of training and it equaled to the performance of the same subjects when the object was removed on the 8th day. However the rats improved substantially on the 10th day when the arena rotated while the region together with the distant object was stationary. A different group of rats (n = 7) was trained in the later version of the task (rotating arena, stationary to-be-avoided region) from the very beginning. These rats improved substantially during the first four days of the training. When these rats were tested on the stationary arena with the to-be-avoided region rotating around the arena their performance was deteriorated. These results indicates that the inertial information available to the subjects in the second version of the task is crucial for the effective solution of the task.

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Are inertial stimuli and/or motor skills sufficient for successful navigation of rats in the AAPA task (moving world)?

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Spatial cognition of rats is usually tested in stable environment. The recently designed AAPA (Active Allothetic Place Avoidance) task is unique in this respect as it requires the animals to distinguish between two subsets of orientation cues, the stable extraarena cues and the continuously rotating intraarena cues. So far published experiments always assumed that rats must use extraarena cues to perform well in the task. As the speed of arena rotation is constant in time, it is however conceivable that inertial stimuli and stereotypic motor behavior can substantially contribute to the solution of the task. The goal of our study was to address this assumption by testing whether rats are able to learn the task in a situation where extraarena cues are eliminated and whether well trained rats are able to solve the task in this situation. Male Long Evans rats (n=16) divided into two groups by 8 were trained in the AAPA task in a 5-day training protocol for 5 weeks with or without extraarena cues available respectively. The 6th day of each week extraarena cues were eliminated for both groups and the rats' strategies were tested. The rats previously trained in environment where extraarena cues were not available did not learn the task, while those that had learned the task using extraarena cues were after two weeks able to find an alternative strategy that was however less efficient. We conclude that the rats' performance in a standard 5-day training protocol depends on the use of extraarena cues and that exposure to extraarena cues is necessary for the rats to develop a successful strategy.

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Strategies used by rats during navigation toward a visible moving target

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Animals do not navigate to stationary goals only but also to moving ones. Preys, predators and members of the same social group are examples of moving objects which can be approached or avoided. In order to study strategies used by rats to navigate toward moving objects we designed a novel spatial task in the water maze apparatus. Rats (n = 7) were trained to navigate to a moving target in a circular swimming pool (diameter = 191 cm). The target moved along the maze wall. Direction of the target movement (clockwise, counter-clockwise), speed (slower than the rat, faster than the rat) and starting position (northwest, northeast, south) changed between trials in a pseudorandom manner but they remained constant within the trials. Well trained rats changed their target-approaching strategy according to the speed of the target. If it moved slower than the rats, the rats swam directly toward the target. If the target moved faster than the rats, the rats swam along a straight line toward the place of collision indicating that they were able to predict future positions of the target.

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Role of posterior parietal cortex of the rat in two tasks involving dynamic environment

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Most spatial tasks require animals to organize their behavior in relation to stable environmental cues. However, cue manipulations such as rotation or transition allow to assess their control exerted over behavioral processes and to examine underlying brain mechanisms.

We used Long-Evans rats with lesion of posterior parietal cortex in two behavioral tasks taking place in dynamic environment. Both of them were conducted on elevated circular arena (d=80cm), in 20 min daily sessions. At first, the rats were trained 6 days in Robot Avoidance Task (RAT), in which the rat must keep safe distance at least 25cm from a programmable robot to avoid a mild footshock. The robot was programmed to move straight forward (15cm/s) until it hit the wall, then it waited for 15s, turned 180 ± 0 to 90 degrees, and ran again. Two weeks after the first experiment, food-deprived rats were trained to asymptotic performance in a modified version of Place Avoidance (PA) task to search for randomly dispersed pellets while avoiding a small sector on the arena. Since the sector is not directly perceivable rats can remember its position either in the coordinate frame of the arena, or in the frame anchored to cues outside the arena. By rotating the arena slowly while the shock is switched off (extinction session), we may examine in which of these two frames the rats maintain their avoidance. These rotation+extinction sessions were made twice (on day 8 and 15) to see temporal consistency of the results.

Results showed that parietal rats (n=8) were slightly but not significantly facilitated (p=0.10) in learning RAT when compared to controls (n=8). In addition, they did not differ in locomotion or in thigmotaxis. Similarly, both groups learned the PA task at the same rate. However, in both extinction sessions, parietal rats chose arena frame more frequently than controls, which rather avoided in the room frame.

These results suggest that parietal rats may not have difficulties when processing proximal cues. This is in contrast with data of other authors obtained in a static environment task - water maze. In those experiments, lesions aimed to posterior cortex disrupted navigation based on proximal cues, while navigation based on distal cues left unaffected. We suggest that this discrepancy would be partly due to different requirements of the tasks, since in water maze the target area is considerably small.

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Study of animal cognitive functions using an active allothetic place avoidance task: assets, problems, questions

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Spatial orientation belongs to the most intensively studied topics in cognitive neuroscience. This type of behavior is often considered as a model of human higher cognitive functions. Roughly ten years ago, a novel behavioral task, active allothetic place avoidance (AAPA), was designed in our laboratory and our efforts to investigate the task intimately date back to this time.

In this task, animals avoid an unmarked shock sector defined in a coordinate frame of experimental room while moving over a rotating arena. It was established that besides navigation with respect to a hidden place, the task requires cognitive coordination, usually explained as an ability to separate spatial stimuli from the environment into coherent representation of an arena and a room, and to select the room frame as the only relevant one for efficient navigation.

We studied the effects of specific receptor antagonists on the behavior of animals in this task and it was found that changes in spatial efficiency are often accompanied by alterations in overall locomotor activity. In this regard, the task has an advantage of simultaneous assessment of both place navigation and locomotor behavior. The analysis of locomotion was found to be important for exclusion of a more general impairment of animals after an experimental manipulation. The results suggest that at least in some cases, the changed locomotion and decreased spatial efficiency occur concurrently, but without a mutual causal relationship. The poster will sum up the existing evidence about modulation of behavior in this spatial task.

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All subtypes of mild cognitive impairment are impaired in episodic-like memory

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Alzheimer's disease (AD) is associated with a loss of episodic memory. It is preceded by a stage of cognitive impairment without dementia, referred to as mild cognitive impairment (MCI). Episodic-like memory concept was developed by Clayton and Dickinson (1998) as the memory for information about 'where' a unique event or episode took place, 'what' occurred during the episode, and 'when' the episode happened. We developed non-verbal test of episodic-like memory for human presented on computer. The test consists of a presentation and a testing phase. In the presentation phase, the subject is shown a computer screen with several abstract pictures on predefined places on the right part of the screen and an empty open chest on the left. S/he is instructed to drag, using the computer mouse pointer, the pictures from the predefined places in a given order slowly into the chest. The subjects should memorize both the order and the position of each picture. After about 10 minutes break, the subject should drag the pictures in the same order to the correct position. Successively, memory for position and order of three, five and seven pictures was tested. We evaluated separately the errors in giving order of the pictures, position of the pictures and order of the predefined positions. Comparison was made among groups diagnosed with non-amnestic, amnestic single domain and amnestic multidomain subtypes of MCI, early stage of AD and a control group. The non-amnestic MCI subjects were impaired in the order of the object, while the amnestic multidomain MCI subjects were impaired in the object position. The amnestic multidomain and AD patients were impaired in both. These results can be useful in predicting the outcome of different MCI subtypes.

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The effect of enforced physical activity and cerebellar transplantation on spatial learning in Lurcher mutant mice

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Lurcher mutant mice represent a model of olivocerebellar degeneration. They suffer from cerebellar ataxia and impaired spatial learning or orientation ability. The aim of the work was to assess the effect of enforced physical activity and transplantation of embryonic cerebellar tissue on spatial learning ability in young and aged Lurcher mutant mice of the B6CBA strain.

Aged Lurcher mice were at least 60 days old (mean age 109 days) and young Lurchers were 12-14 days old at the beginning of the experiment. Solid cerebellar grafts were obtained from wild type mouse embryos and applied into the cerebella of the host mice. To sham-operated control animals only the vehicle vas administered. One part of each group of mice was then trained on the rotarod for 32 days over the course of 7 weeks. The second part of mice of all groups remained inactive. Spatial learning was tested in the Morris water maze from the 60th day after the surgery for 10 consecutive days. The survival of the grafts was examined histologically. Spatial learning ability of both young and aged Lurchers was also compared with wild type mice of the same age and strain, which were not influenced by the training or transplantation.

The graft survived in 74 % of adult untrained mice, 67 % of adult trained, 88 % of young untrained and 100 % of young trained mice. Both young and aged Lurchers showed significantly worse learning ability than wild type mice of the same age. In wild type mice there were no differences in spatial learning ability between young and older animals while aged Lurcher mutants achieved worse results than young ones. Both the enforced training and transplantation led to improvement of spatial learning ability in aged Lurcher mice. When not combined, the training was more effective than the transplantation but trained mice with the graft showed similar results as untrained mice with graft. In young Lurchers no effect of the training or transplantation was observed.

In the contrary to wild type mice, in Lurchers spatial learning ability was decreased by their higher age. Physical activity or transplantation helped older Lurchers to compensate the age related deficit of spatial learning ability. This treatment improved their learning ability almost to the level found in younger Lurchers, but not above it and they remained still worse than wild type mice. Combination of both treatments did not lead to any mutual enhancement of their effects. In young Lurchers, in which the spatial learning test started at the age of 72-74 days, the treatment with the physical activity or transplantation was completely ineffective.

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Retrospective longitudinal study of titres of specific antibodies to ß tubulin class III in patients with MS and depression treated by glatimeracetate and SSRI

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Objective
Hypothesis of the relationship between depression and dysfunction of the immune system in MS patients is supported by psycho-neuroimmunological studies, clinical experience and by neuroimaging methods which detected ceasing of the inflammatory changes in MS patients treated by glatirameracetate and SSRI. IgM and IgG specific antibodies to tubulin ß class III (sabt) were found in samples from patients with NS diseases causing axonal damage.

Methods
This retrospective longitudinal study was planned to compare the changes of titres of IgM and IgG sabt in serum of MS patients treated by glatirameracetate and SSRI. All patients were divided into 2 groups. Group A (n=21, glatirameracetate + SSRI), control group (n=58, glatirameracetate). The titres of sabt were determined in all 79 patients in the 6th month of the therapy, sabt were tested during the whole examination period three times at least.

Results
The titres of sabt (IgG and IgM) decreased significantly from the start of glatimeracetate therapy in group A+ control patients without a significant difference. A significant decrease of IgM sabt titres was detected in the 6th month of glatimeracetate therapy in depressive patients treated by SSRI (Group A) contrary to the patients untreated by SSRI (Control group).

Conclusion
Decrease of IgM titres of specific serum antibodies to ß tubulin class III found in patients treated by glatimeracetate & SSRI supports the hypothesis of neuroprotective effect of SSRI. A further research in MS patients treated with glatimeracetate and SSRI is required.

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Regulation of RGS3 function by 14-3-3 protein

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The regulator of G protein signaling (RGS) proteins serve as negative regulators of G protein-mediated signal transduction. RGS proteins function both as GTPase activating proteins and regulators of G protein-effector interactions. RGS proteins act as GTPase activating proteins specific to Galpha subunit, and thus they play a crucial role in the shutting off process of G protein-mediated cell responses. The 14-3-3 proteins are a family of regulatory proteins that play an important role in the regulation of signal transduction, apoptosis, cell cycle control, and nutrient-sensing pathways. It has been demonstrated that 14-3-3 proteins bind to other proteins in a phosphorylation dependent manner. RGS3 protein is one of the RGS proteins recently found to be 14-3-3 binding partners. The role of 14-3-3 protein in the regulation of RGS3 seems to be an inhibition of its GAP (GTPase activating protein) function on G protein mediated signals. We have prepared RGS3/14-3-3 complex in vitro. Complex stoichiometry and stability was investigated. Various methods of fluorescence spectroscopy have been used to characterize conformational changes of RGS domain induced by 14-3-3 protein. Crystal structure of RGS domain of RGS3 protein (pdb code 2OJ4) was solved and this structure has been used both to design and interpret experiments based on tryptophan fluorescence.

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Does 14-3-3 Protein Affect Conformation of FoxO4 DNA-Binding Domain?

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14-3-3 proteins are abundant regulatory molecules expressed in all eukaryotes. They bind to other proteins in a phosphorylation-dependent manner. Through these interactions they are involved in signal transduction, metabolism control, oncogenesis, or apoptosis. More then 300 different 14-3-3 binding partners were identified up to date (e.g. FoxO, p21CIP1, BAD). Since the 14-3-3 protein molecule seems to be very rigid, it was suggested that 14-3-3 protein functions as molecular äanvilô that modulates both the structure and the function of the bound ligand.
We used fluorescence spectroscopy to investigate whether the 14-3-3 protein changes conformation of DNA Binding Domain (DBD) of transcription factor FOXO4. FOXO4 (AFX) belongs to a family of forkhead transcription factors family and regulates life span, response to stress and apoptosis. AKT/PKB-induced phosphorylation of FOXO4 at Thr28 and Ser198 generates two 14-3-3 protein binding sites. The 14-3-3/FOXO4 complex is subsequently rapidly relocated from the nucleus to the cytoplasm probably as a result of the 14-3-3 protein-mediated hindrance of FOXO4 nuclear localization sequence (NLS). It is also known than 14-3-3 protein inhibits DNA-binding potential of FOXO4 through still not fully understood mechanism.

Main goal of this study was to investigate whether the 14-3-3 protein binding affects the conformation of FOXO4 DBD. AKT/PKB-phosphorylation sites Thr28 and Ser193 were mutated to generate versions of FOXO4 containing only one phosphorylation site. Time-resolved tryptophan fluorescence intensity and anisotropy decays of single and doubly phosphorylated FOXO4 mutants were measured to investigate the 14-3-3 protein-induced changes of FOXO4 DBD structure and mobility. Our data indicate that 14-3-3 protein binding does not induce any significant structural changes in FOXO4-DBD. Thus the 14-3-3 protein-induced inhibition of FOXO4 DNA binding potential results from other processes, e.g. the masking of DNA binding interface.

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Determinants of calmodulin binding site on the C-tail of TRPC6 channel

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Transient receptor potential channel TRPC6 is a non-selective calcium permeable cation channel expressed in many cell types, including sensory receptor cells. TRPC6 consists of four subunits with six membrane-spanning domains and intracellular N- and C-terminal. Calmodulin (CaM) takes part in the calcium dependent regulation of many proteins, including ion channels. There was identified one CaM binding site on the C-tail of TRPC6. The aim of this study is to map in detail C-terminal region of mouse TRPC6 that is capable of interacting with CaM using in-vitro binding assays. The part of sequence of the C-tail (amino acids 801-878) was subcloned into pET15b or pET42b expression vectors and used as a template for site directed mutagenesis. There were performed mutations of several amino acid residues that could potentionally distrupt CaM binding. These residues were chosen on the basis of three-dimensional computer model. All fusion proteins were expressed in E. coli Rosetta (DE3) and purified using nickel-chelating sepharose. The homogeneity of the purified recombinant proteins was confirmed by SDSPAGE electrophoresis and mass spectrometry. The ability of binding of the protein (amino acids 801-878) and its mutants to CaM was tested by fluorescent anisotropy measurements using CaM Alexa Fluor 488 fluorescent probe. Our results show that amino acids R852, Y854, K856, M858, K859, R860, L861, K863, R864 and L867 participate in CaM binding on C-termini of TRPC6.

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ATP binding studies on the C - termimus TRPV1

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Transient receptor potential channel vanilloid receptor subunit 1 (TRPV1) is a non-selective cation channel, with the relative preference for Ca^{2+} and Mg^{2+}, responsible for a heat and chemical – evoked pain response. TRPV1, together with more than 30 homologous channels, belonging to the TRP channels family that could be divided into seven subfamilies. Although ATP by itself doesn't have the function of direct activator, intracellular ATP increases TRPV1 currents by direct interaction with the nucleotide-binding domains, so called Walker A and Walker B motifs, that are homologous to other ATP binding proteins. On the basis of this similarity and using the structure of Fragile histidine triad protein (FHIT) a comparative computer model of the C- terminus was created and molecules of ligands were docked. The main aim of our study was the estimation of the role of single amino acids those create the ATP binding domain and the confirmation of the ability to bind ATP to isolated C-tail TRPV1. In order to do that, the set of single amino acids inside the walker motif A, were selected and using PCR replaced by alanine. Protein was expressed in bacteria E. coli strain BL21. To avoid impurities, two steps of purification protocols were performed. Consequently fluorescence ATP binding studies were undertaken, namely TNP-ATP competitive binding assay, steady-state and time resolved measurement of FITC labeled samples and quenching of FITC fluorescence. Our experimentally gained results are in accordance with our comparative computer model and confirm the ability of isolated CT TRPV1 to bind TNP-ATP and ATP. Mutation of K735 caused the complete inability to bind ATP to the isolated C-terminus TRPV1. K735 could be pointed out as a crucial residue for ATP binding.

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Possible involvement of ryanodine receptor-controlled calcium release in sensitization of GnRH-stimulated IP3 receptor in melatonin-sensitive neonatal gonadotrophs of the rat

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Ca^{2+} mobilization plays an important role in pituitary function by stimulating Ca^{2+}-dependent synthesis and release of pituitary hormones. In addition to inositol-1,4,5-trisphosphate (IP3) mediated Ca^{2+} mobilization, Ca^{2+} release from ryanodine (Ry)-sensitive pools and Ca^{2+}-influx through voltage-dependent Ca^{2+} channels, store-operated channels and TRP channels have been suggested to be important in pituitary Ca^{2+}-signaling. The function of IP3 and Ca^{2+}-conducting channels is relatively well documented. However, the molecular identity of IP3 receptors (IP3R) and function of ryanodine receptors (RyR) are still elusive. We have previously shown that GnRH-stimulated Ca^{2+}-signaling in a subpopulation of neonatal gonadotrophs is sensitive to melatonin, a hormone secreted by pineal gland. Removal of extracellular calcium delays the onset of agonist-stimulated Ca^{2+} oscillations similarly as melatonin, indicating that extracellular Ca^{2+} influx could play an important role in melatonin inhibition of neonatal pituitary gland function. Here we hypothesized that Ca^{2+}-stimulated RyR is important for sensitization of IP3R in neonatal gonadotrophs and contributes to melatonin inhibitory effect on calcium signaling. Using PCR and patch-clamp techniques, functional expression of RyRs and IP3 channels was determined in mixed population of anterior pituitary cells. The mRNA transcripts for RyR type 1, 2, and 3 subunits were identified in 6-days old rats. The specificity of primers was further confirmed by the sequence analysis of PCR products. In patch-clamp experiments on identified neonatal pituitary gonadotrophs, GnRH application induced oscillatory hyperpolarizing current mediated by activation of Ca^{2+}-activated K+ channels, that reflected oscillatory InsP3 mediated Ca^{2+} release. In melatonin-sensitive gonadotrophs, the onset of GnRH-induced current oscillations were delayed by ryanodine and shortened by applications of caffeine (0.5 mM). Neonatal pituitary expressed also all types of IP3R, the IP3R1, IP3R2 and IP3R3. In identified gonadotorphs, intracellular introduction of IP3 stimulated Ca^{2+}-oscillations that were insensitive to Ry application. Our findings suggest that, in addition to InsP3 mediated Ca^{2+} release, Ca^{2+} release from ryanodine-sensitive stores mediated by RyR1-3 might contribute to Ca^{2+} mobilization in neonatal pituitary gland. The expression of functional RyR could represent important components of Ca^{2+} signaling in neonatal pituitary which could explain its specific inhibition by melatonin.

Identification of Transmembrane Residues Contributing to Channel Gating and Interaction with Ivermectin at Rat Purinergic P2X4 Receptor

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Ivermectin (IVM) is a member of class of lipophilic compounds known as avermectins, that are used as an antiparasitic agent in human and veterinary medicine. The therapeutic effects of IVM is mediated by its interaction with glutamate-gated chloride channels expressed by parasite, leading to muscle paralysis and starvation. In addition to glutamate-gated chloride channels, IVM modulates function of several other ligand-gated channels, including GABAA receptors, recombinant glycine-activated chloride channels, and neuronal α7nicotinic receptors. At purinergic P2X receptors (P2XRs), a family of ligand-gated ion channels activated by binding of extracellular ATP, IVM specifically enhances the P2X4R-channel function by interacting with the open conformation state of the channel. IVM increases both the sensitivity of receptor to ATP and the current amplitude in response to supramaximal agonist concentrations. It also greatly prolongs the deactivation of current after agonist washout. We have previously shown that IVM actions are independent of ectodomain structure of P2X4R. In this study we used cysteine scanning mutagenesis of both transmembrane domains (TM) of the rat P2X4R and examined effects of mutations on ATP potency, peak amplitude of currents in response to supramaximal agonist concentrations, and receptor deactivation in the absence and presence of IVM. The receptor function was unchanged in mutants of 28 residues, and among them the IVM effects were altered at Gln36, Leu40, Val43, Val47, Trp50, Asn338, Gly342, Leu346, Ala349, and Ile356 mutants. The substitution-sensitive Gly29, Arg33, and Cys353 mutants could also be considered as IVM-sensitive hits. The pattern of these 13 residues was consistent with helical topology of both TMs, with every third or fourth amino acid affected by substitution. These predominantly hydrophobic-nonpolar residues are also present in the IVM-sensitive Schistosoma mansoni P2X subunit. They lie on the same side of their helices and could face lipids in the open conformation state and provide the binding pocket for IVM. In contrast, the IVM-independent hits Met31, Tyr42, Gly45, Trp46, Val49, Gly340, Leu343, Ala344, Gly347, Thr350, Asp354, and Val357 map on the opposite side of their helices, probably facing the pore of receptor and playing important roles in gating. These results for the first time identified residues of the potential importance for P2X4R channel gating and interaction with IVM.

Disruption of the plasma membrane structure by depletion of cholesterol impairs effectiveness of TRH receptor-mediated signal transduction via Gq/11-alpha protein

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According to the raft hypothesis, sphingolipid-cholesterol microdomains are involved in a number of signaling processes in the plasma membrane. Here we aimed to evaluate the presumed role of these structures in signal transduction mediated by thyrotropin-releasing hormone (TRH) receptors and their cognate Gq/11-alpha proteins. These experiments were conducted in HEK293 cells transfected to express high levels of TRH receptors and G11-alpha protein (clone E2M11). We monitored distribution of TRH receptors and Gq/11-alpha proteins, as well as a functional status of the whole signaling system. Our analyses indicated that disruption of plasma membrane microdomains by cholesterol depletion (through beta-cyclodextrin treatment) did not markedly influence binding parameters of TRH receptors, but altered efficacy of signal transduction. The functional coupling between TRH receptor and Gq/11-alpha was assessed by agonist-stimulated GTPgammaS binding and results of these measurements pointed out to significantly lower potency of TRH to mediate G-protein activation in samples prepared from cholesterol-depleted cells; there was a shift in sensitivity by one order of magnitude to the right. A similar marked shift to the right in the sensitivity to stimulation with TRH was observed in our experiments dealing with determination of the hormone-induced Ca^{2+} response. Moreover, cholesterol depletion prolonged lag phases and somewhat reduced maximal TRH-induced Ca^{2+} responses. Our additional investigations performed by fluorescence confocal microscopy indicated that treatment with beta-cyclodextrin also affected the pattern of Gq/11-alpha distribution in the plasma membrane of E2M11 cells. Subcellular fractionation studies indicated that membrane domains/rafts were degraded by cholesterol depletion in intact cells. Collectively, results of our present study suggest that the intact structure of plasma membranes is intact structure of plasma membranes is necessary for an optimum signal transduction initiated by TRH receptors and mediated by Gq/11-alpha proteins.

Calcium responses to thyrotropin-releasing hormone and angiotensin II. The role of plasma membrane integrity and effect of G11-alpha protein overexpression on homologous and heterologous desensitization

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The molecular mechanisms involved in thyrotropin-releasing hormone (TRH) and angiotensin II (ANG II) receptor-mediated signaling are still not understood in detail. Here we analyzed hormone-induced Ca^{2+} responses and the process of desensitization in HEK-293 cells, which express endogenous ANG II receptors. These cells were transfected to express also high levels of TRH receptors (clone E2) or both TRH receptors and G11a protein (clone E2M11). We observed that the characteristics of Ca^{2+} responses, as well as the process of desensitization of hormone response, both were strongly dependent on the receptor number and G11-alpha protein level. Whereas treatment of E2 cells with TRH or ANG II led to significant desensitization of Ca^{2+} responses to subsequent stimulation, these responses were not desensitized in E2M11 cells expressing high amounts of G11-alpha. Importantly, the process of desensitization and recovery of the Ca^{2+} response was strongly dependent on temperature. Besides that, addition of THR to both cell lines elicited a clear heterologous desensitization of the Ca^{2+} response to ANG II. On the other hand, ANG II did not affect subsequent response to TRH. In addition, ANG II-mediated signal transduction was strongly dependent on plasma membrane integrity, but signaling through TRH receptors was relatively slightly modified by cholesterol depletion. It might be concluded that the level of expression of G-protein-coupled receptors and their cognate G-proteins strongly influences not only the magnitude of cellular response but also the process of desensitization and resilience of transmembrane signaling mediated by these receptors to changes in the plasma membrane structure.

Delayed effects of xanomeline on evoked ACh release from rat brain slices

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Xanomeline binds to the orthosteric site of all muscarinic receptor subtypes with high affinity but in short-term assays selectively stimulates only M1 and M4 receptors. We observed previously that at all receptor subtypes xanomeline also interacts with another distinct site where it binds in a wash-resistant manner. This unusual type of binding caused delayed persistent stimulation of GTP-γ 35S binding at the M1 receptor and with lower efficacy but similar affinity also at the M2 receptor (1). It also causes durable antagonism of M5 receptor activation (2). To further investigate the functional outcome of wash-resistantly bound xanomeline we tested assumed direct and delayed effects of xanomeline on electrically-evoked ACh release from rat brain slices that is autoinhibited by presynaptic M2 and M4 receptors in cortical and striatal slices, respectively. Slices were loaded with tritiated choline to label acetylcholine and then exposed for 15 minutes to xanomeline either 1 hour before or during measurement of electricaly-evoked ACh release. Xanomeline (10 μM) had no direct effect on ACh release in either cortical or striatal slices. In contrast, wash-resistantly bound xanomeline (15-minutes preincubation with 1, 10 or 100 μM xanomeline followed by 1 hour washing before stimulation) concentration-dependently decreased ACh release from both cortical and striatal slices. Xanomeline delayed effects were not additive to the decrease of ACh release caused by the agonist carbachol. Furthermore, xanomeline did not prevent inhibitory effect of carbachol when present together during stimulation. Wash-resistant effects of xanomeline were fully (1 and 10 μM) or partially (100 μM) reversed by 1 μM N-methylscopolamine, a muscarinic antagonist. However, delayed inhibitory effects of xanomeline were not prevented by either presence of N-methylscopolamine during xanomeline treatment or irreversible inactivation of the orthosteric binding site by propylbenzilylcholine mustard prior to xanomeline treatment in cortical slices. These data demonstrate that xanomeline functions as wash-resistant M2 or M4 agonist and that the orthosteric binding site is not necessary for creation of wash-resistant binding. On the other hand, blocking of the orthosteric site can at least partially prevent agonistic effects of wash-resistantly bound xanomeline. Together, these findings strongly indicate that the complicated pharmacological profile of xanomeline action may be also due to not yet well characterized effects of wash-resistant xanomeline at all subtypes of muscarinic receptors thus far tested.

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Cholesterol differentially influences G-protein signalling activated by the muscarinic M2 receptor

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The muscarinic acetylcholine M2 receptor that was originally identified as the predominant muscarinic receptor subtype in the heart is also widely distributed in the central nervous system. Its signal transduction is effected by both the ßγ dimer of heterotrimeric G-protein that activates potassium or inhibits calcium conductance and the α subunit that preferentially inhibits cAMP synthesis. However, we demonstrated that M2 muscrinic receptors expressed in CHO cells (CHO-M2) directly activate signalling pathways of all three major subclasses of G-proteins, i.e. preferred Gi/o subclass and at concentrations higher than needed for standard inhibition of forskolin-stimulated cAMP synthesis also Gs and Gq/11 subclasses to cause stimulation of cAMP synthesis and accumulation of inositolphosphates (IP), respectively (1-3). In the present experiments we investigated influence of membrane cholesterol content on activation of signalling pathways of these three G-protein subclasses in CHO-M2 cells by carbachol, a non-hydrolysable acetylcholine analogue. Treatment of cells with methyl-ß-cyclodextrin decreased cell and membrane cholesterol content by 74% and 39%, respectively, and incubation in the presence of cholesterol-saturated methyl-ß-cyclodextrin increased cholesterol content by 169% and 137%, respectively. The decrease in cholesterol was accompanied by an increase of plasma membrane muscarinic receptor density measured in saturation binding experiments with the tritiated non-permeable antagonist 3H-N-methylscopolamine by 63% in intact cells whereas the increase in cholesterol had no significant effect. Cholesterol depletion increased efficacy of carbachol in inhibiting forskolin-stimulated cAMP synthesis by 35% (Gi/o) and in stimulating cAMP synthesis by 69% (Gs) but decreased its efficacy in stimulating IP accumulation by 35% (Gq/11). Cholesterol enrichment significantly reduced cAMP synthesis inhibition (by 23%) but had no effect on stimulation of cAMP synthesis or IP accumulation. Cholesterol depletion significantly increased the potency of carbachol on the IP response (decrease of EC50 from 83 to 49 μM) but had no influence on its potency in stimulating or inhibiting cAMP synthesis. These findings may be relevant to the impairment of brain cholinergic transmission and cognitive functions in neurodegenerative diseases linked to abnormalities of cholesterol metabolism like Alzheimer´s disease.

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Looking for fluorescent probes for allosteric binding sites at muscarinic receptores: Unusual, but not exclusive, mechanism of interactions of tacrine at muscarinic receptors

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It has been observed repeatedly1[1,2], that the inhibitor of cholinesterases tacrine (1,2,3,4-tetrahydroacridin-9-amine) inhibits the binding of orthosteric muscarinic ligands to muscarinic receptors with unusually steep binding curves (high Hill slopes about value 1.5). Definite explanation of this phenomenon is not available. A mechanistical model has been proposed according to which tacrine binds to the orthosteric site and to the allosteric site simultaneously with homotropic positive cooperativity [3]. At the same time, the binding of tacrine to the allosteric site has a negative efect on the binding of the commonly used radiolabelled muscarinic ligands ([3H]N-methylscopolamine, or [3H]quinuclidinyl benzilate) to the classical site. Another hypothesis suggests that tacrine binds to two spatially separated allosteric sites on muscarinic receptor with positive cooperativity or its binding to the common allosteric site modulates receptor-receptor interactions [4]. We have discovered recently that structurally closely related compounds 7-methoxytacrine, acridin-9-amine, and proflavine (acridine-3,6-amine) also yield characteristically steep binding curves while quinacrine (6-chloro-9-([4-diethylamino]-1-methylbutyl)amino-2-methoxyacridine) behaves like a competitor and displaces the orthosteric ligand [3H]N-methylscopolamine yielding the standard curves with Hill slope close to 1. These experimental findings are rather surprising. First, tacrine has been synthesized as a three-dimensionally voluminous analogue of acridin-9-amine in order to demonstrate that the complete planar acridine nucleus is required for antibacterial activity. Nevertheless, their effects at muscarinic receptors are almost identical. On the other side, allosteric modulators proflavine and acridin-9-amine, together with ‚orthosteric' competitor quinacrine belong to the same category of compounds which possess the very special mechanisms of action: intercalation, stacking and self-aggregation [5]. We hypothesize that the bulky substitution at position 9 on basic acridine skeleton sterically hinders the simultaneous binding quinacrine into orthosteric and allosteric binding sites while the molecules of tacrine, or 7-methoxytacrine, respectively, can stack themselves, as do molecules of proflavine, or acridin-9-amine, inside the receptor pocket.

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M1 receptors participate in the nonquantal acetylcholine regulation via feedback NO action

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Nitric oxide (NO), previously demonstrated to participate in the regulation of the resting membrane potential in skeletal muscles via muscarinic receptors, also regulates non-quantal acetylcholine (ACh) secretion from rat motor nerve endings(1). The muscarinic agonists oxotremorine and muscarine lowered the H-effect and the M1 antagonist pirenzepine prevented this effect. M-agonist ABET, which is more selective for M2 receptors than for M1 receptors and DAMP, a specific antagonist of M3 cholinergic receptors had no significant effect on the H-effect. The oxotremorine-induced decrease in the H-effect was calcium and calmodulin-dependent. The decrease was negated when either NO synthase was inhibited by L-NAME or soluble guanylyl cyclase was inhibited by ODQ. The target of muscle-derived NO is apparently nerve terminal guanylyl cyclase, because exogenous hemoglobin, acting as an NO scavenger, prevented the oxotremorine-induced drop in the H-effect. Oxotremorine and non-quantal ACh selectively inhibit the non-quantal secretion of ACh from motor terminals acting on post-synaptic M1 receptors coupled to Ca^{2+} channels in the sarcolemma to induce sarcoplasmic Ca^{2+} dependent synthesis and the release of NO. It seems that a substantial part of the H-effect can be physiologically regulated by this negative feedback loop, i.e., by NO from muscle fiber and take place during synapse fomation, maintenance and degradation; there is apparently also Ca^{2+} and calmodulin-dependent regulation of ACh non-quantal release in the nerve terminal itself, as calmidazolium inhibition of the calmodulin led to a doubling of the resting H-effect.

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Photoperiodic entrainment of the circadian molecular clock in the mice SCN

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The circadian rhythms are controlled by clock located in two suprachiasmatic nuclei (SCN) of the hypothalamus. The basic molecular core clock mechanism responsible for generation of the SCN rhythmicity became partly elucidated with cloning of mammalian clock genes. The underlying mechanism is based on interlocked feedback loops between transcription of clock genes and translation of their protein products. The SCN rhythmicity and molecular core clockwork are affected by duration of daylength, i.e., photoperiod that changes with the season in the temperate zones.

The aim of this study was to characterize the effect of the rectangular (abrupt light on/off transition) and twilight (gradual light on/off transition) photoperiod on the molecular time keeping system within the mice SCN. Mice were maintained under a long (18h of light and 6h of darkness, LD 18:6) or a short (6h of light and 18h of darkness, LD 6:18) photoperiod with rectangular or twilight light/dark transition. Daily locomotor activity was monitored. On the day of the experiment, mice were released into constant darkness and sacrificed every 2h throughout the whole circadian cycle. Daily profiles of mRNA of clock gene, namely Per1, Per2, Cry1, Cry2, Bmal1, Clock, and immediate-early gene c-fos, as well as of proteins, namely PER1, PER2 and c-FOS, were determined in the SCN by in situ hybridization and immunohistochemistry respectively. The photoperiod affected phase, waveform, and amplitude of the rhythmic profiles of gene and protein expression as well as phase relationship between the profiles.

The data indicate that the complex molecular clockwork in the mice SCN is photoperiod modulated and hence may differ according to the season of the year. The modulation is also is significant under nature-like conditions of photoperiod with twilight.

Subunit composition of hippocampal NMDA receptor in heuristic (animal) model of schizophrenia

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The pathophysiology of schizophrenia is poorly understood but is likely to involve alterations in excitatory glutamatergic signaling that may be related to dysregulation in the expression of the N-methyl-D-aspartate receptor (NMDA-R) subunits in cortical-subcortical brain areas. Heterogeneity of the postmortem findings is an occasion for validation of the NMDA-R hypofunction hypothesis using heuristic (neurodevelopmental) animal models of schizophrenia. As prenatal inflammation belongs to risk factors in the etiopathogenesis of schizophrenia, we used an animal model of schizophrenia based on the intraventricular (i.c.v) infusion of quinolinic acid (QUIN), a potentially excitotoxic metabolite of tryptophan, levels of which are increased during brain inflammations. The QUIN infusion on postnatal day 12 (PND 12) resulted in a reduction of NMDA-sensitive [3H]glutamate binding to the hippocampal membranes of rat males on PND 50 [1]. Further analysis of the decreased NMDA-R density revealed changes in the laterality and protein expression of main subunits forming the receptor NR1/NR2 tetramer. We showed that there is an asymmetrical expression of protein for hippocampal NR1 subunit in intact/controls which disappeared in rats with model psychosis representing a significant decrease in the subunit NR1 protein in the left hippocampus. The hippocampal NR2 subunit were well-detectable in Western blots except the NR2C protein. The subunit NR2A protein exhibited insignificant elevation in the left hippocampus, but was significantly less expressed in the hippocampi of neonatally QUIN-treated rats. In contrast, the NR2B subunit was more expressed in the right hippocampus of naive rats and highly significant decreases were found in both hippocampi of rats with model psychosis. Upon neonatal QUIN treatment the expression of NR2D subunit decreased by one/third in the both rat hippocampi. Interestingly, the asymmetry of the NR2D expression was reversed in the neonatally QUIN-treated animals with a higher NR2D density in the left hippocampus. This is the first report demonstrating right/left asymmetry in the protein expression of NR1 and NR2D subunits of NMDA receptors in the rat hippocampus. Based on our data we propose that the alteration of the left/right asymmetry of NMDA receptor expression may play a role in schizophrenia.

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Acute and subchronic administration of quinolinic acid and psychotic-like behaviour in neurodevelopmental model of schizophrenia

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Schizophrenia (SCZ) is considered to be a serious mental disease with compromise working capacity, social interactions and family life. Its etiology is still largely unknown but increasing evidence suggests that SCZ results from an interaction between genetic background and early environmental factors. The adverse environmental factors, infectious agents and consequently released substances like cytokines, are involved in abnormal brain development resulting from the affected neuronal proliferation, migration and formation of synaptic connections. The constructed animal model was based on the simulation of pro-inflammatory processes as one of environmental factors asserting in early periods of the brain development. Previous results have suggested that neonatal exposures of rats to quinolinic acid, a cytokine-like product of activated microglia/macrophages (QUIN), alters developmental processes and can induce behavioural changes comparable to schizophrenia. Male Wistar rat pups were daily injected with QUIN in a dose of 1 mg and/or 10 mg/kg intraperitoneally (i.p.) between postnatal day 4 and 8 (PND 4-8) whereas controls were injected with saline only; intact (naive) pups were without manipulation. On PND 50 the prepulse inhibition (PPI) of acoustic startle (ASR), a measure of sensorimotor gating, was carried out to study the mechanisms acting as a filter of environmental stimuli. Clinical findings have reported that the PPI is regularly disrupted in schizophrenic patients and, therefore, it was used to investigative the psychopathology of SCZ. The PPI was measured, after a 5-min habituation, in pseudo-randomly arranged 50 trials with the intensity of 3, 5 and 10 dB above "white" noise (70 dB). Results showed that QUIN administered in early neonatal period (PND 4-8) did not change basal startle amplitudes (ASR) when injected systematically at daily doses of 1 and 10 mg/kg i.p., respectively. Also any significant alteration of PPI levels was observed in 50-day-old rats. However, impairment of PPI was found after the intracerebroventricular (i.c.v.) infusion of QUIN application as it was shown in previous experiments. We conclude that the systemic administration of this cytokine-like compound is not sufficient to affect negatively the development of sensorimotor gating owing to (1) low doses of QUIN used and/or to (2) the blood-brain barrier functioning. In contrast, the i.c.v. administration of QUIN documents an existing vulnerability of the immature rat brain to infectious stimuli as a risk factor for manifestation psychotic-like behaviour.

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Protein expression of NMDA-NR1 subunit and acoustic startle in genetic (animal) model of schizophrenia

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Decreased expression of ubiquitous NMDA-NR1 subunit in some schizophrenic brain regions has been related to several single nucleotide polymorphisms (SNP) in the subunit gene GRIN1 [1]. Also the neurodevelopmental (animal) model of schizophrenia-like behaviour based on the neonatal treatment of 12-day-old rat pups with quinolinic acid (QUIN; 250 nmol/0.25 μL 0.9% NaCl into each lateral cerebral ventricle), exhibited a decrease in the hippocampal subunit NR1 protein together with an impairment in the sensorimotor gating (a deficit in the prepulse inhibition of startle reaction) accompanying schizophrenia-spectrum disorders [2]. However, the changed levels of the subunit NR1 protein went together with modified expression of NR2 subunits, it was impossible to reveal a direct relationship between the decreased NR1 expression and changed prepulse inhibition (PPI) of the acoustic startle (ASR). Therefore, we tried to decrease selectively the expression of NR1 subunit using a single dose (5 nmol/2.5 μL H2O) of (a) antisense oligodeoxynucleotide (aODN; the 18-mer of NR1 sequence corresponding to bases 4-21 [3]), (b) sense oligodeoxynucleotide (sODN) specific for NR1 subunit and/or (c) distilled H2O infused into ventral hippocampi of 50-day-old rat males. Twenty-four hours later, we determined a statistically significant decrease of the NR1 protein levels in right hippocampi (cca 20 %), but PPI of the ASR remained unchanged. At the next stage of NR1 subunit suppression we infused the aODN, sODN (20 nmol/24 h) and/or H2O for 7 days with the Alzet mini-osmotic pumps (Model 2001, rate 1 μL/h). The aODN-induced decrease in the NR1 protein expression was similar to its acute application, but the PPI was not changed. Our findings can be interpreted as follows: (1) the aODN-induced decreases in the NR1 protein expression are not sufficient to change PPI values and/or (2) the changed NR1 expression is not directly related to PPI deficits.

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Lateralization of 17beta-hydroxysteroid dehydrogenase type 10 in hippocampi of demented and psychotic patiens

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Multifunctional mitochondrial enzyme 17beta-hydroxysteroid dehydrogenase type 10 can play a role in the development of Alzheimer disease via its high-affinity binding to amyloid beta peptides. In the brains of Alzheimer disease patients, enzyme overexpression has been found. We evaluated specificity of alterations in enzyme mRNA levels in human autoptic right and left hippocampi. We observed marked right/left laterality in nondemented nonpsychotic controls and in people with multi-infarct dementia. In Alzheimer disease and schizophrenia, however, shifts to left/right asymmetry were found and the changes were associated with the increase especially in the dominant left hemisphere. Our results are in agreement with the studies reporting the involvement of mitochondria in modulation of intracellular levels of sex steroids and in behavioral laterality. Moreover, our experiments support the studies reporting overexpression of some mitochondrial enzymes and increased vulnerability of the dominant left hemisphere in Alzheimer disease. However, the similar -although smaller - changes observed in the hippocampi of patients with schizophrenia are surprising and suggest that overexpression of enzyme should not be associated only with the intracellular amyloid beta peptide pathway. We suppose that the enzyme influences the development of sex-dependent brain asymmetry and that its levels e.g. in CSF could be used as a biomarker to distinguish Alzheimer disease from multi-infarct dementia. Nevertheless, a direct estimation of laterality in activity/enzyme expression should be performed in future. Another subtypes of vascular dementia and another dementias remain to be elucidated

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Absorption of high-frequency electromagnetic radiation by the mouse brain; theoretical model and real experiment

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High frequency electromagnetic field (HF EMF) became a common part of our environment because it is produced by many artificial sources as radars, transmitters and especially cellular (mobile) phones. In the presenting study we demonstrate a possible methods for theoretical and real measurement of HF EMF absorption using 900 and 1800 MHz microwave source.

Experimental animals were exposed to HF EMF with frequency of 900 and 1800 MHz. Output power of the HF EMF generator after amplifying was aproximately 10 W or 1 W respectively. Mice were placed into a plastic box just before the orifice of the waveguide. Control mice were kept in analogous conditions without the HF EMF. In the first phase model experiments arranged for SAR (Specific Absorption Rate) detection were performed. The value shows a absorption dose of radiation in the animal tissue. In the distance 0.3 m from the orifice it is practically impossible to measure SAR directly and only an estimation (from the gradient of the field) suggests the whole-body exposure which correspond to the triple power of classical GSM.

In the second part a computerized modeling of electromagnetic field absorption in the animal body was created. To estimate SAR in experimental animals we have done basic 3D calculation of electromagnetic energy distribution in a simplified dielectric model of an adult mouse. The model consists of a homogenous lossy dielectric material mimicking muscle tissue and it has cylindric shape (radius 3 cm and high 9 cm terminated to cone) with dielectric properties er = 54, conductivity s = 0.8 S/m, density r = 1000 kg/m3. The calculations were done with the aid of 3D electromagnetic field simulator SEMCAD which used FDTD (Finite Different Time Domain). The method is based on the fact that original continuous function is replaced by the set of discrete function‘s values. Maxwell‘s equations are discretized using a 2nd order finite-difference approximation both in space and in time in an equidistantly spaced mesh. Several simulations for different positions of mice were done. Calculations of SAR performed in connection with the simulation of absorption electromagnetic energy by experimental animals using the arteficial dielectric model showed the dependence of the absorption on the real topical position of the mouse against the waveguide and was in the range of 0.05 to 1.44 mW/g.

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Calretinin-contaning neurons in normal and epileptic human temporal neocortex

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The calcium binding protein Calretinin (CR) labels a specific interneuronal subpopulation in cerebral neocortex. CR immunoreactive (CR-ir) neurons typically innervate dendritic shafts and to a lesser degree dendritic spines of pyramidal neurons. At least in superficial cortical layers, they also innervate and thus inhibit the other interneuronal subpopulations and provide so the desinhibition of pyramidal neurons.

The aim of this study was to evaluate the distribution of CR-ir neurons in the normal human temporal neocortex.

Human brain tissues included in the study were obtained shortly post mortem during autopsies of 6 patients, who died due to diseases non-affecting the nervous system. Temporal pole and neighbouring parts of superior, middle and inferior temporal gyri were examined. 4 μm thick paraffin-embedded sections, monoclonal anti-CR antibodies, biotin streptavidin detection system and DAB for chromogenic development were used.

Two-dimensional counting method was used, the counts of immunopositive neurons were expressed as cell density per μm2. The highest average cell density was found in supragranular layers- I. 27 neurons/μm2, II. 138 neurons/μm2, III. 59 neurons/μm2. Density in IV. layer was 20 neurons/μm2. In infragranular layers, the cell density was markedly lower – 4 neurons/μm2 in V.+VI. layers. Occasionally, few CR-ir neurons were located in white matter. CR immunoreactivity of neuropil was highest in I. cortical layer, high in II. and III. layers and low in V. and VI. layers. The majority of CR-immunoreactive neurons exhibited bipolar and bitufted somatodendritic morphology. The results are in accordance with similar, previously published studies on human as well as on animal experimental material. The results of this study will be used as a control in the study of CR-immunoreactive neurons in temporal cortex in patients suffering from farmacoresistant epilepsy. After preliminary evaluation of material acquired from patients with various types of temporal lobe epilepsy, it seems that there is no significant change of CR-ir neurons density comparing to healthy controls.

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Changes in the permeability of blood brain barrier: Cortical photothrombosis and epileptic seizure induced by flurothyl inhalation

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We evaluated local and remote changes of blood brain barrier (BBB) permeability in the model of cortical photothrombosis (PT) and tested the influence of the choice of anaesthetic agent on our results. Also, we evaluated overall changes in BBB permeability after flurothyl (FT) induced seizure.

Male Wistar rats were used for all the experiments (w = 200-220g). We have described the PT model in our previous publications. After i.v. application of Rose Bengal (RB), three stereotactically defined spots on the scull overlaying the left sensorimotor cortex were irradiated by a laser beam. Each spot was irradiated for 6 minutes. The animals were divided in two subgroups: in the first group, the rats were anaesthetised with pentobarbital (PB; N=3), in the latter, ketamine/xylazine was used instead (K/X; N=4). Animals of the control group received saline in place of RB (N=4).

In another group of animals (N=4), generalized epileptic seizure was induced by FT inhalation. FT was applied at a constant rate on a filtration paper located at the top of an airtight chamber, in which the rats were placed. At seizure onset, the air in the chamber was rapidly exchanged.

Immediately before irradiation in the PT model and before FT inhalation in the FT seizure model, the rats received i.v. Evans Blue (EB; 0.04 g/kg/2ml). In case of BBB disruption, the EB-albumin complex escapes from the blood vessels, and causes a blue stain of the adjacent nervous tissue. 24 hours after experimental manipulations, the brains of all animals were fixated, sectioned and evaluated with a fluorescence microscope.

24 hours after PT, all experimental animals showed large ischemic lesions located in the left sensorimotor cortex. An area of tissue showing BBB disruption surrounded the lesions. In this area, brightly red cells accumulating EB (EB+) were observed. The lesions were larger in the K/X group (mean lesion volume - MLV = 16.26 mm3), but the area of BBB disruption was relatively small (mean volume of tissue showing BBB disruption - MPV = 2.62 mm3). In the PB group, the lesion core was smaller (MLV = 15.11 mm3), however, a large area of BBB disruption (MPV = 9.46 mm3) surrounded the lesion. Moreover, in the PB group, EB+ cells were also observed in remote areas (incl. cerebellum).

Epileptic seizure induced by FT inhalation did not lead to lasting significant changes in the BBB permeability.

In the PT model, both the volume of the lesion core and the volume of tissue with BBB impairment can be influenced by the choice of anaesthesia. In addition, in the PB group, remote neuronal accumulation of EB was observed (to our knowledge, we are the first to report such results). Isolated FT seizure does not lead to lasting changes of BBB permeability.

Preconditioning effect of normobaric intermittent hypoxia on behavioral consequences of chemically induced seizures in rats

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Flurothyl vapors evoke tonic-clonic epileptic seizures in rats causing worsening of learning in water maze, despite absence of morphological changes. We presume involvement of free radical release in this. Earlier, we described protective effect of hypobaric hypoxia induced prior flurothyl seizures (FS). Intermittent normobaric hypoxia (INH), as occurs in obstructive sleep apnea, causes cognitive changes due to extreme production of free radicals. Present study was aimed to investigate whether INH would exhibit cross-preconditioning on FS in Wistar rats. To reveal cognitive changes we use Morris water maze (MWM). Three experiments were performed. First group was exposed to INH and flurothyl 3 days afterwards; second – to hypoxia alone, third – to flurothyl alone. Results were compared with controls (MWM tested only). INH was conducted in chamber (4L) in which oxygen concentrations were cycled between 21 and 8% every 30s during one hour. INH, FS alone or combination of both caused increase in latency and distance moved in WMW, in comparison to control animals. However, there was significant improvement of performance of animals preconditioned by INH in contrast to FS group. Thus on one hand, these findings confirm our hypothesis that INH has protective preconditioning effect, an increase of further insult tolerance. On the other, despite this effect it still causes some worsening of learning and memory comparing to our previous results with hypobaric hypoxia.

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The Effect of ET-1 on the Excitability of the Rat Cortical and Hippocampal Slices in Vitro

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In the mammalian CNS, endothelin (ET-1) potent vasoconstrictor is produced in neurons, endothelium and in the glial cells. It has been suggested that ET-1 may induce a wide range of physiological actions in CNS. In our lab, we apply direct injection of ET-1 into brain parenchyma as a model of focal ischemia in rats in vivo. Intracerebral application of ET1 produces seizures and starts epileptogenesis (Mateffyova, EJN 2006). Our aim was to conclude if epileptogenesis occurs due to the ischemia or if ET-1 itself also has an impact on excitability of the tissue. Experiments were performed in 15 adult, male Wistar rats (120-180 g). Animals were decapitated under deep anaesthesia, the brain cut in coronal and hippocampal (400 μm) slices. The slices were incubated in the incubation chamber containing buffered ACSF, ET-1 in concentration 20μM was added in ET-1 group. After one hour the slice was placed into interface recording chamber (perfusion 3ml/min) where the slices were maintained at 33 ± 1 °C in humidified, carbogenated (95% O_2, 5% CO_2) gas atmosphere. Glass microcapillary extracellular recording electrode (5–10 MΩ) filled with 1 M NaCl was positioned in the third layer of the somatosensory cortex of the cortical slice and in the stratum radiatum in the hippocampal slice, bipolar tungsten stimulating electrode was placed below the recording electrodes at the border of the white and gray matter in cortex and in the CA1 pyramidal cell layer in hippocampus. 10min after replacement of the slice into the recording chamber we determined the stimulus threshold. Every ten minutes up to 70 minute I/O curve was obtained by gradually increasing stimulus intensity in the range of T-4T in cortical slice and in the range of T-2.5T in hippocampus. Slope of the I/O curve was calculated from linear regression coefficient. For statistical comparison of thresholds and slopes t-test or ANOVA were used where appropriate. Cortical results: The evoked responses in both cortical groups have shown the same shape. The slope of the I/O curve does not significantly changed between measurements in both groups (contr P=0.9; ET-1 P=0.99). The slope of the control group was 26.1±0.97° and 29.93±3.5° in ET-1 group. There was no statistically significant difference between the groups (P = 0.62). Hippocampal results: The pop spikes have had the same shape in both groups. The slope of the controls was 48.69±3.3° and 48.15±3.1° of ET-1group.There was no statistically significant difference between the groups (P=0,907). In conclusion, our data shows that ET-1 does not directly influence excitatibility of both the rat cortical and hippocampal slices.

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Alteration of regulation of the regional cerebral blood flow in chronic epileptic rats

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Regulation of regional cerebral blood flow (rCBF) is important function of the nervous tissue which has to be finally ôtuned upö to cover metabolic demands of the tissue. However, in epileptic patients is relative hypoperfusion observed after the epileptic seizure as revealed by postictal SPECT. Thus it suggests that regulation of the rCBF might be altered. Thus we performed in vivo cortical measurement of blood flow in chronic epileptic rats. Broadly accepted model of the temporal lobe epilepsy (LiCl-pilocarpine) was used (Otahal et al, Epilepsia, 2005) Briefly, at postanatal day 12 (P12) males (n=9) of wistar rats were intaperitonealy injected with pilocarpine (40mg/kg) to produce status epilepticus. Peripheral cholinergic side effect of pilocarpine was minimized by pretreatment with scopolamine (1mg/kg, 1hr before). Day before were animals injected with LiCl (127mg/kg, i.p.) to decrease the dose of pilocarpine. Two hours after the SE developed it was stopped by application of the paraldehyde (0.3ml/kg, i.p.). Controls (n=10) obtained all treatment except pilocarpine. In part of experimental animals spontaneous epilepsy developed during 3 weeks. Part of the animals from both groups was EEG monitored for 72hours to confirm presence of spontaneous seizures. To assess rCBF rats were 3-6months after initial SE anesthetized with urethane (1g/kg, i.p.) Laser doppler probe was placed over the senzorimotor cortex. Two epidural stimulation silver electrodes were implanted contralaterally. EEG recording electrodes were implanted over the both hemispheres. The temperature of the animal was kept constant. Spontaneously breathing animals underwent series of stimulation to assess I/O relation. Changes of the rCBF were monitored during transcalosal stimulation (20Hz, 10s) with increasing currents (1-8mA). All results are expressed as means±S.E.M. For statistic comparison t-test was used where appropriate and ANCOVA was used to compare linear regression of I/O curves. EEG monitoring revealed presence of spontaneous seizures in 25% of animals. The stimulation of the contralateral senzorimotor cortex produced significant increase of the cerebral blood flow in both groups. The shape was monophasic in both groups with latency of the maxima 9.8±1.2s in controls and 9.8±0.8s (P=0.72) experimental group respectively. The rCBF fall back to zero in 36±3.5s and 29±3.4s (P=0.13) respectively. When comparing slopes of the I/O curve ANCOVA reveals significant increase in pilocarpine group ($P<0.05$). In conclusion, our data suggest that in chronic epileptic rats is regulation of the rCBF significantly altered.

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Effects of metabotropic glutamate receptor 5 antagonist MPEP on learning in developing rats

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Highly selective mGluR5 antagonist MPEP was used to elucidate the role of mGluR5 subtype of metabotropic glutamate receptors in certain forms of learning and memory. This subtype of receptors is distributed in various brain regions such as hippocampus, amygdala and cortex. In our laboratory, MPEP was found to exhibit anticonvulsant action in adult and immature rats without inducing side effects in motor performance, therefore we studied its effects on learning in the immature rats.

The experiments were performed in 12-, 18-, and 25-day-old Wistar albino rats. Experimental animals received i.p. 20 or 40 mg/kg of MPEP, controls received saline and were tested for on a simple olfactory learning in the homing response test:

Instead of true homing, a single pup was placed in an empty cage connected with the home cage occupied by littermates. A number of twelve trials with a 60s inter-trial intervals was chosen for 12-day-old rats. Ten trials were chosen for 18- and 25-day-old rats with a 180-s and 300-s inter-trial intervals respectively. A correct homing response was considered if the pup was able to reach the home cage within the 60s interval. Twelve trials for 12-, and 10 trials for 18- and 25-day-old were used. The following criteria were evaluated: the ratio of correct homing responses to the total number of trials, the mean latency to homing and occurrence of five consecutive correct homing responses.

Either dose of MPEP impaired the homing response in 12-day-old rats in all measured behavioral parameters if compared with the controls. Higher MPEP dose exhibited a similar effect in 18-day-old rats whereas lower dose did not compromise homing. In 25-day-old rats, neither of MPEP doses affected the homing response.

In conclusion, MPEP affected the homing response of developing rats in a dose- and age- dependent manner. The inability of 12- and 18-day-old pups, treated with MPEP, to return into their home cage suggests an impairment of their olfactory spatial learning.

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Does paraldehyde treatment participate in short-term effects of status epilepticus in immature rats?

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Purpose

To study participation of paraldehyde and/or lithium-chloride administration in short-time consequences of status epilepticus (SE) in immature rats.

Methods

SE was elicited by pilocarpine (40 mg/kg i.p.) in 12-day-old rats pretreated 24 hours before with LiCl (3 meq/kg, i.p.). Paraldehyde (0.3 ml/kg i.p.) was administered after two hours of continuous SE. Some drugs were replaced by saline in other experimental groups so that drugs they received were only LiCl, only paraldehyde or a combination of LiCl and paraldehyde. Controls received three saline injections. Cortical stimulation and registration electrodes were implanted 3 days after SE. After one-hour recovery cortical epileptic afterdischarges (ADs) were elicited by 15-s series of low-frequency (8 Hz) pulses. Intensity was increased stepwise from 0.2 to 15 mA, interval between stimulation series was at least 10 minutes. Threshold intensities for movements during stimulation, spike-and-wave ADs, clonic seizures, transition to limbic (mixed) ADs and recurrent ADs as well as durations of spike-and-wave (SW) and mixed ADs were measured.

Results

Only threshold intensities for movements were increased 3 days after SE, thresholds for other measured phenomena remained untouched. Mixed type of seizures and recurrent ADs failed to appear after SE at this interval. Administration of either LiCl or paraldehyde as well as their combination also led to an increase of threshold for movements. Changes in duration of Spike-and-Wave ADs caused by LiCl are prone to those in SE group.

Conclusions

Administration of LiCl or/and paraldehyde plays a role in short-time effect of SE on cortical excitability but they do not influence elicitation of mixed and recurrent afterdischarges.

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Software for synchronous electrophysiologic and image recordings and high level mathematical analysis

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In present, in neuroscience is increasingly important to combine synchronous optical and electric signals detection and analysis. However, majority systems reachable on the market are tools specialized for either imaging or electrophysiology not combining both in one environment. Moreover, high level mathemathical analysis is currently done separately usually in home based scripts written in Matlab. The aim of present study was to develope free software for broadly used windows based computer systems with low cost NI data acquisition cards series M and standard set of videocameras which will provide simple user interface for both types of recordings, triggering external devices like stimulator and powerfull matlab interoperability.

The software was programmed using Microsoft .NET Framework 2.0 library and C# programming language and thus it is suitable for any MS Windows based computer. For digitalization of voltage signals M series PCI data acquisition boards (National Instruments) were chosen for their high temporal and bit resolution. The communication with the card is done using low level NI-DAQmx driver programming allowing optimalization of hardware tasks. For image acquisition we have developed software components which make possible to acquire from standard webcams, DV cams and selected high sensitive cameras for microscopy use (UI, Q-Imaging, etc.). Communication with matlab is based on two different approaches. The first is based on compilling user matlab scripts into .NET dll libraries using Matlab .NET builder to calculate partial results for later processing or display in the software, the second approach is based on communication with COM Automation server object of Matlab which allows transferring of data to Matlab for further processing or displaying in its own environment.

The software was tested with PCI-6221 (National Instruments, Czech Republic) data acquisition board (16bit, 250kS/s, 16 AI and 2AO channels) with homemade EEG four channel amplifiers for signal preconditioning. For video recording we have tested simultaneous image acquisition with high resolution webcam (Philips 900NC), industrial cameras UI-2230C and 2230M (ids imaging, Germany) and high sensitive cooled camera Retiga 2000R (Q-imaging).

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Effect of methamphetamine exposure and postnatal care on sensorimotor development of rat pups

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Our previous studies demonstrated that methamphetamine (MA) administered during gestation and lactation periods impairs maternal behavior as well as the postnatal development of rat pups. The present study tested the hypothesis that the postnatal care of adoptive mother influences the development of rat pups. The aim of our study was to distinguish the extent of the drug-induced effect and the extent of the effect induced by impaired maternal care. Mothers were daily exposed to injection of MA (5 mg/kg) or saline (S) approximately for 9 weeks: about three weeks prior to impregnation, throughout the entire gestation period and during lactation until the weaning period. As an absolute control (C) females with no injections were used. On postnatal day (PD 1), pups were cross-fostered so that each mother received some of her own and some of the pups of mother with the other two treatments. Based on the prenatal and postnatal treatments 9 experimental groups (CC, CS, CM, SC, SS, SM, MC, MS, MM) were tested. Pup's development and sensorimotor coordination was examined between PD 1 and PD 23. Following behavioral tests were used: negative geotaxis (PD 9 and 11), tail pull on cellulose cotton wool and on grid (PD 10, 12 and 14), righting reflex on surface (PD 12), righting reflex on mid-air (PD 17), rotarod (PD 23) and bar-holding (PD 23). Further, the pups were examined for physiological maturation (weight gain during lactation, startle response, tooth eruption and ear and eye opening). Our results showed that the birth weight in prenatally MA-exposed pups was lower than controls or saline-exposed pups regardless of sex. Prenatally MA-exposed pups gained less weight than controls or saline-exposed pups regardless of postnatal treatment and sex. On the other hand, prenatally MA-exposed pups fostered by control or saline-exposed dams gained more weight during lactation. MA-exposed pups regardless of postnatal drug exposure and sex had displayed startle response, ear and eye opening later than control or saline-exposed pups. Further, our data demonstrated that pre- and postnatal MA exposure impairs sensorimotor functions in these tests: negative geotaxis, tail pull, righting reflex on surface, righting reflex in mid-air and rotarod. On the other hand, postnatal care of control mothers at least partially suppressed the negative effect of prenatal MA exposure. Our hypothesis, that the care of adoptive mother may affect postnatal development of pups, was confirmed.

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Effect of methamphetamine on social and locomotion behaviors of adult male rats

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Psychostimulants have been shown to affect human behavior in serious manner; specifically they induce aggressive behaviors and impair social interaction. Increased anxiety caused by psychostimulants was found in several types of tests. The aim of the present study was to assess the effect of low dose of methamphetamine (MA) on social interaction and locomotion in adult male rats. Test of social interaction (SIT) was used as a test of anxiety. SIT was tested in three different levels of stressful conditions: low stress, middle stress, high stress, depending on lighting intensity and known/unknown arena. Rats were or were not, respectively, habituated in an open field arena for 10 minutes on 2 consecutive days. Thirty minutes prior to SIT half of the animals were injected subcutaneously (s.c.) with MA (1.0 mg/kg). The other half of the animals did not receive any drugs. Social interaction (SI) was tested in the open field for five minutes and recorded by using a video camcorder. Animals of the same treatment and weight were assigned to one pair. Each pair was assessed as a unit and time spent by social interaction and locomotion was recorded and analyzed using a One-way ANOVA (drug treatment) for each condition separately. Locomotion (swimming activity) was further tested in the Morris water maze (MWM). The tests consisted of eight trials a day and lasted for five days. The same dose of MA (1 mg/kg) was given to half of the rats after finishing all 8 trials each day. The other half were not administered any drugs and were assigned to be a control group. A One-way ANOVA (drug treatment) was used for data analysing. Our results showed that acute MA administration decreased SI while increasing locomotion in the open field test relative to controls. This effect of MA depended on the level of stressful condition. Further, acute MA administration increased the speed of swimming in individual trials. Thus, our study indicates that application of MA at low doses decreases SI, thereby has anxiogenic effect, and increases locomotor activities tested in both Open field and MWM tests.

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Effect of acute methamphetamine administration on seizures in adult male and female rats prenatally exposed to the same drug

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The aim of the present study was to investigate whether sensitivity to seizures after acute methamphetamine (MA) administration (5mg/kg) is different in prenatally MA-exposed adult rats compared to controls without prenatal drug exposure. Adult rats with respect to sex and female estrous cycle (prenatally MA-exposed, prenatally saline-exposed and controls) were divided into groups with or without acute MA (1mg/kg) administration. Two models of seizures were used. Seizures induced by inhalation of flurothyl, gas that is assumed to inhibit GABAA receptors; and seizures induced by injection of 250 mg/kg of N-Methyl-D-Aspartate (NMDA), agonist of NMDA receptors. In flurothyl-induced seizures: prenatal MA exposure decreased threshold of the first clonus relative to prenatally saline-exposed and control animals. Acute MA administration increased threshold to the first clonus and clonic seizures and shortened duration of clonic seizures regardless of prenatal drug exposure. Prenatally saline-exposed animals with acute MA administration decreased threshold in all measures (first clonus, clonic seizures and tonic-clonic seizures) relative to prenatally MA-exposed and control rats with acute MA injection. In NMDA-induced seizures, acute MA administration increased latency to onset of stereotypic behavior in control and prenatally MA-exposed rats, while decreased latency to onset of clonic-tonic seizures in prenatally saline-exposed rats. The length of clonic-tonic seizures induced by NMDA was longer after acute MA pretreatment relative to animals without acute MA injection in all experimental groups. In both models, there were sex and/or estrous cycle-induced differences. Our study suggests that acute MA exposure changes seizure susceptibility in respect of prenatal drug exposure and hormonal status in two models of epileptic seizures.

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Analgesic effect of acute methamphetamine in prenatally stressed and methamphetamine-treated rats

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Recently we have shown that prenatal methamphetamine (METH), stress, and fostering affect differently nociception in adult animals (1). As METH possesses an analgesic effect, we decided to test its acute effect in adult animals with prenatal experience with the same drug.

Methods: Nociception was tested during the 85-90 postnatal day in three groups of Wistar male rats (N=27). Two groups consisted of animals, whose mothers were during gestation and lactation daily treated with METH (5mg/kg; s.c.) or saline (STRESS). The control group consisted of intact animals without any prenatal and postnatal intervention. Latencies of withdrawal reflexes of forelimbs, hind limbs and the tail on thermal nociceptive stimuli (Plantar Test, Ugo Basile, Comero, Italy) were repeatedly measured in 15-min intervals after the application of 1mg/kg s.c. of METH. Last measurement was performed 75 min after the injection.

Results: There were no group differences either in baseline nociception or in absolute level of METH-induced analgesia. In all groups, analgesia increased in cranio-caudal direction, with the smallest values observed in forelimbs (124% of the baseline values) and the highest values observed in the tail (249% of the baseline values). Analgesia in the METH group was already present 15 min after the injection. The slowest progress of analgesia occurred in the control group and it was dependent on measured site; it took 30 min for hind limbs and the tail and 60 min for forelimbs. In forelimbs of STRESS group, METH had a biphasic effect with hyperalgesia followed by mild analgesia.

Discussion: METH is a stimulatory drug which increases brain dopamine, serotonin and noradrenalin levels. All these neurotransmitters are important modulators in descendent antinociceptive system. As the main group differences were observed in the speed of analgesic process instead of its absolute values, we suppose that prenatal exposition to METH may have sensitization effect especially on dopaminergic brain reward system, which also can represent a common-final pathway of both opioid and non-opioid analgesia. Thus, our results show that the long-term consequences may be induced even by prenatal exposition to the drug.

(1) Yamamotová A, Šlamberová R, Fifth Conference of the Czech Neuroscience Society, Prague November 19-21, 2005, p.146.

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Grooming induced by intraperitoneal application of malanotan II, a dirivative of α-MSH

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Melanocortin II (MTII) is a cyclic peptide analog of αMSH; it acts on MC4 receptor subtype, which is believed to participate on stress responses and stress-induced behaviors (1,2,3). In rodents MTII-stimulated receptors cause the grooming. We investigated the involvement of MTII on stress-induced behaviors, such as activity, exploration and anxiety, in order to find whether i.p. application of this peptide will influence these parameters. Further, we were interested whether MTII will influence grooming and whether it will be changed at the simultaneous action of restraint stress. Male Wistar rats (Velaz, Czech Republic) (290–305g) were used. Rats were exposed to 60 min immobilization (IMO) (4,5). MTII (2mg/kg b.w.) given i.p. immediately after stress termination; testing in the open-field was performed one hour later. Behavior of rats was video-monitored by an activity monito-ring system (AnyMaze, Stoelting, USA) in a circular arena with the diameter of 150 cm.

We have demonstrated in the open-field test that a derivative of αMSH MTII stimulated grooming and strongly increased grooming induced by immobilization stress. Other behavio-ral parameters, namely activity (locomotion), exploration (rearing) and anxiety (entry into the inner zone of the device) were not substantially influenced. The results support the notion that melanocortins influence some of the behavioral outcomes of stress, supposedly by acting on MC4 receptors. Under the used experimental conditions MTII selectively increased grooming without affecting the spatio-temporal structure of locomotor behavior in the open field.

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Carbetocin improves deterioration of stress-induced behavior in the open-field device

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Carbetocin (1-butanoic acid-2-/O-methyl-Ltyrosine/1-carbaoxytocin) is a long-acting analogue of a neurohypophyseal nonapeptide hormone oxytocin that is used to control postpartum bleeding and is also indicated for the treatment and prevention of breast cancer and psychiatric disorders like autism and obsessive-compulsive disorder. There are only very limited data on the central effects of carbetocin after its application peripherally.

The aim of this study was to compare the effects of oxytocin and carbetocin on Wistar rat´s behavior in the open-field device after administration of restraint/ immobilization stress (IMO), which is known to change basic behavioral parameters in the open-field test (1,2). We used male Wistar rats (Velaz, Czech Republic), and IMO was applied for 60 min. Saline, oxytocin or carbetocin (0,3 or 1,0 mg/kg b.w.) were administered i.p. immediately after stress termination. After 60 min lasting pause the tests were performed in the large (150 cm diameter) circular arena and behavior was recorded by AnyMaze software (Stoelting Co, USA).

When compared to controls IMO alone reduced locomotion as well as exploratory activity and revealed increased sings of anxiety. Oxytocin applied after IMO slightly decreased behavioral parameters when compared to IMO alone. Carbetocin (1 mg/kg) antagonized the behavioral parameters induced by IMO and returned them nearly to control values. Lower dose of drugs produced similar changes, however no sedative effect of oxytocin was observed. The experiment with higher dose was repeated three times in three consecutive days and the effect of carbetocin was strongest on the third day. In summary, carbetocin was shown to have strong antagonistic effect on behavioral manifestations of stress; we speculate that this drug can be used for treatment of some stress-induced effects.

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