9th Conference

Electrophysiological techniques in examination of bioelectric phenomena: from ion channels to neuronal nets

5–6 June 2014

Lublin

Abstracts of lectures and posters

Maria Curie-Skłodowska University
Faculty of Biology and Biotechnology
Department of Biophysics

Polish Biophysical Society

9th Conference

Electrophysiological techniques in examination of bioelectric phenomena: from ion channels to neuronal nets

Lublin, 5–6 June 2014

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LECTURES

A1. Ion channels of the skin mitochondria

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Potassium channels have been found in the inner mitochondrial membranes of various cells. The activation of these channels is cytoprotective. Hence, the identification of ion channels present in the inner mitochondrial membrane of skin cells is important in distinguishing possible protective mechanisms. In our study, inner membrane mitochondrial channels of the keratinocyte and fibroblast were investigated using a patch-clamp. In the inner mitochondrial membrane of fibroblast we detected large-conductance Ca^{2+}-regulated and ATP-regulated potassium channels. In keratinocytes mitochondria we observed potassium-selective channel with a conductance of 83 pS. The channel activity was inhibited by acidic pH and lidocaine. Additionally, channel knockdown decreased viability after UVB radiation exposure. Our findings indicate presence of the potassium channels in the inner mitochondrial membranes of human skin and their possible role in protection.
Study was supported by grant MERIS PBS1/B8/1/2012

A2. Theta oscillations in the posterior hypothalamic area – *in vivo* approach

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Numerous studies showed that occurrence of hippocampal theta rhythm is critically dependent on the integrity of a number structures localized at the level of brainstem and diencephalon. In detailed electrophysiological studies, conducted on anesthetized rats, we demonstrated that the posterior hypothalamic area (PHa), is not only a modulator of hippocampal formation theta but also could generate theta activity independently. The most effective areas in generation of examined EEG pattern were the supramammillary and posterior hypothalamic nuclei. Pharmacological manipulation reviled that PHa theta activity had a cholinergic (muscarinic) profile. Local injection of orexin A and B never induced theta activity in PHa. Finally, we showed that mineralocorticoid receptor but not gap junction play crucial role in generation of the posterior hypothalamic theta rhythm.
Studies supported by NCN grant no. 2011/01/B/NZ4/00373.

A3. Effect of auxin (IAA) on SV channels and volume changes in vacuoles isolated from red beet (*Beta vulgaris* L.) taproots

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The plant cell vacuoles play an important role in many physiological functions i.a. regulation of turgor pressure, which is essential for auxin-induced cell elongation growth. In the vacuolar membrane of higher plants two major ion currents are mediated by non-selective slowly (SV) and fast (FV) activating cation channels. The aim of the present study was to determine the effect of indole-3-acetic acid (IAA) on SV channel activity and volume changes in red beet taproots vacuoles. The SV channels were studied by the patch-clamp technique. The diameter of vacuoles was measured by means of microscope equipped with camera. It was found that in symmetrical 100 mM KCl, IAA at 1 μM inhibited SV channels activity. We have also showed that the incubation of vacuoles in the presence of IAA caused an increase in volume of vacuoles.

A4. Unique fingerprint of intergeniculate leaflet neurons among lateral geniculate body – the variability of low-threshold currents

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The intergeniculate leaflet (IGL) is a small but important structure of the mammalian biological clock located in the thalamus. It has been previously classified as an integral part of lateral geniculate nucleus (LGN) but there are many histochemical and functional differences. In this study we have characterized the diversity of low-threshold ionic currents what helped us to distinguish different IGL neuronal subpopulations. The *in vitro* patch clamp recordings in voltage and current clamp mode were performed on the brain slices from Wistar rats. After each experiment, the immunohistochemical study was carried out to verify the location and the biochemical nature of the recorded cell. Our results show a group of IGL neurons expressing inward calcium currents, similar to the ones recorded from LGN interneurons, but characterized by much lower amplitude. Moreover, some IGL neurons show low-threshold outward currents what is unique feature among LGN cells.

A5. Development of integrated multi-electrode probe to study ion transport across epithelial cell layer

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Our present construction of multisensory system is a result of six years of mostly unsuccessful attempts. At first, we had try to grow cells on round filter and then sandwich filter with cells between to flat multiprobe electrodes. This failed because handling the thin and flexible filter during assembling of the device usually damaged the cell monolayer. Secondly, we tried to exchange the medium by the tubes parallel to the cells plane. This failed due to difficulties in sealing of the system. Thirdly, we tried to use solid state electrodes. This attempt also failed because working electrodes stick over the surface of the probe by more than 100 μm.

A6. Control of membrane potential in medial prefrontal cortex (mPFC) pyramidal neurons by α_2-adrenergic receptors in young rats

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The aim was to clarify the effect of α_2-adrenergic receptor activation on membrane potential, determine the cellular effector and the signal transduction pathway responsible for membrane potential changes. Recordings were made in slices in layer V mPFC pyramidal neurons in perforated-patch configuration at 33.8°C. Clonidine evoked a dose-dependent membrane hyperpolarisation. It was affected neither by the adenylyl cyclase inhibitor, protein kinase A inhibitor, phospholipase C inhibitor nor the protein kinase C inhibitor, but it was attenuated by the G-protein βγ-subunit inhibitor. The blocker of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels abolished the hyperpolarisation and the α_2-receptor antagonists reduced it. We conclude that α_2-adrenergic receptor activation evokes hyperpolarisation in mPFC pyramidal neurons due to HCN channel inhibition in a membrane-delimited fashion by the $G_{\beta\gamma}$ subunit released from the G-protein.
Supported by NN401584638, NN301572940.

A7. Intensification of permethrin activity by alpha scorpion toxin – electrophysiological studies on insect nervous system

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Insecticide permethrin causes paralysis of insects by modification of membrane sodium channel (VGNa). It binds to site 7 on VGNa which shows positive allosteric interaction with site 3 for a-toxin from the venom of the scorpion. Alpha toxin accelerates depolarizing effect of permethrin on insect (cockroach) isolated axon. We assumed that the interaction between toxin and permethrin will be observed also on the cockroach whole nervous chain activated by mechanostimulation of cercus. In toxin presence the activity of chain observed after permethrin was higher than after permethrin alone, decreased slower and often salves of spontaneus discharges appeared. Such results correspond well to observations performed on locomotor activity of the cockroach. Alpha toxin accelerated insect paralysis induced by permethrin.

A8. α1F64 influences $GABA_AR$ gating through flipping mechanism

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$GABA_AR$s are crucial for neuronal inhibition. Using patch-clamp technique with ultrafast perfusion we found that mutations of hydrophobic residue at GABA-binding site affected not only binding affinity but also kinetics of macroscopic desensitization. Non-stationary variance analysis indicated that α_{1F64C} mutation reduces maximum open probability. To obtain further information about the role of α_{1F64} we used two different agonists. Experiments with a partial agonist, P4S, suggested an impact of α_{1F64C} mutation on the channel gating efficacy. Application of muscimol (with higher affinity than GABA) entailed a partial rescue of rapid desensitization in $\alpha_{1F64L}\beta_1\gamma_2$ receptors but in cysteine mutants – did not. Model simulations show that observed effects result from changes in flipping mechanism which links binding and gating. We conclude that α_{1F64} plays a crucial role in signal transduction from binding site to the channel gate.
Supported by NCN Grant 350/B/P01/2011/40 to JWM.

A9. Method and research equipment for synchronous SSVEP signal recording

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Stimulation of the sense of sight with a steady light signal evokes in the nervous system, and particularly in the primary visual cortex, the phenomenon called Steady State Visualy Evoked Potentials (SSVEP). Presented method and equipment allow synchronous recording of stimulatory and evoked (SSVEP) signals. As the real-time controller a DSM-51 unit was used. This controller serves two functions: (1) generates a sinusoidal signal influencing the

brightness of the two three-color LED sets, (2) decides on one of the 37 light colors of the LED sets. A special adapter has been developed to convert signals coming from the DSM-51 into signals suitable to control the LED sets. The adapter generates an additional signal fed directly to an EEG amplifier. This signal is recorded synchronously with the EEG signal coming from the electrocap. As the EEG amplifier a 16-channel Mindset 1000, produced by Nolan Computer Systems, was used. The proposed registration method allows precise identification and mutual matching of the stimulus and evoked signals, and thus their effective analysis.

A10. Theta oscillations in the posterior hypothalamic area – *in vitro* approach

Kowalczyk T., Caban B., Bocian R., Kłos-Wojtczak P., Kaźmierska P., Konopacki J.
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Hippocampal formation (HPC) theta rhythm is the largest rhythmical waveform generated by the mammalian brain. Studies performed in rodents revealed that the ascending brainstem-hippocampal synchronizing pathway originates in the nucleus reticularis pontis oralis (RPO), next RPO fibres ascend to posterior hypothalamic area (PHa) and then PHa neurons project to medial septum, known as a hippocampal theta "pacemaker". Posterior hypothalamic area forms a critical part of that pathway, serving as a modulator of theta rhythm recorded in the HPC. However, two years ago we have discovered that the PHa is also capable of independent generation of local theta. The first aim of the present work was to review the data concerning local theta rhythm in the posterior hypothalamic area *in vitro*. The second aim was to perform the comparison between basic features of theta activity observed in the PHa and hippocampal formation in *in vitro* conditions.

Supported by NCN grant 2011/01/B/N24/00373.

A11. *Dioaea muscipula* nutrient transporters employ channel-like properties

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Two Venus flytrap transporters, ammonium *Dm*AMT1 and sodium transporter 1, were found to be abundant in traps and up-regulated when glands were stimulated for digestion. Neither *Dm*AMT1 nor sodium transporter 1 acted as H^+ symporters. An electrochemical gradient empowered cation uptake exclusively. Moreover, a weak temperature dependence (Q_{10}=1.3) additionally pointed to a passive transport mechanism. Hence, both transporters exhibited the hallmark properties of channels. K_m decreased systematically with an increasing negative clamp-voltage. Consequently, in case of *Dm*AMT1 we noted a voltage dependent shift toward high affinity transporters in hyperpolarized cells. From our findings we conclude that membrane potential readjustments in glandular cells provide for effective nutritional strategy in flesh-eating plants.

A12. Effect of muscarinic receptors activation on membrane potential in medial prefrontal cortex (mPFC) pyramidal neurons

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The aim of this study was to clarify the mechanisms involved in regulation of membrane potential in mPFC pyramidal neurons by muscarinic receptors. Recordings of membrane potential were performed with the gramicidin perforated-patch method on mPFC pyramidal neurons in slices obtained from 20-day-old rats. It was determined that muscarinic receptor stimulation by carbachol (100 μM) evoked membrane potential depolarization (10.0±1.3 mV), which was completely eliminated by the M_1 receptor antagonist pirenzepine (2 μM), by removing Na^+ ions from the extracellular solution, in the presence of a TTX-resistant Na^+ channel blocker amitriptyline (100 μM) and significantly reduced by the inhibitor of G-protein βγ-subunits gallein (20 μM). We conclude that muscarinic M1 receptor-dependent depolarisation in mPFC pyramidal neurons is evoked by activation of TTX-resistance Na^+ currents by G-protein βγ-subunits.

Supported by grants no: NN401584638 and NN301572940.

A13. Mutation at GABA binding site (α1F64) affects both binding and gating properties of $GABA_A$ receptors

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In spite of broad knowledge of $GABA_AR$ pharmacokinetics, molecular mechanisms of conformation transitions remain elusive. Intriguingly, GABA binding site is distant from the channel gate (ca. 5 nm). In this study we searched for residues at GABA binding site involved in conveying binding energy to the channel gate of α1β2γ2 $GABA_ARs$. Mutation at α1F64 decreased the receptor affinity (shifted dose-response and reduced binding rate in racing protocol) and, in addition, strongly influenced onset, deactivation and desensitization of currents elicited by saturating

agonist, indicating gating modification. Non-stationary variance analysis of GABA- or pentobarbital-evoked currents showed that the cysteine mutation strongly decreased the open channel probability confirming its impact on receptor gating. We conclude that α1F64 residue, although located at the binding site, strongly affects gating properties of $GABA_A$ receptors.
Supported by NCN Grant 350/B/P01/2011/40 to JWM.

A14. Considering the ticking network of the intergeniculate leaflet neurons of the rat – *in vitro* studies
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The intergeniculate leaflet (IGL) is a small part of the biological clock mechanism. The well known IGL function is the integration of photic and non-photic information and then such integrated message is delivered to the master biological clock – suprachiasmatic nuclei. IGL consists of two main neuronal populations: enkephalinergic (ENK) and neuropeptide Y (NPY) positive neurons. In both populations GABA is expressed. During our study using patch clamp technique we investigated how the two kinds of IGL non-photic inputs: orexin and serotonin, can modulate the functioning of this structure. Moreover, we checked how local neurotransmitter (GABA) can affect IGL neuronal network. We observed that both orexin and serotonin can mainly activate target IGL neurons. In case of GABA we observed both inhibition and depolarization. Our data indicates that various IGL neurons can differently respond to non-photic signals what probably leads to modulation of output information.

A15. The grounds for successful neurofeedback
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The use of neurofeedback (NFB) in medical treatments, sport and art skills, rapidly increases. However, the mechanisms underlying NFB, its reliability and efficacy are still unclear. We reviewed 48 papers describing NFB training of adult, healthy subjects and performed our own, quantitative analysis on those which included control group (22). The analysis comprised: number of affected bands, group size, modality and intensity of training stimuli, number and position of electrodes. Correlation was measured with Kendall's T between factors and training success in groups: 'general' (22) and 'EEG' (17). We found negative correlation for number of manipulated bands, and electrodes position at Cz. Positive correlations were found for number of electrodes. Our results suggests that: (1) NFB training targeted for many EEG bands is less efficient; (2) The detection of effects following NFB training requires multiple recording sites.
Supported by Polish National Science Centre Grant UMO-2012/07/B/NZ7/04383

A16. Inactivation of the GluN1 subunit of the NMDA receptor modifies functions of dopaminoceptive neurons in the rat nucleus accumbens
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Adaptive behavior, such as regulation of appetitive motivation or reward processing, is associated with plasticity of the brain's dopamine system. We examined different types of adaptive behavior in the transgenic mouse line ($NR1^{D1CreERT2}$) where the GluN1 subunit is selectively inactivated in dopaminoceptive neurons. We used electrophysiological recordings to investigate if functions of D1-expressing neurons are modified. In transgenic animals the amplitude of field potentials in the NAcc was not different form controls, however, LTP was reduced from 138% in control to 118% in D1ERT2 [Tg-N/0-N] animals. Recordings of spontaneous EPSCs from medium spiny neurons of the NAcc demonstrated that approximately 50% of the cells lacked the NMDAR-dependent component of sEPSCs. These results suggest that the mutation significantly alters functions of dopaminoceptive neurons.

A17. Regulation of mitochondrial potassium channels
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Potassium channels have been described in the inner mitochondrial membrane: the ATP-regulated potassium channel, the calcium activated potassium channels, the voltage-gated potassium channel and the twin-pore domain TASK-3 potassium channel. Mitochondrial potassium channels play an important role in cardioprotection and in neuroprotection. Potassium channels in the inner mitochondrial membrane are modulated by inhibitors (such as 5-hydroxydecanoic acid or iberiotoxin) and activators (potassium channel openers such as diazoxide) previously described for plasma membrane potassium channels. The mitochondrial potassium channels are regulated by variety of endogenous modulators such as reactive oxygen species or free fatty acids. 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 and regulation.
Supported by a grant MERIS PBS1/B8/1/2012 from the National Centre of Research and Development.

A18. β-receptor agonist increases voltage-gated Na^+ currents in medial prefrontal cortex pyramidal neurons

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Voltage-gated Na^+ currents were recorded in cell-attached configuration from mPFC pyramidal neurons in slices obtained from 3-weeks-old rats. Isoproterenol (2 μM) did not influence maximal amplitude of Na^+ currents (43.7±8.6 pA in control and 41.6±8.1 pA in the presence of the drug, $P>0.05$, $n=9$). The β-receptor agonist however increased submaximal Na^+ currents (145±6.0% after β-receptor stimulation vs. 100% in control, $P<0.001$, $n=10$). This effect was abolished by β-receptor antagonist propranolol (30–60 μM). Isoproterenol (2 μM) depolarized the membrane potential recorded in perforated-patch configuration (−71.7±3.5 mV in control and −69.5±3.9 mV in the presence of the drug $P<0.05$, $n=3$). This depolarization was absent when cesium ions (3 mM) were added to the bath. It is concluded that isoproterenol increses submaximal voltage-gated Na^+ currents in mPFC pyramidal neurons.

A19. The patch-clamp studies on the influence of selected polycyclic compounds on the activity of Kv1.3 channels in normal and cancer cells

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The influence of selected heterocyclic compounds on the activity of voltage-gated potassium channels Kv1.3 have been studied by the whole-cell patch-clamp technique. Studies were performed on the channels in the model system- human T lymphocytes isolated from peripheral blood of healthy donors – and in cancer cell line Jurkat T. The studied compounds were flavonoids, chalcones, substituted stilbenes and their natural and synthetic derivatives. This presentation summarizes results of the recent studies. It is shown that some of the studied compounds are inhibitors of Kv1.3 channels in the model system and in cancer cells. These compounds may be applied in the future to support therapy of some tumor diseases characterised by an overexpression of Kv1.3 channels.

A20. Ion channels in plant cell vacuoles

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Plant vacuoles occupy up to 90% of mature plant cells. They participate in turgor regulation, cell signaling, storage of ions and other compounds. Ion channels in the tonoplast play an important role in these processes. A number of ion channels have been characterized both at the electrophysiological and molecular level. The most abundant channels are cation-permeable: slow vacuolar channels, SV/TPC, vacuolar potassium channels, VK/TPK, and anion-selective channels belonging to ALMT and CLC groups. SV channels are permeable to both mono- and divalent cations and activate at high cytoplasmic Ca^{2+} concentrations $[Ca^{2+}]_{cyt}$ (10–100 μM). They rectify from the cytoplasm to the vacuole. SV channels are regulated by phosphorylation, 14-3-3 proteins, redox potential, and heavy metals. VK channels are K^+-selective, operate at physiological $[Ca^{2+}]_{cyt}$, and are outwardly rectifying. VMAL channels transport mainly malate from the cytosol to the vacuole. CLC-type proteins exist as channels or NO_3^-/H^+ transporters.

A21. Rapid, reversible $GABA_B$R-mediated circuit rewiring

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About 10% of neocortical pyramidal neurons in layer 2/3 are connected to each other with strong and reliable synapses, displaying near-zero failure rates under the condition of very low network activity. However, *in vivo* many cells, including inhibitory neurons, exhibit elevated spontaneous firing activity. Here we investigated the effect of network activity on connection probability, strength, and synapse reliability between layer 2/3 pyramidal neurons. Under the condition of network activity, failure rates are two-fold higher. We find that $GABA_B$ receptors are tonically active during spontaneous network activity and that these receptors profoundly influence release probability. Using optogenetic tools we examined what type of inhibitory neurons is responsible for $GABA_B$ inhibition. Our data suggest that neocortical networks may be dynamically rewired based upon presynaptic $GABA_B$ activation, and that this phenomenon may be state-dependent.

A22. Matrix metalloproteinase activity regulates LTP through L-type voltage-gated calcium channel modulation

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Long-term synaptic plasticity at hippocampal CA3-CA1 synapses exhibit NMDAR- and L-type calcium channel (VDCC)-dependent components. To address the role of MMP proteases in mechanism of LTP induction, we used field potentials

recordings in acute mice brain slices. LTP was induced by 200 Hz tetanus. We have found that MMP inhibitor (NNGH, inhibits mainly MMP-3) disrupts 200 Hz-LTP (CTR: 164±11%, n=9; NNGH: 117±7%, n=7, P<0.01). Next, we dissected two components of 200 Hz-LTP using nifedipine (L-type blocker) and APV (NMDAR inhibitor). MMP blockade with NNGH prevented only vdccLTP (nmdaLTP in the presence of nifedipine: 140±6%, n=8; nifedipine+NNGH: 141±8%, n=6 P=0.93; vdccLTP in the presence of APV: 168±27%, n=7; APV+NNGH: 110±13%, n=6, P<0.05). Our observations show that MMP activity (presumably MMP3) specifically regulates VDCC-dependent component of hippocampal LTP.

Supported by NCN grants: NN401541540 and ETIUDA 2013/08/T/NZ3/00999.

A23. Impact of matrix metalloprotease inhibitor NNGH on long-term plasticity of NMDARs-mediated EPSPs in mouse CA1 hippocampal region

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The matrix metalloproteinases (MMPs) play a key role in synaptic plasticity, learning and memory. However, the underlying mechanisms remain poorly understood. Here we studied the role of MMPs inhibitor NNGH (10 μM) in shaping NMDAR-mediated component of synaptic plasticity by recording field potentials in Sch-CA1 hippocampal synapses in mice (P30-P45) brain slices. NMDAR-mediated EPSPs were isolated with AMPA/kainate receptors antagonist DNQX (20 μM) in Mg^{2+}-free solutions. We found that NNGH completely abolished NMDAR-mediated LTP (178.7±12% vs. 75.8±2.9% of baseline at 1 hour post 4×100 Hz stimulation, n=6, P<0.05). The role of specific MMPs in this process is currently investigated. In conclusion, MMPs regulate NMDAR-mediated LTP and most likely postsynaptic calcium influx during long-term neuronal plasticity.

Support: Ministry of Science and Higher Education grant NN401541540.

POSTER SESSION

P1. Somatosensory evoked potentials in rat's zona incerta

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Ventral sector of zona incerta (ZIv) was postulated to provide a significant GABA-ergic input to the higher-order thalamic nuclei. The aim of this study was to characterize, at the population level, the electrical activity evoked by a whisker stimulation in ZIv. In anesthetized rats microelectrodes were positioned into several locations encompassing ZI. EPs were recorded in response to deflection of mystacial vibrissae. The strongest response occurred in the posteriomedial part of ZIv with EPs starting at 3 ms post-stimulus with a large phasic negative wave followed by two low-amplitude slow positive and negative waves. The known connectivity of ZI suggest that short latency negative waves result from glutamatergic input from the spinal trigeminal nuclei, whereas longer-latency negative waves could originate from the cortical projections. Positive waves might reflect the activation of inhibitory input from anterior pretectal nucleus.

Supported by the polish National Science Centre grant N N401 533040

P2. The impact of hydrogen peroxide (H_2O_2) to the bioelectric signals in the liverwort *Conocephalum conicum*

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Plants are able to generate electrical responses to various mechanical, electrical, chemical, or light stimuli causing the disturbance of the balance between cation and anion fluxes through the plasma membrane. The aim of the research was to determine the impact of H_2O_2 (11–20 mM) on activation of ion channels in the plasma membrane of the liverwort *Conocephalum conicum*. The study was conducted using intracellular microelectrodes. Application of hydrogen peroxide in concentrations higher than 11 mM resulted in vanishing series of action potentials (APs), leading to permanent depolarization of the membrane potential. Potassium channel inhibitor (TEA, 10 mM) and anion channel inhibitor (A9C, 2 mM) caused the suppression of AP series evoked by H_2O_2.

P3. Local theta rhythm mapping studies of posterior hypothalamus *in vitro*

Caban B., Kłos-Wojtczak P., Kowalczyk T., Bocian R., Kaźmierska P., Konopacki J.

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Theta rhythm is one of the finest examples of synchrony in the mammalian brain. Hippocampal formation (HPC) theta is best described in rats and this EEG pattern consists of very regular, almost sinusoidal waves in the frequency range of 3–12 Hz. It is well-known that HPC theta rhythm is a result of the ascending brainstem-hippocampal synchronizing pathway activa-

tion, which originates in the pons and then projects to posterior hypothalamus, medial septum, and finally to the HPC. Recently, we have shown that administration of cholinergic agent – carbachol (75 μM), induces theta rhythm in *in vitro* maintained posterior hypothalamic area (PHa), specifically supramammillary nucleus (SuM) and posterior hypothalamic nuclei (PH). The purpose of this mapping study was to present the topography of areas capable of generation local theta rhythm in PHa slices as a result of carbachol administration.
Supported by NCN grant 2011/01/B/N24/00373

P4. Analysis of decay of Steady State Visual Evoked Potentials
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Electroencephalography (EEG) is one of the methods of examining an electrical activity of the brain. One can analyze everyday functioning or response for particular stimuli. Such responses are called Evoked Potentials (EP), and when the stimuli are given with stationery frequency, they are Steady State Evoked Potentials (SSEP). In my presentation I will concentrate on Steady State Visual Evoked Potentials (SSVEP), which are commonly used in Brain – Computer Interfaces (BCIs). After characterizing the phenomenon I will describe the results of SSVEP experiment. I examined the decline of this potential caused by end of stimulation. Such research may help in improving the BCIs and in understanding the mechanism of creating SSVEP in brain.

P5. The evaluation of selected functional connectivity between neurons generating resting discharge in postganglionic neurons before and after deafferentation of aortic baroreceptors
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The neurons generating resting discharge in postganglionic neurons are constantly apprised by activity of aortic baroreceptors. We evaluated influence of denervation of baroreceptors on appearance and size of parameters of shared input accompanied with reciprocal activation observed in activity of sympathetic renal fibers of anaesthetized rabbits. The number of examined interaction was significantly reduced an hour after baroreceptor deafferentation in comparison with control conditions (χ^2=5.738; P=0.017). However the evaluation of value of parameters of cross-correlation (width of shared input, height of shared input, mean amplitude and width of reciprocal activation and the distance between peaks) carried out during intact baroreceptor innervation and one hour after severing aortic nerves did not show any significant differences.

P6. Post-stimulus beta oscillations in the barrel system of awake rat
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Beta band (15–25 Hz) activity increases within thalamo-cortical pathways in non-sleeping animals and in attentive states. In order to investigate this activity, we recorded LFP and EPs elicited by whisker stimulations in the barrel cortex and somatosensory thalamus of non-anesthetized rats. In a fraction of trials (up to 40%), whisker stimulations evoked phase-locked beta (~20 Hz) oscillations in a form of 300–400 ms bursts of characteristic trapezoid shape clearly distinguished in cortical signals, but hardly discernible in the thalamic recordings. Our data confirm that strong, natural sensory stimuli evoke high amplitude beta oscillations in the rat barrel cortex. As simultaneously recorded beta activity in thalamus is weak, it can hardly be considered as an important generator of these oscillatory events.
Research supported by the polish National Science Centre grant N N401 533040

P7. Is orexin A involved in generation of the hippocampal theta rhythm? *In vivo* approaches
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Orexins are mainly synthesized in lateral hypothalamus but orexinergic projections are present in many brain areas including cerebral cortex, thalamus, brain stem and the hippocampal formation (HPC). The study was designed to check wheather intrahippocampal injections of orexin A and blockers of orexin receptors exert effect on theta. The studies were performed on anasthetized rats. Initially spontaneous theta rhythm recorded from HPC was blocked by the intravenous injections of atropine. When the HPC theta disappered, animals were subjected local injections of orexin A and the mixture of: orexin A and SB, orexin A and TCS and orexin A with both blockers in

separate studies. Theta activty was only observed in the first and second case but with a lower parameters in comparison to theta recorded after orexin A injection. Obtained results indicates crucial role of orexinergic receptors underlying theta HPC rhythm production.

P8. The effect of amphotericin B on the cell membrane tightness and ion channel activity in protoplasts of *Candida albicans*

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The patch clamp measurements carried out on the patch of the cell membrane showed that amphotericin B (AmB) applied at a low concentration (0.05 and 0.1 μM) causes a decrease in seal resistance, indicating loss of membrane tightness. Application of AmB also decreased the number of active outward rectifying potassium channels TOK1, but did not change the unitary conductance of the channels. Interestingly, AmB did not significantly change the open probability of the single channel. Such results indicate a lack of direct interaction of AmB and the channels. Membrane permeability changes after AmB were also observed by fluorescent microscopy. This research allowed us to conclude that integrity of the membrane between the pores formed by AmB is maintained.

Supported by NCN grant DEC-2012/05/BNZ1/00037

P9. Cation-permeable vacuolar channels in the tonoplast of *Marchantia polymorpha*

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The object of our patch-clamp study was the liverwort *Marchantia polymorpha*. Basic solutions were symmetrical in the bath and in the pipette and contained 100 mM KCl and 0.5 mM $CaCl_2$. Whole vacuole recordings mainly showed slowly activating outward rectifying currents, typical for SV (slow-vacuolar) channels. The unitary conductance registered from isolated patches was 74.7±4.0 pS at 100 mV. Replacement of K^+ with Na^+ caused reduction of the unitary conductance to 59.8±6.87 pS. Tenfold decrease of the KCl shifted the reversal potential close to E_K and caused reduction of the SV unitary conductance to 31.5±5.5 pS. Moreover, the gradient of KCl revealed the current at the negative potential; the unitary conductance at −100 mV was 43.3±0.5 pS, whereas the gradient of Na^+ did not evoke such an effect. This may suggest that inwardly rectifying K^+ channels exist in the tonoplast of the liverwort.

Supported by NCN grant 2013/09/B/NZ1/01052

P10. Action potentials generated in tomato after electrical and light stimulation

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Multichannel extracellular measurements of electrical potential changes allowed comparison of the electrical activity in two types of tomato: wild type (MT*wt*) and ABA-deficient, draught-sensitive mutant (MT*sit*). After electrical stimulation of the stem, MT*sit* generated action potentials (APs) with an amplitude of 40 mV and half-time ($\tau_{1/2}$) 19 s compared to MT*wt* 20 mV and 19 s, respectively. Intracellular microelectrodes were used to detect APs triggered by light in the leaves and cotyledons of MT*wt*. APs were characterized by long latency and their amplitudes were 77±2 mV and $\tau_{1/2}$ 58±2 s in leaves, and 67±14 mV, $\tau_{1/2}$ 13±2 s in the cotyledons. Turning off the light evoked 25 mV hyperpolarization in the leaf and 10 mV in the cotyledon.

P11. Regulation of hippocampal theta rhythm by the ventral tegmental area in freely moving rats

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Ventral tegmental area (VTA) is thought to be an important component in the mesocorticolimbic system involved in the regulation of theta rhythm in the hippocampus. In this study we investigated the effect of pharmacological inactivation of the VTA on theta rhythm parameters in fear conditioned rats. Animals were implanted with bilateral recording electrodes into the dorsal hippocampus and bilateral injection cannula into the VTA. Hippocampal LFP was recorded throughout the experiment, in pre- and post-injection condition during freezing behavior and active locomotion (avoidance). We found that intra-VTA injection of procaine temporary suppressed avoidance response and decreased the power of hippocampal theta rhythm during freezing in comparison to control (water). Our results indicate that inactivation of neuronal activity in the VTA reduces hippocampal theta rhythm as well as avoidance response in fear conditioned animal.

Supported by MNiSW Grant IP2011 034371.

P12. Influence of proteins on model lipid membrane studied with application of the BLM-Black Lipid Membrane method

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Black Lipid Membrane (BLM) technique was used to examine effects of BSA (Bovine serum albumin) on properties on the model membrane. BLMs were formed with EYPC (L-α-Phospatydylocholine from eggs yolk) in n-dekane. BLM studied were performed in phosphate buffer (pH 7.4). Various concentrations of BSA (between 0.01 mg/ml and 2.5×10^{-3} mg/ml) were used in order to optimize experimental conditions. BSA concentrations above 0.01 mg/ml proved too high as they caused collapse of the lipid membrane. Supplementation of the protein resulted in change of resistance of the lipid membrane. The experiment indicates that the protein incorporates into the lipid membrane.

P13. Time-lapse video and electrophysiological methods in studies of circumnutation in plants

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In specialised sensitive plants such as *Mimosa*, *Dionaea*, and *Aldrovanda*, rapid organ movement is observed and the excitation-turgor loss mechanism is the basis for rapid leaf or trap closure. In non-specialised ubiquitous plants, slow movement named circumnutation is common and it is driven by turgor. We examine whether transmembrane potential changes such as oscillations and long distance signal action and variation potentials are involved in this movement. Video camera recordings combined with extracellular measurement of electrical potential changes are applied. Additionally, intracellular microelectrodes and patch-clamp measurements are engaged. Novel software Circumnutation Tracker has been developed to track organ movement and standardisation of circumnutation parameters. *Helianthus annuus* and *Arabidopsis thaliana* are studied and a model of the circumnutation mechanism is proposed.

P14. Phase angle measured by bioelectrical impedance analysis in a healthy population

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Background: Bioelectrical impedance analysis (BIA) by obtaining information about the electrical properties of tissues by using raw bioelectrical impedance measurements [resistance, reactance, phase angle (PA)] is widely used in evaluating body-composition. The objective of this study was to investigate phase angle in a healthy population. Methods: In this study BIA examinations were conducted on 75 healthy population (67 men and 8 women). The mean age of the group was 56.56±12.05 years, BMI 26.12±4.19 kg/m^2 and height 171.23±8.65 cm. Results: At 50 kHz the value of resistance was 511.02±65.35 ohm, reactance 48.48±8.18 ohm and PA 5.44±0.75º. Conclusions: Further observations of a larger group of individuals are needed to assess reference ranges of impedance values (resistance, reactance, phase angle) for the Polish population.

P15. Parameters of shared input and modulations of activity of pairs of sympathetic renal neurons

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This study was carried out on sympathetic renal fibers of anaesthetised rabbits. Shared input assessed by cross-correlation analysis was found in 159 pairs of neurons in control conditions and in 128 during increased blood pressure. The width of cross-correlogram peak was 237.57±5.54 ms in control conditions and 249.97±7.13 ms during elevated blood pressure. The peak height was respectively 4.03±0.14 spikes/s and 4.39±0.23 spikes/s. Geometric mean of frequency of discharge of neuronal pairs was 1.17±0.04 spikes/s and 0.93±0.04 spikes/s. Geometric mean of cardiac modulation was weak 33.56±1.18% and significantly correlated with geometric mean of frequency of discharge and correlogram width in control conditions. The respiratory modulation of pairs of neurons was strong – respectively 78.63±0.85% and 88.29±0.95% – and was significantly related with geometric mean of frequency of discharge, correlogram width and peak height in resting conditions.

P16. Indoxacarb affects insect sodium channel inactivation

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Oxadiazine insecticide, indoxacarb is known to block voltage-gated Na channels (Nav) in insects but the mechanism of block is not yet well understood. There are indications that indoxacarb shifts slow inactivation curve in the hyperpolarizing direction. The conclusion coming from our experiments performed on cockroach DUM neurons is similar because strong artificial hyperpolarization eliminated effects of indoxacarb. In second series of experiments, the discharges in whole cockroach nerve cord under

mechanostimulation of cercus were recorded. Indoxacarb (10^{-6} M) significantly decreased the cord activity. Pretreatment of preparation with scorpion alpha toxin (blocking Nav inactivation) abolished effect of indoxacarb. Thus, our results confirm the effects of indoxacarb on Nav steady state inactivation.

P17. Post-stimulus gamma oscillations in the barrel system of awake rat

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We investigated the dynamics of gamma oscillations in thalamo-cortical part of the rat vibrissa-barrel system. LFPs were recorded by multiwire arrays implanted chronically in the barrel cortex and somatosensory thalamus in non-anesthetized rats at two arousal levels: quiescence (induced by long lasting habituation process) and arousal (elicited by contextual aversive stimuli). As compared to quiescence, in an aroused state we observed enhancement of power in gamma frequency (45–90 Hz) band arranged in a specific temporal pattern: the high gamma (73–90 Hz) bursts appeared ca. 230 ms after stimulus onset and developed into the lower gamma (53–73 Hz) activity at ~300 ms after stimulus. The effect was observed in 7 out of 14 examined sessions (in four animals) in which novelty component was raised by the first introduction of new arousing stimuli.

Research supported by the Polish National Science Centre grant N N401-533040

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