8th Conference
„Electrophysiological techniques in bioelectricity research: from ion channels to neural networks”

Wroclaw Medical University
June 1-2, 2012

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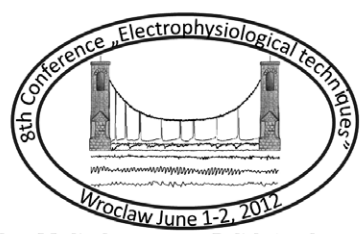

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1. GABA, A NEUROTRANSMITTER CRUCIAL FOR THE DEVELOPMENT OF NEURONAL CIRCUITS: INVOLVEMENT IN AUTISM SPECTRUM DISORDERS

Cherubini E.
Neurobiology Department, International School for Advanced Studies (SISSA), Trieste, Italy

The construction of the brain relies on genetic and environmental factors "nature and nurture". While nature provides a set of genes that control the general organization of the brain, nurture ensures that genetically built neuronal circuits adapt to the environment. Learning constitutes an adaptive process involving activity-dependent mechanisms regulating several developmental steps, including synapses formation and elimination. One of the main actors in these processes is the neurotransmitter GABA, which inhibits adult neurons but depolarizes and excites immature ones because of high intracellular chloride concentration. In synergy with glutamate GABA gives rise to coherent network oscillations which are instrumental in enhancing synaptic efficacy at emerging glutamatergic and GABAergic pathways. A premature shift of GABA from the depolarizing to the hyperpolarizing direction severely impairs the morphological maturation of cortical cells. In addition, a dysfunction of GABAergic signaling early in postnatal development leads to an excitatory/inhibitory unbalance a condition that may account for some of the behavioral deficits observed in neuro-developmental disorders such as Autism Spectrum Disorders (ASD). ASD comprise a heterogeneous group of pathological conditions, mainly of genetic origin, characterized by stereotyped behavior, marked impairment in verbal and non-verbal communication, social skills and cognition. Interestingly, in a small number of cases, ASD are associated with single mutations in genes involved in synaptic transmission, including single mutations of genes encoding for synaptic cell adhesion molecules of the Neurexin-Neuroligin families and for Shank3. Although rare, these mutations provide crucial information on the synaptic abnormalities which possibly affect ASD patients and point to synapses dysfunction as a possible site of autism origin.

2. MITOCHONDRIAL POTASSIUM CHANNELS

Szewczyk A.
Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland

Mitochondrial potassium channels play an important role in cytoprotection. The following potassium channels have been described in the inner mitochondrial membrane: the ATP-regulated potassium channel, the large conductance calcium activated potassium channel, the voltage-gated potassium channel and the twin-pore domain TASK-3 potassium channel. Potassium channels in the inner mitochondrial membrane are modulated by inhibitors and activators (potassium channel openers) previously described for plasma membrane potassium channels. The majority of mitochondrial potassium channel modulators exhibit a broad spectrum of off-target effects. These include uncoupling properties, inhibition of the respiratory chain and effects on cellular calcium homeostasis. Therefore, the rational application of channel inhibitors or activators is crucial to understanding the cellular consequences of mitochondrial channel inhibition or activation. In this paper, recent observations on three fundamental issues concerning mitochondrial potassium channel will be discussed: (1) their molecular identity, (2) their interaction with potassium channel openers and inhibitors and (3) their functional role.

This study was supported by the European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09)

3. A ROLE OF POLYUNSATURATED FATTY ACIDS IN THE REGULATION OF MITOCHONDRIAL POTASSIUM CHANNELS

Kajma A., Szewczyk A.
Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland

Polyunsaturated fatty acids (PUFA), have been implicated in the modulation of a variety of biochemical processes in the cells. They were able to interact with the membrane proteins including ion channels in normal and pathological conditions. The aim of the project was to determine whether the neuroprotective mechanism of the large-conductance calcium-activated potassium (mitoBKCa) channel from the inner mitochondrial membrane can be explained by mutual interaction between the channels and polyunsaturated fatty acids. The study was performed using patch-clamp technique and mitochondria isolated from rat astrocytes. We analyzed effect of arachidonic acid (AA), eicosatetraynoic acid (ETYA) a non-metabolizable analog of AA, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The open probability of the channel did not change significantly after application of 10 μM ETYA, but after adding 10 μM AA open probability increased. This suggest that channel is affected by metabolites of AA rather than fatty acids itself. Application of 30 μM DHA, and 30 μM EPA increased open probability of the channel. Also number of the open channels in the patch increased in the presence of 30 μM EPA. Summarising, our results indicate that neuroprotective PUFAs, like DHA and EPA activate mitoBKCa channel, while proapoptotic and proinflamatory AA had no effect on mitoBKCa channel.

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4. MITOCHONDRIAL POTASSIUM CHANNELS IN *DICTYOSTELIUM DISCOIDEUM*

Laskowski M.[1], Kicinska A. [2], Jarmuszkiewicz W.[2], Szewczyk A.[1]
[1]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland; [2]Department of Animal Physiology and Development, Adam Mickiewicz University, Poznan, Poland

The strict control of inner mitochondrial membrane permeability and selective ion transport is essential for mitochondria functioning. Potassium ions homeostasis is an important process for mitochondrial optimal functioning. Currently we characterize mitochondrial potassium channels from one of *Dictyostelium* species. Preliminary experiments are focused on biophysical and pharmacological characterization of mitochondrial ion channels. Purified inner mitochondrial membranes (submitochondrial particles) were reconstituted into planar lipid bilayer. To form model membranes asolectin from soybean mixture of phospholipids was used. We observed two types of potassium selective ion channels in submitochondrial particle samples: a large- and small-conductance channels. Experiments were performed both in gradient solution 50/150 mM KCl (cis-trans) and in symmetrical solution 150/150 mM KCl at voltages from −50 to 50 mV. Regulation of the channel activity by divalent cations such as Ca^{2+} and Mg^{2+} was explored. Additionally, interaction of the ATP with mitochondrial potassium channels was characterized. The knowledge on mitochondrial ion channels may contribute to understanding molecular mechanism of *Dictyostelium discoideum* functioning.

5. INTEGRATED ELECTRODE SYSTEM TO STUDY ION FLUXES ACROSS THE MONOLAYER OF EPITHELIAL CELLS GROWN ON POROUS SUPPORT

Toczyłowska-Mamińska R.[1], Zając M.[1], Lewenstam A.[2], Dołowy K.[1]
[1]Department of Biophysics, Warsaw University of Life Sciences – SGGW, Warsaw, Poland, [2]Laboratory of Analytical Chemistry, Process Chemistry Center, Åbo Akademi University, Åbo/ Turku, Finland

Cystic fibrosis is the most common fatal human genetic disorder. It is caused by mutation of chloride channel protein called CFTR. CFTR channel plays a role in osmotic driven water transportacross epithelial cell monolayer. The defective water transport leads to dense viscous mucus secretion which blocks pancreatic juice production and the clearance of lung fluid. The viscous mucus leads to opportunistic infections in lungs which deteriorates its function. The simple explanation of the mechanism of fluid secretion and absorption in different epithelial tissue failed. Either there are different mechanisms for different epithelial tissues or other ion channels beside CFTR are involved in water transport. From that what is known from experimental studies across the lung epithelium sodium, potassium, protons, chloride and bicarbonateions are transported. To solve the mechanism of the mechanism of water transport we need to measure all the ion concentration changes, as fast as possible. Materials and methods: Silver wires were mounted in poly(methyl metacrylate)-based modules. Solid state ion selective electrodes and reference electrode has been used. In the preliminary studies the cell layer was replaced by a porous membrane of resistance of approximately 800 $\Omega \bullet cm^{-2}$ similar to resistances of cell monolayers (300–1500 $\Omega \bullet cm^{-2}$). Results show that we are able to detect ion flow across artificial membrane. Our integrated electrode system can be used to study epithelial cells.
This work was supported by a grant from National Center of Science (NCN) grant No. N N401 182839.

6. SLOWLY-ACTIVATING CATION CHANNEL IN THE RED BEET VACUOLES IN THE PRESENCE OF ORGANOLEAD: ANALYSIS OF MICROSCOPIC CURRENT

Trela Z.,[1] Miskiewicz J.,[1] Burdach Z.,[2] Karcz W.,[2] Przestalski S.[1]
[1]Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland; [2]Department of Plant Physiology, Faculty of Biology, University of Silesia, Katowice, Poland

Patch-clamp technique was used to examine effect of trimethyllead chloride (Met_3PbCl) on the SV channel activity in the red beet (*Beta vulgaris* L.) taproot vacuoles. It was found that in the control bath macroscopic currents showed typical slow activation and strong outward rectification of the steady-state currents. The addition of Met_3PbCl to the bath solution blocked in a concentration-dependent manner SV currents in red beet vacuoles. The time constant, τ, increased several times in the presence of 100 μM trimethyllead chloride at all voltages tested. Trimethyllead chloride decreased significantly (by about one order of magnitude) the open probability of single channels. The recordings of single channel activity obtained in the presence and in the absence of Met_3PbCl showed that organolead only slightly (by ca. 10%) decreased the unitary conductance of single channels. It was also found that Met_3PbCl diminished significantly the number of SV channel openings, whereas it did not change the opening times of the channels. Within the present work the existence of spatial correlation beetwen ion channels of *Beta vulgaris* vacuolar membrane was shown. Moreover, the comparison of data collected in the control and in the presence of trimethyllead shows that organolead compound disturb not only the ion transport through the membrane, but also spacial correlations among channels.
This work was supported by the KBN grant N305 336434

7. HOW EXPERIMENTAL CONDITIONS CAN INFLUENCE THE EFFECTS OF TOXINS BINDING TO "SITE 3" ON SODIUM CHANNEL – ELECTROPHYSIOLOGICAL OBSERVATIONS

Dąbrowski M.[1,2], Nowak W.[1], Jankowska M.[2], Stankiewicz M.[2]
[1]Institute of Physics and [2]Institute of General and Molecular Biology, N. Copernicus University, Toruń, Poland.

Toxins binding to "site 3" on Na channel inhibit the inactivation of channel and often induce the plateau action potentials (AP). Effects of scorpion toxin LqhαIT and sea anemone toxin ATX II were observed on insect axonal preparation. Duration of AP increased progressively with time after toxin application. Time of plateau AP development was shorter in higher toxin concentration and length of AP was bigger. Although extension of plateau AP decreased with high frequency stimulation (HFS), the effect of HFS was depressed by higher toxin concentration (10^{-6} M). Elevated Ca^{2+} concentration has been determined as another factor which induced limitation of plateau AP duration. Modifications of sodium current under toxins correspond well to all observations done in current-clamp configuration. Toxin-induced late sodium current had smaller amplitude with high frequency depolarizing pulse application – such an effect was partially eliminated by increased toxin concentration. High Ca^{2+} concentration diminished current modification induced by toxins. The electrophysiological data were additionally compared with data obtained by theoretical binding calculations using ligand docking to all atom ion channel model.
Supported by MNiSW (grant N N303 320637); Dąbrowski M. is a beneficiary of PhD grant: Project No POKL.04.01.01-00-081/10

8. EFFECTS OF D1 RECEPTOR AGONIST ON VOLTAGE-GATED SODIUM CURRENTS IN mPFC PYRAMIDAL NEURONS IN RATS OF DIFFERENT AGES

Szulczyk B., Grzelka K.
The Medical University of Warsaw, The Department of Physiology and Pathophysiology, Warsaw, Poland

We recorded voltage-gated Na+ currents from mPFC pyramidal neurons in slices. The recordings were made in cell-attached configuration (macropatches) in young (3-weeks-old) and adult (9-weeks-old) rats. Fast synaptic transmission was blocked. The maximal Na^+ currents amplitude varied in different recordings because of different number of channels in different patches. The mean amplitude of Na^+ currents was 16.7 ± 1.4 (n=45) in young rats and 8.4 ± 1.2 pA (n=45) in adult rats (P<0.001). Sodium currents of big amplitude were selected to test the effects of a D1 receptor agonist (SKF 38393 10 μM). In young rats the maximal sodium current amplitude was 28.9 ± 3.7 pA in control and 29.7 ± 3.8 pA in the presence of SKF 38393 (P>0.05, n=7). Moreover in young rats the potential of half-maximal activation (V0.5) was not significantly different in control and with SKF 38393 in the bath (−11.5 ± 1.9 mV and −14.7 ± 1.2 mV respectively, n=7, P>0.05).In adult rats the maximal sodium current amplitude was 33.5 ± 10 pA and 32.5 ± 8.9 pA in control and in the presence of SKF 38393 respectively (P>0.05, n=5). Also in adult rats the potential of half-maximal activation was −11.9 ± 1.6 mV in control and −14.2 ± 1.8 mV with SKF 38393 in the bath (n=5, P<0.05). We suggest that D1 receptor agonist does not influence maximal Na^+ currents and exerts small effects on their kinetic properties in rats of different ages.
Supported by grant no. NCN/MNiSzW- N N401 030037.

9. PROPERTIES OF SINGLE NONINACTIVATING K^+ CHANNEL CURRENTS IN MEDIAL PREFRONTAL CORTEX (mPFC) PYRAMIDAL NEURONS

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

Distorted activity of mPFC neurons occurs in age-dependent, widespread neuropsychiatric disorders. Single noninactivating K^+ channel currents were recorded in cell attached configuration from freshly dispersed mPFC pyramidal neurons. Neurons were obtained from young (20 days old, 185 channels analyzed), periadolescent (40 days old, 71 channels analyzed) and adult rats (60 days old, 84 channels analyzed). There were found 3 major groups of channel currentsin tested neurons: (1) Ca^{++} dependent K^+ channel currents; these channel currents when recorded in cell attached configuration expressed only outward conductance (150 pS); in inside out configuration they expressed also inward conductance when Ca^{++} ions applied to the cytoplasmatic side of the membrane; (2) large conductance leak channel currents showed outward (150 pS) and inward (150 pS) conductance in cell attached configuration; these channels were strongly mechanosensitive; (3) small conductance leak channel currents had outward and inward conductance about 30–40 pS. Each category of tested channel currents had similar properties biophysical properties in neurons obtained from rats of different age. Smaller proportion of Ca^{++} dependent K^+ channel currents and larger proportion large conductance leak channel currents were found in pyramidal neurons obtained from preadolescent rats comparing to young and adult rats (see table).
Supported by NN401584638 and NN401076537.

age	20 days old	40 days old	60 days old
n	185	71	84
Ca^{++} dependent K^+ channel currents	38.9%	12.7%	22.5%
large leak channel currents	38.9%	64.8%	55%
small leak channel currents	16,8%	18.3%	15%
other channels	5%	4.2%	7.5%

10. INHIBITION OF THE ACTIVITY OF Kv1.3 CHANNELS IN HUMAN JURKAT T-CELLS BY 8-PRENYLNARINGENIN

Gąsiorowska J., Teisseyre A., Uryga A., Michalak K.
Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland

Voltage-gated potassium channels of Kv1.3 type are expressed in the plasma membrane of many cells. It has been shown that these channels are involved in proliferation of lymphocytes and other cell types including some cancer tissues. Blocking of Kv1.3 channels may inhibit cell proliferation at early phase of this process. In this study, the influence of 8-prenylnaringenin – a prenylated flavonoid isolated from common hop – on the activity of Kv1.3 channels in human leukemic Jurkat T cell line was investigated applying the "whole-cell" patch-clamp technique. 8-prenylnaringenin was identified as the most potent plant phytoestrogen, it may also modulate the processes of cancer cells proliferation, inflammation and angiogenesis. Application of 8-prenylnaringenin in the range from 2–7 μM reduced the current amplitude significantly in a dose-dependent manner. The currents were blocked completely at 10 μM concentration. The inhibitory effect was reversible. It was accompanied by a significant acceleration of the currents' inactivation rate without changing their activation rate. We suggest that 8-prenylnaringenin may block potassium channels *via* an "open channel block" mechanism.

11. GROWTH OF POTASSIUM TRANSPORT DEFICIENT *ESCHERICHIA COLI* STRAINS EXPRESSING POTASSIUM LEAKY MUTANTS OF THE MECHANOSENSITIVE CHANNEL MsCS AS A TOOL FOR INVESTIGATION ITS STRUCTURE-FUNCTION RELATIONSHIP

Koprowski P., Kubalski A.
Department of Cell Biology, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Previously, it was shown that expression of potassium channels in potassium transport deficient *E. coli* strains restores growth defect in low potassium media (<5 mM) and allows for isolation of mutations that result in channel activation (1). Using similar principle we isolated potassium leaky mutants of mechanosensitve non-selective channel MscS (2) The mutations that result in potassium leak could be grouped into two major categories: (i) mutations that cause channel to open easier (gain-of-function, GOF) located on the pore-forming helix (TM3); (ii) mutations that result in inactive channels (loss-of-function, LOF) located in peripheral crevices in between TM3 and peripheral helices TM1/2. It raises the question whether potassium leak pathways are the same in GOF and LOF mutants. Here we present analysis of growth temperature dependence of potassium transport deficient *E. coli* strains expressing GOF and LOF mutants that allows us to identify channel pore as a common pathway for both types of mutants. The results shed also new light on the gating principles of the MscS channel.

(1) Sun S, Gan JH, Paynter JJ, Tucker SJ (2006) Physiol Genomics 26: 1–7.
(2) Koprowski P, Grajkowski W, Isacoff EY, Kubalski A (2011) J Biol Chem 286: 877–888.

12. A HYDROPHOBIC RESIDUE IN $GABA_A$ RECEPTOR LIGAND BINDING SITE STRONGLY INFLUENCES RECEPTOR GATING

Szczot M.[1], Kisiel M.[1], Czyżewska M.M.[1], Mozrzymas J.W.[1,2]
[1]Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University, Wrocław, Poland; [2]Department of Animal Molecular Physiology, Institute of Zoology, Wroclaw University, Wroclaw, Poland

Despite broad knowledge on $GABA_A$ receptors ($GABA_ARs$) structure, the mechanism of ligand induced conformational transitions remains poorly understood. To address this issue we have examined the activity of a recombinant $\alpha_1/\beta_1/\gamma_2$ $GABA_AR$ with point mutation introduced at the ligand binding site. Currents were elicited by ultrafast GABA applications and measured using patch-clamp technique. We show that cystein mutation of single hydrophobic residue not only weakened the agonist binding but also abolished fast desensitization and slowed the onset of currents evoked by saturating GABA. Non-stationary variance analysis showed that the mutation does not affect single channel conductance, but reduces maximal open probability, further indicating a change in gating properties. Ratio of current amplitudes elicited by pentobarbital (which activates $GABA_AR$ by different pathway than GABA), and by GABA was higher for mutant receptors, supporting interference with receptor gating. Our data show that the considered residue may strongly influence conformational transitions of $GABA_ARs$ thus indicating this residue as a key element in transduction of free energy supplied by agonist binding to the conformational transitions.

13. PRESYNAPTIC VOLTAGE FACILITATION OF SYNAPTIC TRANSMISSION BETWEEN CA3–CA3 PYRAMIDAL NEURONS

Białowąs A., Rama S., Zbili M., Fronzaroli-Molinières L., Ankri N., Carlier E., Debanne D.
INSERM UMR 1072 Unité de Neurobiologie des canaux Ioniques et de la Synapse (UNIS), Aix-Marseille Université, Faculté de Médecine – campus Nord, Marseille, France

Synaptic transmission in the brain generally depends on action potentials. However, subthreshold variation in presynaptic membrane potential also determines spike-evoked transmission. We show here that excitatory synaptic transmission at CA3–CA3 connections depends on the membrane potential of the presynaptic neuron. Connected CA3 pyramidal neurons were recorded and synaptic transmission was tested when the presynaptic neurone was held at −61 mV, −77 mV or −48 mV. The presynaptic voltage facilitation (PVF) of synaptic transmission quantified by normalizing the postsynaptic responses obtained at −48 mV to those measured at −77 mV amounted to 135 ± 14% (n=13). PVF was associated with a decrease in the paired-pulse ratio and was totally occluded by bath application of the Kv1 channel blocker dendrotoxin (DTX). Using confocal laser scanning microscopy, we measured calcium transients evoked by the propagated spike in axons (50–250 µm from the soma) of CA3 neurons loaded with Fluo-4. Depolarization of the cell body from −75 to −50 mV enhanced spike-evoked calcium transients by ~17%. We conclude that PVF is a short-term plasticity at excitatory CA3–CA3 synapses resulting from the increase in spike-evoked calcium transients in the axon caused by voltage-inactivation of Kv1 channels.

14. DISSECTING ACTIVITY OF INDIVIDUAL CELL POPULATIONS WITH KERNEL CURRENT SOURCE DENSITY METHOD AND INDEPENDENT COMPONENTS ANALYSIS

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

Local field potentials (LFP), the low-frequency part of extracellular electric potential, reflect dendritic processing of synaptic inputs to neuronal populations. Today one can easily record simultaneous potentials from multiple contacts. Due to the nature of electric field each electrode may record activity of sources millimeters away which leads to significant correlations between signals and complicates their analysis. Whenever possible it is convenient to estimate the current source density (CSD), the volume density of net transmembrane currents, which generate the LFP. CSD directly reflects the local neural activity and CSD analysis is often used to analyze LFP. We present here a general, nonparametric method for CSD estimation based on kernel techniques, which can take into account known anatomy or physiology of the studied structure. Using data from a simulated large scale model of thalamo-cortical column we also show how CSD analysis combined with independent component analysis (ICA) can reveal information on activity of individual cell populations.
Research supported by grants 5428/B/P01/2010/39, POIG.02.03.00-00-003/09, POIG.02.03.00-00-018/08.

15. GLUTAMATERGICALLY-INDUCED THETA OSCILLATIONS RECORDED IN BOTH *IN VITRO* AND *IN VIVO* MAINTAINED POSTERIOR HYPOTHALAMIC PREPARATIONS

Kowalczyk T., Bocian R., Caban B., Gołębiewski H., Kaźmierska P., Konopacki J.
Department of Neurobiology, University of Lodz, Lodz, Poland

Theta rhythm is the best synchronized EEG activity recorded from the mammalian brain. In rodents this EEG pattern consists of high-voltage, almost sinusoidal oscillations in the range of 3–12 Hz. Hippocampal formation (HPC) is considered to be the main structure involved in the generation of this activity. Anatomical and electrophysiological studies have revealed that the ascending brainstem hippocampal synchronizing pathway, which originates in pontine region, constitutes a major source of extensive inputs to the HPC. Fibres from pontine nuclei ascend to the posterior hypothalamic (PH) area and then PH neurons projects to medial septal area. There are evidences from physiological and pharmacological studies supporting the view that the PH region forms a critical part of the ascending synchronizing pathway linking the rostral pontine region with the septo-hippocampal pathways. In our previous studies we have demonstrated that posterior hypothalamic region was able to generate the cholinergically-induced type 2 theta rhythm in both *in vitro* and *in vivo* conditions. The purpose of the present study was to evoke theta-band oscillations in PH area using the glutamatergic agonist – kainic acid. We have shown that application of kainic acid resulted in the generation of well-synchronized theta activity in PH slices maintained *in vitro* as well as enhancement of spontaneous theta recorded from posterior hypothalamic region of anaesthetized rat. Mechanisms underlying the generation of glutamatergically-induced theta oscillations in both *in vitro* and *in vivo* posterior hypothalamus are discussed.
Supported by NCN grant no. 2011/01/B/NZ4/00373.

16. PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL PROFILE OF THETA ACTIVITY RECORDED FORM THE POSTERIOR HYPOTHALAMUS IN ANESTHETIZED RATS

Bocian R., Kowalczyk T., Gołębiewski H., Kaźmierska P., Konopacki J.
Department of Neurobiology, University of Łódź, Łódź, Poland

Theta rhythm is the largest, most prominent and best synchronized rhythmical waveform generated by the mammalian brain. Commonly, theta activity has been associated with hippocampal formation (HPC). Pharmacological and behavioral studies have shown that there were two distinct types of hippocampal theta activity in rodents. The first type (type 1) occurs during voluntary motor behavior and is atropine-resistant. The second one (type 2) occurs during immobility and is atropine-sensitive. Interestingly, a number of *in vitro* reports have revealed that HPC is not the only limbic cortical region involved in the production of theta activity. Theta oscillations have also been recorded from the entorhinal and cingulate cortex in freely behaving or anesthetized rats. In our recent studies we provided for the first time, evidences that atropine-sensitive theta rhythm could be recorded also from posterior hypothalamic (PH) region. Current research has been devoted to determining the pharmacological and electrophysiological profile of PH theta. In separate experiments the effects of carbachol (0.5 μg/μl) and orexin B (0.2 μg/μl) administrations were examined. Microinjection of cholinergic agonist (carbachol) into PH induced well-synchronized theta with increased amplitude and power. However, the local injection of orexin B did not affect PH rhythmic activity. These results confirm cholinergic nature of posterior hypothalamic theta in anesthetized rats.

Supported by NCN grant no. 2011/01/B/NZ4/00373.

17. RECIPROCAL INHIBITION AND SLOW CALCIUM DECAY IN RECURRENT THALAMIC INTERNEURONS EXPLAIN CHANGES IN SPONTANEOUS FIRING OF THALAMIC RELAY CELLS AFTER CORTICAL INACTIVATION

Rogala J., Waleszczyk W.J., Łęski S., Wróbel A., Wójcik D.K.
Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Most of recent studies of the role of cortical feedback in thalamo-cortical loop focused on its effect on thalamo-cortical relay (TCR) cells of the dorsal lateral geniculate nucleus (LGN). In a previous, physiological study we showed in cat visual system that cessation of cortical input decreased spontaneous activity of TCR cells and increased spontaneous firing of recurrent inhibitory interneurons located in the perigeniculate neucleus (PGN). To identify underlying mechanisms we studied several networks of point neurons with varied membrane properties, synaptic weights and axonal delays in NEURON simulator. We considered six network topologies. All models were robust against changes of axonal delays except for the delay between LGN feed-forward (f-f) interneuron and TCR cell. The best representation of physiological results gave models including reciprocally connected PGN cells driven by the cortex assuming slow decay of intracellular calcium. This indicates that thalamic reticular nucleus plays an essential role in the cortical influence over thalamo-cortical relay cells while the thalamic f-f interneurons are not essential in this process. The models revealed also that dependence of the PGN activity on the rate of calcium removal can be one of the key factors determining TCR response to diminished cortical input.

18. STIMULUS DEPENDENT OSCILLATIONS IN VISUALLY EVOKED ACTIVITY OF CAT'S SUPERIOR COLLICULUS

Foik A.[1], Silva A.[1], Wypych M.[1], Mochol G.[1,2], Waleszczyk W.J.[1]
[1]Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland; [2]Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain

Visual information is transferred from retina to higher order cortical areas by few parallel pathways and encoded in different ways. In this study, we focused on encoding of visual information in the superior colliculus, which is the first stage of extrageniculate pathway. One of ways in which neurons encode information is rate coding based on the change in a number of action potentials in response to stimulus presentation. The other coding scheme is temporal coding, which include information about temporal patterns of neuronal spiking, present, for example, in the form of oscillations. The aim of this study was to reveal the presence of stimulus dependent oscillations in visually evoked activity of neurons in the superficial layers of the cat's superior colliculus. Neuronal activity was recorded from anesthetized (isoflurane in N_2O/O_2) animals during visual stimulation by spot of light moving in broad range of velocities as well as during presentation of light spot flashing in random locations within the receptive field of recorded neuron. Oscillations were identified by means of auto-correlation and spectral analyses. We found stimulus dependent oscillations in half of tested neurons. Moreover, oscillatory activity appeared to depend on the stimulus velocity. Different measures of oscillations strength (SO), such as *z*-score, oscillation score or *F*-statistics revealed positive correlation between SO and velocity. Stimulus dependent oscillations appeared also in responses to onset of the light spot. These results suggest importance of oscillations for processing of information about fast changes within receptive fields of neurons in the extrageniculate pathway. Supposedly presence of oscillations in responses of collicular cells can increase probability of information transfer to higher level of visual processing.

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19. THE EFFECTS OF STRESS ON SYNAPTIC TRANSMISSION AND PLASTICITY IN RAT FRONTAL CORTEX

Bobula B.[1], Wabno J.[1], Tokarski K.[1], Gądek-Michalska A.[1], Hess G.[1,2]

[1]Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland; [2]Institute of Zoology, Jagiellonian University, Kraków, Poland

Chronic stress and elevated level of corticosterone have been implicated in a variety of pathophysiological processes including mood disorders. A growing body of evidence links cognitive dysfunctions with abnormalities of excitatory transmission in the brain. We studied glutamatergic transmission and synaptic plasticity in the frontal cortex after exposure of rats to two different types of stress: crowding and restraint. Additionally, repeated corticosterone administration was employed as a model of non-adaptive stress. Electrophysiological experiments were performed on *ex vivo* frontal cortical slices prepared 24 h after last stress session. In slices originating from stressed and corticosterone-treated animals the amplitude of extracellular field potentials recorded in cortical layer II/III was increased and the magnitude of long-term potentiation (LTP) was decreased. Whole-cell recoding from layer II/III pyramidal neurons demonstrated corticosterone treatment-induced increase in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs). These data point to an enhancement of excitatory transmission in the frontal cortex resulting from an increased glutamate release as a common effect of different types of stress.

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20. NEONATAL MATERNAL SEPARATION ATTENUATES LTP IN CORTICAL AND THALAMIC INPUTS TO LATERAL AMYGDALA OF THE RAT

Danielewicz J.[1], Hess G.[1,2]

[1]Institute of Zoology, Jagiellonian University, Krakow, Poland; [2]Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Maternal separation (MS) procedure is an experimental paradigm for studying disturbances in brain functions that result from adverse events occurring during development. However, the effects of early life stress on synaptic plasticity in the amygdala of adolescent animals are poorly understood. This study investigated the effects of MS on LTP in the cortical (CoI) and the thalamic input (ThI) to the lateral amygdala (LA) of rats. Wistar dams with their offspring were housed under 12:12 L/D conditions. On each postnatal day (PND) 1–21 rats were subjected to MS (3h/day). For electrophysiological *ex vivo* experiments rats between PND 35 and PND 55 were used. The animals were anesthetized and brain slices containing LA (450 μm) were cut. Field potentials (FPs) were evoked by the stimulation of CoI or ThI to LA. LTP was induced using theta-burst stimulation protocol. In slices prepared from control, animal facility reared (AFR) rats, the amplitude of FPs after LTP induction in CoI amounted 185.9 ± 17.04. In slices obtained from MS-subjected rats LTP in the cortical input was weaker (117.7 ± 7.7%). Amplitude of FPs after LTP induction in ThI, amounted 128.99 ± 9.47%, while in MS-subjected rats amplitude of FPs recorded after LTP induction amounted 110.41 ± 8.91%. These results demonstrate that MS impairs LTP in the cortical and in the thalamic input to LA.

21. RESTRAINT STRESS ENHANCES EXCITATORY BUT NOT INHIBITORY SYNAPTIC TRANSMISSION IN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS OF THE RAT

Kusek M.,[1] Tokarski K.,[1] Bobula B.,[1] Gądek-Michalska A.,[1] Hess G.[1,2]

[1] Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland; [2]Institute of Zoology, Jagiellonian University, Kraków, Poland

The paraventricular nucleus of the hypothalamus (PVN) is the main regulatory center of the hypothalamic-pituitary-adrenal (HPA) axis. One important function of PVN neurosecretory neurons is the release of corticotropin releasing hormone (CRH) to the pituitary gland. This study was aimed at establishing the effects of restraint stress on GABAergic and glutamatergic inputs to parvocellular neurons of the PVN. Male adult rats were subjected to restraint in metal tubes lasting for 10 min, 2 times daily (repeated for 3 consecutive days). Whole-cell recording were performed from parvocellular neurons. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded from cells which were voltage clamped at 0 mV and spontaneous excitatory postsynaptic currents (sEPSCs) were recorded at −76 mV. The amplitude and the frequency of sIPSCs and sEPSCs were measured. Restraint stress resulted in an increase in the frequency of sEPSCs while the amplitude of sEPSCs was not altered. Also the parameters characterizing sIPSCs remained unaltered. Thus, restraint stress selectively enhances excitatory input to the paraventricular nucleus of the rat, most likely *via* a presynaptic mechanism.

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22. FINE-TUNED MMP-9 ACTIVITY LEVEL IS A PERMISSIVE FACTOR FOR INDUCTION AND MAINTENANCE OF LTP IN HIPPOCAMPAL MOSSY FIBER-CA3 PATHWAY

Wiera G.[1,2], Dzięgiel P.[2], Kaczmarek L.[3], Mozrzymas J.W.[1,2]
[1]Wroclaw Medical University, [2]Wroclaw University, Wroclaw, Poland; [3]Nencki Institute of Experimentak Biology PAS, Warsaw, Poland

Matrix metalloproteinases have been implicated in physiological and pathological functions of neuronal networks. To get an insight into the specific role of protease MMP-9 in the plasticity of the mossy fiber-CA3 pathway (mf-CA3), we used field recordings to measure LTP in slices from wild type, MMP-9 KO and overexpressing (OVX) animals. Both lack of protease (KO) and its overexpression impaired the early and maintenance phase of mf-CA3 LTP (128% vs. 181% of respective baseline in KO and WT mice; and 136% vs. 189% in OVX and WT rats respectively, $P<0.05$). Importantly, impaired LTP in transgenic rodents could be rescued by administration of recombinant MMP-9 to the KO slices (152% of respective baseline). Moreover, autoactive MMP-9 protein application induced a stable autopotentiation of synaptic response in KO and WT mice. β-dystroglycan is a synaptic membrane protein and its MMP-mediated cleavage has been described during pathological, epileptic activity. In mf-CA3 pathway we observed a MMP-dependent significant decrease in 30 kDa β-dystroglycan digestion product as early as 5 minutes after LTP induction. Altogether, these results suggest that in mf-CA3 pathway during first minutes after tetanus fine-tuned level of MMP-9 activity acts as a permissive factor for LTP establishment.

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23. MATRIX METALLOPROTEASES REGULATE THE EXTENT OF SYNAPTIC PLASTICITY AND SPIKE POTENTIATION IN HIPPOCAMPAL CA3 ASSOCIATIONAL NETWORK

Wójtowicz T., Mozrzymas J.W.
Laboratory of Neuroscience, Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland

Matrix metalloproteinases (MMPs) activity support spatial and associative learning *in vivo* however the underlying mechanisms are poorly understood. It has been shown that MMPs inhibition impairs the maintenance of long-term potentiation (LTP) in hippocampal Sch-CA1 synapses and in mf-CA3 pathways. However, it is not known whether MMPs activity influences the scaling of postsynaptic neuronal responses (population spikes, PS) typically associated with synaptic LTP. Therefore, we investigated the plasticity of evoked synaptic transmission (input) and resulting PS (output) in CA3 associative network by simultaneous double electrode extracellular field potentials recording. We found that high-frequency-induced (4 × 100 Hz) LTP of EPSPs and PS was significantly impaired in the presence of broad spectrum MMPs inhibitor FN439 while the EPSP-to-Spike (E-S) curves were right-shifted indicating that less neurons were recruited to fire for a given input. Additionally, these effects could be mimicked by application of MMP-3 inhibitor (NNGH). MMPs inhibitors did not affect input–output function of the CA3 neurons or various forms of short term-plasticity. In conclusion, MMPs and particularly MMP-3 may play an important role in the maintenance of E-S potentiation in the CA3 network and thereby may regulate formation of memory traces in this hippocampal region.

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24. THE MAGNITUDE OF THE MOVEMENT-RELATED CORTICAL POTENTIALS DURING FAST AND SLOW VOLUNTARY KNEE EXTENSOR DEACTIVATION

Kisiel-Sajewicz K.[1,2], Siemionow V.[2,3], Zhang L.D.[2], Mencel J. [1], Yue G.H.[2,3,4], Jaskólska A.[1], Jaskólski A.[1]
[1]Department of Kinesiology, Faculty of Physiotherapy, University School of Physical Education, Wrocław, Poland; Departments of [2]Biomedical Engineering and [3]Physical Medicine and Rehabilitation, Cleveland Clinic, Cleveland, OH, USA; [4]Kessler Foundation Research Center, West Orange, NJ, USA

The aim of the study was to learn cortical signal characteristics for controlling voluntary deactivation of human skeletal muscles with different speeds. This study investigated whether MRCP measures for fast knee extensor (KE) deactivation were different from those for slow deactivation. Twenty-seven healthy volunteers (22.4 ± 4.5 years) participated in the study. They performed isometric KE contractions [2 sets (slow and fast) of 45 trials] using the right leg at a target level of 20% maximal voluntary contraction (MVC) force. In each trial, subjects held the force at the target for 10s after reaching it and then relaxed the force slowly (rate of force descending ~4% MVC/s) or as quick as possible to the baseline during fast set. Cortical signals for deactivating the muscles were quantified by estimating amplitude of the MRCP derived by force-triggered averaging of the 45-trial EEG data. Mapping of the MRCP based on data of the 128 channels was obtained to evaluate distribution of brain activity associated with slow and fast KE deactivation. MRCP controlling KE deactivation was

significantly higher for fast than slow deactivation ($P<0.05$). A higher level of cortical activation is required for controlling fast muscles deactivation. These findings indicated that control of KE deactivation at the cortical level is modulated by a rate of force decrease. This could be related to a varied impact of feedback and feedforward mechanisms during slow and fast deactivation and this may result in differential corticospinal projections to the motoneuron pools of the synergist and antagonist muscles during both conditions.

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25. DESYNCHRONIZATION OF ALPHA RHYTHM IN MOTOR IMAGERY TASK AFTER A KINESTHETIC TRAINING

Zapała D., Zabielska E., Cudo A., Krzysztofiak A., Francuz P.
Department of Experimental Psychology, Faculty of Social Sciences, The John Paul II Catholic University of Lublin, Lublin, Poland

Is has been shown that motor kinesthetic imagery in contrast to motor visual imagery serves better to control brain-computer interfaces which relay on ERD/ERS patterns. However many people still have problems with producing an adequate brain activity pattern and some of them are not familiar with kinesthetic motor imagery concept. The possible way to overcome this problem is by training of motor skills. Subject's concentration on kinesthetic feelings may help in acquiring a better control of ERD/ERS patterns. To address this issue we replicated the experimental procedure for hand motor imagery task proposed by Hwang et al. (2009) but with modified training protocol. In the original study the neurofeedback-based training was used. We propose a physical hand training during which concentration on kinesthetic feelings is emphasized. The purpose of the experiment was to determine whether after a physical training focused on a kinesthetic experience of hand movement a significant differences in ERD/ERS patterns can be found. Ten healthy, right-handed males performed hand movement imagery task. The EEG data was recorded twice, once before and once after the physical training of hand movements focused on kinesthetic experience.

Hwang H, Kwom K, Im Ch-H (2009) Neurofeedback-based motor imagery training for brain–computer interface. J Neurosci Methods 179: 150–156.

26. BRAIN BIOELECTRICAL ACTIVITY STIMULATED BY NEUROFEEDBACK EEG STATIC TRAINING IN EXPERIENCING THE STATE OF READINESS FOR SPORT ACTIVITY

Mikicin M., Kowalczyk M.
Interfaculty Laboratory of Neuropsychophysiology, University of Physical Education in Warsaw, Warsaw, Poland

The authors attempted to assess the brain activity in athletes and the related mental states. The study was based on a Neurofeedback EEG (NEEG) static training with respect to what is termed the state of readiness for sport activity. The QEEG method (mapping, EEG) was used to analyse changes in brain bioelectrical action potential which remain in close temporal relation to particular events e.g. relax, concentration. The participants were athletes. In addition to sport training, the subjects (25 people) participated in 20 NEEG static training sessions aided with audiovisual brain stimulation (alpha). The results obtained for individual brain areas before and after 20 static training sessions showed that the activity of neurones was elevated in the visual and auditory areas, whereas it decreased in the motor area, and, what is worth emphasizing, the alpha activity was rising in general. Neurone activity in other brain areas was at similar levels before and after training sessions. Conclusion: NEEG static training excites bioelectrical activity of the brain in the area involved in perception from the stimulated senses, silences the non-involved areas and intensifies the state of relax (alpha). We find this training useful as a particular form of stimulation and control of mind, both in sport and in other domains of life.

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