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## XI ELECTROPHYSIOLOGICAL CONFERENCE

**NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY, WARSAW, POLAND**
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# 11TH CONFERENCE ON ELECTROPHYSIOLOGICAL TECHNIQUES IN BIOELECTRICITY RESEARCH: FROM ION CHANNELS TO NEURAL NETWORKS

## Warsaw

## 25-26 of May 2018

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## PROGRAMME

### Friday 25th of May 2018

**9:00** **Registration**

**10:45** **Conference opening**

**11:00** **S1. Membrane channels and receptors**

**S1.1. Therapeutic concentrations of valproic acid inhibit fast TTX-sensitive voltage-gated sodium channels in prefrontal cortex pyramidal neurons**
Szulczyk B
*Department of Drug Technology and Pharmaceutical Biotechnology, The Medical University of Warsaw, Poland, Department of Physiology and Pathophysiology, The Medical University of Warsaw, Poland*

**S1.2. Flurazepam modulates gating of spontaneous and agonist-evoked GABAA receptor activity via distinct mechanisms**
Jatczak-Śliwa M[1,2], Terejko K[2], Brodzki M[1,2], Michałowski MA[1,2], Czyzewska MM[2], Nowicka JM[2], Andrzejczak A[1,2], Srinivasan R[1,2], Mozrzymas JW[2]
*[1]Department of Molecular Physiology and Neurobiology, Wroclaw University, Poland, [2]Department of Biophysics, Laboratory of Neuroscience, Wroclaw Medical University, Poland*

**S1.3. Source of voltage-dependency in hydrocortisone block of nicotinic acetylcholine receptor**
Dworakowska B, Nurowska E, Dołowy K
*Medical University of Warsaw, Laboratory of Physiology and Pathology, Centre for Preclinical Research and Technology, Warszawa, Poland*

**S1.4. Electrophysiological evidence for synergism between carbamate (bendiocarb) and an essential oil component (menthol)**
Jankowska M, Stankiewicz M
*Nicolaus Copernicus University, Toruń, Poland*

**12:00** **Coffee break**

**12:15** **S2. Neuromodulation**

**S2.1. Noradrenaline modulates the membrane potential in medial prefrontal cortex pyramidal neurons via β1-adrenergic receptors and HCN channels**
Grzelka K, Gawlak M, Szulczyk P
*Department of Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland*

**S2.2. Dopaminergic signalling in nucleus incertus – unexpected inhibitory and excitatory effects of D2 activation**
Szlaga A, Guguła A, Sambak P, Błasiak A
*Institute of Zoology and Biomedical Research, Jagiellonian University in Kraków, Kraków, Poland*

**S2.3. The effect of norepinephrine on rat intergeniculate leaflet neurons**
Sanetra A, Paluc-Chramiec K, Chrobok Ł, Lewandowski MH
*Jagiellonian University, Kraków, Poland*

**S2.4. Carbachol-induced NMDA-independent complex bursting of midbrain dopaminergic neurons – in vivo electrophysiological and pharmacological studies on NR1DATCreERT2 mice**
Walczak M[1], Szumiec Ł[2], Rodriguez Parkitna J[2], Błasiak T[1]
*[1]Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Cracow, Poland, [2]Department of Molecular Neuropharmacology, Institute of Pharmacology of the Polish Academy of Sciences, Cracow, Poland*

**13:15** **Coffee break**

**13:30** **S3. Synaptic transmission and neuronal excitability**

**S3.1. Mechanisms responsible for 5-HT7 receptor-mediated effects in the rat hippocampus**
Siwiec M, Sowa JE, Kusek M, Hess G, Tokarski K
*Institute of Pharmacology of the Polish Academy of Sciences, Kraków, Poland*

**S3.2. 5-HT7 receptor increases GABAergic transmission in mouse basolateral amygdala**
Sowa JE[1], Kusek M[1], Siwiec M[1], Hess G[1,2], Tokarski K[1]
*[1]Institute of Pharmacology, Polish Academy of Sciences, Department of Physiology, Kraków, Poland, [2]Institute of Zoology, Jagiellonian University, Department of Neurophysiology and Chronobiology, Kraków, Poland*

**S3.3. Prenatal stress in rats effects synaptic transmission in the dorsal raphe nucleus of their adolescent offspring**
Sowa JG[1], Hess G[1,2]
*[1]Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland, [2]Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

**14:30** **Lunch**

**15:30** **Lecture**

**L1. Impact of astrocytes on neuronal activity**
Butcher JB[1], Sims RE[2], Jenkins S[1], Parri RH[2], Głażewski S[1]
*[1]School of Life Sciences, Keele University, Keele, UK, [2]School of Life & Health Sciences, Aston University, Birmingham, UK*

**16:30** **S4. Neuronal plasticity**

**S4.1. Synaptic plasticity in the central amygdala during addictive and natural learning**
Bijoch Ł, Pękała M, Kaczmarek L, Beroun A
*Laboratory of Neurobiology, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland*

**S4.2. Long-term GABAergic synaptic plasticity in hippocampus strongly depends on the activity of matrix metalloproteinase-3**
Brzdąk P[1,2], Lebida K[1], Lech A[2], Nowak D[1,2], Wiera G[1,2], Mozrzymas JW[1,2]
*[1]Laboratory of Neuroscience, Dept. Biophysics, Wroclaw Medical University, Wroclaw, Poland, [2]Department of Physiology and Molecular Neurobiology, Wroclaw University, Wroclaw, Poland*

**S4.3. Learning increases intrinsic excitability of neocortical somatostatin-expressing interneurons**
Kanigowski D, Urban-Ciecko J, Kaczmarek L
*Nencki Institute of Experimental Biology, Warsaw, Poland*

**17:15** **Coffee break**

**17:30** **S5. Neuronal oscillations**

### S5.1. Cellular correlates of kainate-induced posterior hypothalamic theta rhythm in vitro

Caban B[1], Staszelis A[1], Kaźmierska P[1], Siwiec M[2], Sowa JE[2], Kowalczyk T[1]

*[1]Neurobiology Department, Faculty of Biology and Environmental Protection, University of Lodz, Poland, [2]Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*

### S5.2. Hippocampal theta rhythm as a bio-indicator of the efficiency of vagal nerve stimulation

Kłos-Wojtczak P[1,2], Broncel A[1], Bocian R[2], Konopacki J[2]

*[1]Neuromedical, Research Department, Łódź, Poland, [2]Department of Neurobiology, Faculty of Biology and Environmental Protection, The University of Lodz, Łódź, Poland*

### S5.3. The olfactory bulb generates and imposes ketamine-associated high frequency oscillations in the ventral striatum of rodents

Hunt MJ[1], Adams NE[2], Średniawa W[1], Wójcik DK[1], Simon A[2], Kasicki S[1], Whittington MA[2]

*[1]Nencki Institute of Experimental Biology, Warsaw, Poland, [2]University of York, Heslington, York, United Kingdom*

### S5.4. Reliable estimation of current sources from multielectrode LFP recordings with kernel CSD and L-curve

Średniawa W[1,2], Hunt MJ[1], Wójcik DK[1]

*[1]Nencki Institute of Experimental Biology, Warszawa, Poland, [2]University of Warsaw, Warsaw, Poland*

**19:30** **Get together dinner**

## Saturday 26th May 2018

**10:00** **S6. Neuronal systems**

### S6.1. Retinal or cortical? Which input to the rat dorsal lateral geniculate nucleus is more 'important'?

Jeczmien-Lazur J[1,2], Orlowska-Feuer P[1,2], Smyk M[1,2], Lewandowski MH[1]

*[1]Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland, [2]The Malopolska Centre of Biotechnology (MBC), Jagiellonian University, Kraków, Poland*

### S6.2. The combination of electrophysiology and optogenetics for the in vivo study of the influence of nucleus incertus on the activity of midbrain dopaminergic neurons in the rat

Pradel K, Błasiak T

*Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Cracow, Poland*

### S6.3. Electrophysiological characterization of rat nucleus incertus neurons – in vivo studies using multi-channel recording technique

Trenk A, Walczak M, Błasiak T

*Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Cracow, Poland*

**S6.4. Retinal sheet transplantation in rats with retinal degeneration restores visual cortical responses**

Foik TA[1], Lean GA[1], McLelland BT[2], Mathur A[2], Aramant RB[2], Seiler M[2], Lyon DC[1]

*[1]Anat. and Neurobio., [2]Dept. of Physical Med. & Rehabil., Univ. of California, Irvine, CA*

**11:00** **Coffee break**

**11:15** **Lecture**

**L2. Mapping and stimulation of memory in the human brain – can engrams be electrically enhanced?**

Kucewicz M

*Mayo Clinic, Rochester MN, USA, Gdansk University of Technology, Gdansk, Poland*

**12:15** **S7. Human cognition**

**S7.1. Time-frequency analysis of auditory working memory**

Duda-Goławska J, Żygierewicz J

*Biomedical Physics Division, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland*

**S7.2. Strategies of anticipatory attention**

Paluch K, Jurewicz K, Wróbel A

*Nencki Institute of Experimental Biology, Warsaw, Poland*

**S7.3. EEG estimates of attentional control during modified Multi-Source Interference Task (MSIT+)**

Dzianok P[1], Antonova I[1], Wojciechowski J[1,2], Rogala J[1,3], Kublik E[1]

*[1]Department of Neurophysiology, Nencki Institute of Experimental Biology of Polish Academy of Science, Warsaw, Poland, [2]Bioimaging Research Center, World Hearing Center of Institute of Physiology and Pathology of Hearing, Kajetany, Poland, [3]Centre for Modern Interdisciplinary Technologies Nicolaus Copernicus University, Toruń, Poland*

**S7.4. Amplitude of the resting state beta band oscillations predicts ERP characteristics and behavioural performance during attention tasks**

Rogala J, Kublik E, Wróbel A

*Nencki Institute of Experimental Biology, Warsaw, Poland*

**S7.5. Unconscious detection of one's own image**

Wójcik M[1], Nowicka M[1], Bola M[2], Nowicka A[1]

*[1]Laboratory of Psychophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland, [2]Laboratory of Brain Imaging, Nencki Institute of Experimental Biology, Warsaw, Poland*

**13:30** **Lunch**

**14:30** **S8. EEG analysis and practical application**

**S8.1. Properties and efficacy of a novel method for assessing phase to amplitude cross-frequency coupling**

Bernatowicz G, Żygierewicz J

*University of Warsaw, Faculty of Physics, Warsaw, Poland*

### S8.2. EEG Spectral Fingerprints as a method for classifying different regions of the human brain – initial results

Komorowski M[1,2], Wojciechowski J[2,3], Nikadon J[1,2], Piotrowski T[1,2], Dreszer J[2,4], Duch W[1,2]

*[1]Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University in Toruń, Toruń, Poland, [2]Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University in Toruń, Toruń, Poland, [3]Institute of Physiology and Pathology of Hearing, Bioimaging Research Centre, Kajetany, Poland, [4]Faculty of Humanities, Nicolaus Copernicus University in Toruń, Toruń, Poland*

### S8.3. Recent advances in brain-computer interfaces from BrainTech.pl

Chabuda A[1,2], Dovgialo M[1,2], Różański P[1,3], Wieteska M[1,4], Pawlisz M[1], Duszyk A[2,5], Żygierewicz J[2], Rudkiewicz T[6], Durka P[1,2]

*[1]BrainTech Ltd, ul. Jeziorna 23, 02-911 Warsaw, Poland, [2]University of Warsaw, Faculty of Physics, Warsaw, Poland, [3]University of Warsaw, College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences (MISMaP), Warsaw, Poland, [4]Warsaw University of Technology, Warsaw, Poland, [5]University of Social Sciences and Humanities, Warsaw, Poland, [6]NC.ART, Studio projektowe wzornictwa przemysłowego, Raszyn, Poland*

### W1. Brain-computer interfaces from BrainTech.pl – practical workshop

### Coffee

**16:15** **Conference closing remarks**

# LECTURES

## Friday 25th of May 2018

### L1. IMPACT OF ASTROCYTES ON NEURONAL ACTIVITY

Butcher JB[1], Sims RE[2], Jenkins S[1], Parri RH[2], Głażewski S[1]

*[1]School of Life Sciences, Keele University, Keele, UK, [2]School of Life & Health Sciences, Aston University, Birmingham, UK*

In recent years, it has emerged that astrocytes play a far greater role in brain function than previously envisaged. This includes their participation in the regulation of brain blood flow, formation of blood-brain and CSF-brain barriers, homeostasis of interstitial fluid, removal of metabolites from the interstitial spaces, formation of astrocytic scars due to brain injury, neurotransmitter uptake, and even sleep. Astrocytes are also increasingly acknowledged as active partners with neurons in synaptic communication. They sense the same synaptic inputs as neurons and respond with intracellular Ca2+ elevations, which in turn may elicit the release of gliotransmitters such as ATP, D-serine, GABA, and glutamate. Release of gliotransmitters has been described in various in vitro models of synaptic plasticity, including short- and long-term potentiation (LTP), long-term depression (LTD), and heterosynaptic depression. We have recently addressed the impact of astrocytes on neuronal activity and plasticity in the barrel cortex of mouse. The neocortex exhibits two general forms of neuronal plasticity. One form, termed Hebbian plasticity, concerns changes in synaptic transmission at individual inputs to neurons and their connectivity and is thought to be involved in the coding of external stimuli. The second, called homeostatic plasticity, serves to maintain a restricted dynamic range of neuronal activity. Here we demonstrate that: (1) optogenetic and chemogenetic stimulation of astrocytes via the IP3 (inositol triphosphate) pathway potentiates neuronal firing in vitro and in vivo; (2) IP3R2 deficiency (type 2 receptor for IP3 that is present in astrocytes, but not in neurons) results in diminished DHPG ((S)-3,5-Dihydroxyphenylglycine, a selective group 1 metabotropic glutamate receptor agonist)-evoked astrocytic, but not neuronal [Ca2+] in vitro, with unchanged spontaneous and evoked neuronal firing in vivo; (3) IP3R2 knockout results in impairment of experience-dependent Hebbian depression and homeostatic up-regulation and finally (4) an LTD-inducing protocol evokes LTP in slices deficient in IP3R2 and in wild type slices after filling astrocytes with the Ca2+ chelator BAPTA ((1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid). These results demonstrate that astrocytes regulate neuronal firing, Hebbian depression, and homeostatic up-regulation in the barrel cortex.

## Saturday 26th of May 2018

### L2. MAPPING AND STIMULATION OF MEMORY IN THE HUMAN BRAIN – CAN ENGRAMS BE ELECTRICALLY ENHANCED?

Kucewicz M

*Mayo Clinic, Rochester MN, USA, Gdansk University of Technology, Gdansk, Poland*

Direct electrical stimulation of the human brain can elicit sensory and motor perceptions as well as recall of memories. Stimulating higher order association areas of the lateral temporal cortex in particular has been reported to activate visual and auditory memory representations of past experiences. We hypothesized that this effect could be utilized to modulate memory processing. Recent attempts at memory enhancement in the human brain have focused on the hippocampus and other mesial temporal lobe structures, with a few reports of memory improvement in small studies of individual brain regions that have not been reproduced. We investigated the effect of stimulation in four brain regions thought to support declarative memory: hippocampus, parahippocampal neocortex, prefrontal cortex and temporal cortex. A classic verbal memory task was used to assess the effect of bipolar 50 Hz stimulation during encoding of word lists on subsequent free recall in human patients implanted with intracranial electrodes. We found enhanced recall of words from lists with electrical stimulation in the lateral temporal cortex, but not in the other three brain regions tested. This selective enhancement was observed on the level of individual subjects, subjects stimulated in the temporal cortex, and across the four brain regions studied. In effect, more words were remembered with than without stimulation in the lateral temporal cortex. Stimulation targets in the other brain regions had a negative effect on memory compared to targets in the temporal cortex. These differential behavioral effects were paralleled by modulation of neural activities in the high gamma frequency band (60-120 Hz) during memory encoding. These activities were proposed to reflect coordinated firing of neuronal assemblies – the hypothetical neural substrate for engrams. We conclude

that electrical stimulation in specific brain areas can modulate neural processes induced during encoding of word engrams and enhance their recall. Gamma frequency activities provide a useful biomarker to map memory engrams and guide human brain stimulation.

# SESSIONS

## S1. MEMBRANE CHANNELS AND RECEPTORS

### S1.1. THERAPEUTIC CONCENTRATIONS OF VALPROIC ACID INHIBIT FAST TTX-SENSITIVE VOLTAGE-GATED SODIUM CHANNELS IN PREFRONTAL CORTEX PYRAMIDAL NEURONS

Szulczyk B

*Department of Drug Technology and Pharmaceutical Biotechnology, The Medical University of Warsaw, Poland, Department of Physiology and Pathophysiology, The Medical University of Warsaw, Poland*

Valproic acid is a widely used antiepileptic and mood-stabilizing drug. Its mechanism of action has not been fully elucidated. The aim of this study was to test the effect of different therapeutic concentrations of valproic acid on fast TTX-sensitive voltage-gated sodium channels. These channels play a very important role in neuronal physiology and in epileptic activity. Voltage-gated sodium currents were recorded using the voltage-clamp technique. Dispersed prefrontal cortical pyramidal neurons were tested. At a –90 mV holding potential, valproic acid (200 μM) slightly inhibited the maximal amplitude of sodium channels. The effect of the drug on the maximal amplitude of sodium currents was much more pronounced when a –50 mV depolarized holding potential was applied. At this holding potential, three doses were tested: 200 μM, 2 μM and 0,02 μM. Valproic acid did not influence activation but shifted the inactivation curve of fast voltage-gated sodium channels towards hyperpolarization. This means that the sodium channels were inhibited by valproate over the range of membrane potentials. Recovery from inactivation was the same in the control and in the presence of valproic acid. Use-dependent blockade was tested at 10 Hz and 50 Hz. In every condition, valproic acid did not influence the use-dependent blockade. The main finding of this study is that therapeutic concentrations of valproic acid inhibit fast voltage-gated TTX-sensitive sodium currents when they are evoked from a depolarized holding potential. Thus, fast voltage-gated sodium channels contribute to the pharmacological effect of valproate.

### S1.2. FLURAZEPAM MODULATES GATING OF SPONTANEOUS AND AGONIST-EVOKED GABAA RECEPTOR ACTIVITY VIA DISTINCT MECHANISMS

Jatczak-Śliwa M[1,2], Terejko K[2], Brodzki M[1,2], Michałowski MA[1,2], Czyzewska MM[2], Nowicka JM[2], Andrzejczak A[1,2], Srinivasan R[1,2], Mozrzymas JW[2]

*[1]Department of Molecular Physiology and Neurobiology, Wroclaw University, Poland, [2]Department of Biophysics, Laboratory of Neuroscience, Wroclaw Medical University, Poland*

GABAA receptors mediate inhibitory transmission in the adult mammalian brain and are modulated by many clinically used drugs such as benzodiazepines. It has previously been demonstrated that benzodiazepines affect binding and gating transitions. However, the mechanism of their modulation is still not fully understood. In our present study we address this problem by examining modulation of spontaneous activity by the benzodiazepine flurazepam and its cross-talk with ligand-evoked activity of wild-type and mutated (at α1F64 position located in the GABA-binding site, shown to affect preactivation/flipping transition) α1β2γ2 GABAA receptors. We used patch-clamp technique to measure macroscopic and single-channel currents mediated by wild-type and mutated (Leu, Ala or Cys substitution at the α1F64 position) GABAA receptors. Spontaneous activity was measured using a BioLogic Perfusion System and picrotoxin application. We also performed experiments for saturating GABA and partial agonist applications using an ultrafast perfusion system (theta-glass). We used flurazepam pretreatment and co-application (flurazepam with GABA) protocols, which allowed us to observe the cross-talk between spontaneous and ligand-induced activity. Model simulations were performed in ChaneLab software. α1F64 mutants exhibited larger spontaneous activity compared to wild-type receptors and flurazepam potentiated this activity to the same extent for all considered receptor types. Our single-channel analysis showed prolonged openings upon flurazepam treatment. For saturating [GABA] applications in a pretreatment protocol, we found a significant correlation between the increase of the overshoot (amplitude above the baseline after agonist removal) and the amplitude of currents upon flurazepam application. Flurazepam potentiates the amplitude of currents mediated by mutants after GABA and partial agonist application and affects their kinetics. Our model simulations indicate that flurazepam affects opening/closing transitions of spontaneous activity but affects preactivation and desensitization transitions of ligand-induced activity. Flurazepam's

mechanism of GABAA receptor modulation is different for spontaneous and ligand-induced activity. Moreover, spontaneous openings clearly affect agonist-evoked responses. Altogether, flurazepam alters the GABAA receptor gating transitions in a manner dependent on the receptor ligation. Supported by NCN grants: 2013/11/B/NZ3/00983 and 2015/18/A/NZ1/00395.

## S1.3. SOURCE OF VOLTAGE-DEPENDENCY IN HYDROCORTISONE BLOCK OF NICOTINIC ACETYLCHOLINE RECEPTOR

Dworakowska B, Nurowska E, Dołowy K

*Medical University of Warsaw, Laboratory of Physiology and Pathology, Centre for Preclinical Research and Technology, Warszawa, Poland*

Corticosteroids have been shown to exert direct inhibitory action on the muscle-type nicotinic acetylcholine receptor (AChR) and therefore can promote pharmacological muscle denervation. The mechanism of hydrocortisone (HC) blockade of AChR has not been fully established. It is uncommon for an electrically neutral molecule, e.g. HC, to induce voltage-dependent changes in AChR kinetics. Our experiments aimed to determine the source of voltage-dependency in HC action. Wild-type (WT) and αD200Q receptors were transiently expressed in HEK293 cells. Recordings were performed in either the presence or absence of HC. We showed that the D-to-Q substitution is capable of suppressing voltage dependency in the HC-induced block. We conclude that the distance between αD200 and the agonist binding site depends on the membrane potential. The voltage-dependent changes of the αD200 position have not been determined yet. To our knowledge, the ability to induce voltage-dependency in blocker action has not been shown previously for an amino acid located outside the transmembrane portion of the receptor. Possible mechanisms of HC block (allosteric and knocking) in WT and αD200Q receptors are proposed.

## S1.4. ELECTROPHYSIOLOGICAL EVIDENCE FOR SYNERGISM BETWEEN CARBAMATE (BENDIOCARB) AND AN ESSENTIAL OIL COMPONENT (MENTHOL)

Jankowska M, Stankiewicz M

*Nicolaus Copernicus University, Toruń, Poland*

Bendiocarb is an acetylcholinesterase inhibitor belonging to the carbamate class of insecticides. It causes rapid knock-down of an insect. However, its effective concentrations are relatively high ($10^{-4}$ M). In our previous toxicity tests performed on American cockroach (Periplaneta americana), we have shown that the presence of menthol, a compound found in some essential oils, improved the effectiveness of bendiocarb. The aim of our study was to investigate the interaction between bendiocarb and menthol on the cockroach nervous system in order to clarify their combined mode of action. Experiments were performed using an extracellular electrodes technique on the P. americana connective nerve leaving the terminal abdominal ganglion. Two types of bioelectric signals were evaluated: the size of response to stimulation of cerci mechanoreceptors and the total activity of the nerve, including spontaneous activity and response to stimulation. Bendiocarb did not modify the size of response to stimulation but it increased the total activity of the nerve. It caused bursts in spontaneous activity in a concentration-dependent manner, which resulted in an increase of total activity compared to control. 60 min after application of bendiocarb ($10^{-6}$ M) activity was measured at 3.21 times higher than control. Coapplication of menthol potentiated this effect, as 60 min after application of the mixture of bendiocarb ($10^{-6}$ M) and menthol ($10^{-6}$ M) total activity was 6.71 times higher than control. Statistical analysis performed on data obtained with different bendiocarb concentrations ($10^{-7}$, $5x10^{-7}$, $2x10^{-7}$, $10^{-6}$ M) in the presence and absence of menthol revealed that the addition of menthol decreased the ED50 value by two fold and increased the maximum effect. The combinational index calculated via Chou and Talalay was much lower than 1, which indicates strong synergism between compounds. We assumed that the strengthening effect of menthol takes place via octopaminergic receptors. Menthol decreased the total activity of the nerve; the same effect was observed for octopamine. Phentolamine, an octopamine receptor antagonist, abolished the effect of menthol and eliminated the effect of the menthol + bendiocarb mixture. Menthol increased the effectiveness of bendiocarb in electrophysiological experiments and a possible mechanism of action for menthol is via activation of octopamine receptors. The project was supported by the National Science Center, Poland under grant: NCN 014/15/N/NZ9/03868.

# S2. NEUROMODULATION

## S2.1. NORADRENALINE MODULATES THE MEMBRANE POTENTIAL IN MEDIAL PREFRONTAL CORTEX PYRAMIDAL NEURONS VIA β1-ADRENERGIC RECEPTORS AND HCN CHANNELS

Katarzyna Grzelka, Maciej Gawlak, Paweł Szulczyk

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

The noradrenergic system is essential in medial prefrontal cortex (mPFC) physiology: noradrenaline (NA) acting via adrenergic receptors (α1, α2 and β) plays a significant role in the regulation of cognitive brain functions and affective processes. Impaired modulation of the mPFC

by NA has been implicated in many neuropsychiatric diseases, e.g. posttraumatic stress disorder, attention deficit hyperactivity disorder, and depression. While the presence of all adrenergic receptor subtypes has been reported in the mPFC, little is known regarding the mechanisms by which NA modulates mPFC neurons. The aim of this study was to investigate which adrenergic receptor subtype controls the resting membrane potential and holding currents in mPFC neurons. Secondly, we wanted to define the cellular effector(s) and signaling pathway(s) involved in the action of NA. To answer these questions, we recorded the membrane potential and holding current using patch-clamp techniques. Gramicidin perforated-patch and classical whole-cell recordings were obtained from layer V mPFC pyramidal neurons in slices isolated from young rats. Distribution of the adrenergic receptor subtypes in mPFC was visualized with fluorescent immunohistochemistry. NA evoked depolarization and inward current in the tested cells. Stimulation of α1- and α2-receptors failed to evoke similar effects. Meanwhile, the nonselective β-receptor agonist, as well as the selective β1-receptor agonist, mimicked the effect of NA on holding current. The NA-dependent inward current was considerably reduced by the selective β1-receptor antagonist. The effect of NA was also attenuated, though to a smaller degree, by the selective β3-receptor antagonist. At the same time, application of two different selective β3-agonists evoked inward currents in the tested neurons. Expression of β1- and β3-receptors in mPFC was confirmed with confocal microscopy. The β1-related inward current was greatly decreased in the presence of Cs+ ions and ZD7288, a selective blocker of HCN (hyperpolarization-activated cyclic nucleotide–gated) channels. It was not affected by selective blockers of different signaling pathways known to be responsible for mediating the effects from G-protein-coupled receptors (e.g. adenylyl cyclase-PKA and phospholipase C-PKC). However, it was significantly diminished by blockers of the βγ subunit-dependent transduction system. We conclude that NA modulates the membrane potential and holding current of mPFC pyramidal neurons preferentially via β1-receptors. The effects occur due to HCN channel activation and are probably mediated in a membrane delimited fashion by a βγ subunit released from the G-protein. Stimulation of β3-receptors may be partially responsible for the NA-related effects. Supported by National Science Centre, Poland, grant 2014/15/N/NZ4/04760 and FW5/PM2/16.

## S2.2. DOPAMINERGIC SIGNALLING IN NUCLEUS INCERTUS – UNEXPECTED INHIBITORY AND EXCITATORY EFFECTS OF D2 ACTIVATION

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Nucleus incertus (NI) is a brainstem structure involved in stress response, arousal, and food intake control. NI is a main source of relaxin-3 in the brain, and relaxin-3 was shown to alter reward and stress related behaviours. Recently, dopamine receptors were identified in the NI, however the effect of their activation on NI neuronal activity was unknown. Therefore, the current study aimed to characterise the effect of dopamine receptor activation on NI neuronal activity, as well as identify the source of tyrosine hydroxylase (TH)-positive fibres in the structure. Whole-cell patch-clamp recordings were used to assess the responsiveness of NI neurons to selective D1R and D2R agonist application. Track-tracing combined with anti-TH immunohistochemical staining was used to define the source of TH immunoreactive fibres in the NI. D1R agonist SKF-81297 (10 μM) caused depolarization of NI cells by 5.01±0.75 mV (mean change ± SEM). Depolarization persisted in the presence of tetrodotoxin and glutamate/GABA receptors antagonists which indicates direct postsynaptic action of SKF-81297. Interestingly, activation of D2Rs with quinpirole (20 μM) induced both inhibitory and excitatory effects on NI neuron activity. In 56% of NI neurons an outward current (13.87±6.55 pA), decrease in action potential firing frequency (3.47±1.33 Hz), and hyperpolarization of quiescent cells (2.16±1.01 mV) was observed after quinpirole administration. In 28% of the neurons an increase in inward current amplitude (13.73±2.08 pA) and increase in frequency of action potentials (0.75±0.37 Hz) was recorded after quinpirole application. Both excitatory and inhibitory action of quinpirole persisted in the presence of tetrodotoxin and GABA/glutamate receptors antagonists. Results of track-tracing experiments allowed identification of A11 and A13 cell groups as a source of dopamine innervation in the NI. D1R and D2R are localised postsynaptically on NI neurons. Surprisingly, D2R activation exerted both direct inhibitory and excitatory effects on NI neurons, suggesting a diverse action for dopamine receptor agonists on neurochemically and/or functionally distinct cell classes in this structure. Identification of A11 and A13 dopamine cells groups as a potential source of TH immunoreactive fibres in the NI allows us to conclude that dopaminergic innervation of the NI may be involved in the control of alertness and sensorimotor response to salient stimuli.

## S2.3. THE EFFECT OF NOREPINEPHRINE ON RAT INTERGENICULATE LEAFLET NEURONS

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The intergeniculate leaflet (IGL) of the thalamus is an important structure of the circadian timing system. Its primary role is the integration of both photic and non-photic stimuli relevant for the regulation of the

sleep-wake cycle. The consolidated information is then transmitted to the main generator of circadian rhythms – the suprachiasmatic nuclei (SCN). As the non-photic cues are delivered to the IGL via non-specific projections of the brainstem, we chose to investigate how one projection, the norepinephrine (NE) system from the locus coeruleus (LC), influences IGL neuronal activity. Moreover, we divided recorded cells into two groups, anticipating that they reflect two different projections arising from the IGL. The first group, expressing T-type calcium current (T-type cells), putatively comprises the connection between the leaflets located in both hemispheres, while A-type potassium current expressing neurons (or A-type cells) most likely transmit information to the SCN. The influence of NE on IGL neurons was tested by patch clamp recordings in the current clamp mode on 250 µm brain slices from 2/3-week-old male Wistar rats. In each instance NE (20 µM) was applied twice, the second application in the presence of tetrodotoxin (TTX, 0.5 µM), to determine if the observed effect was postsynaptic or presynaptic. Both substances were applied by bath perfusion. After the experiment, slices were immunostained against the neuropeptide Y to confirm that the recorded cell was within the borders of the IGL. For this purpose, ExtrAvidinCy3 (1:250) and anti-NPY antibodies (1:8000) were used. NE was shown to elicit a direct effect on a majority of IGL neurons. The effects were diverse; however, all A-type cells, and most T-type cells, responded with depolarization. Some neurons within the T-type group showed no response and, interestingly, a few neurons were hyperpolarized. Our study shows for the first time the electrophysiological effects of NE on the neuronal activity of single IGL neurons. The variety of responses, probably through activation of different receptors, requires further study. We predict that the diverse responses reflect the differential impact of NE on neurons foming different projections, but the variety of responses from the T-type cells indicate that this group may be more complex and consist of additional subpopulations.

## S2.4. CARBACHOL-INDUCED NMDA-INDEPENDENT COMPLEX BURSTING OF MIDBRAIN DOPAMINERGIC NEURONS – IN VIVO ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES ON NR1DATCreERT2 MICE

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Dopamine plays a key role in the control of behaviour and motor functions. The amount of neurotransmitter released into a synapse depends on the firing pattern of dopaminergic neurons, which occurs as a continuum between regular and bursting modes of activity. The latter mode results in a phasic increase of dopamine release, whereas a basal level of neurotransmitter is maintained by non-bursting (tonic or irregular) firing of dopaminergic neurons. While functional NMDA receptors are considered crucial for evoking dopaminergic neurons' bursts of action potentials, it remains an open question whether other neurotransmitters also evoke this type of activity. Therefore, the aim of our research was to determine the effect of cholinergic receptor stimulation on the activity of dopamine neurons lacking functional NMDA receptor. We used a genetically modified strain of mice (NR1DATCreERT2), which allowed us to induce a deletion of the NR1 NMDA receptor subunit selectively on dopaminergic neurons of adult animals. Experiments were performed on urethane anaesthetised animals. We used multi-barrel glass micropipettes (five barrels), allowing us to combine single unit extracellular recordings of midbrain dopaminergic neurons' activity and iontophoresis, local application of drugs (a nonspecific agonist of cholinergic receptors – carbachol; muscarinic and nicotinic receptor antagonists – scopolamine and mecamylamine, respectively; and NMDA). Loss of NMDA receptors on dopaminergic neurons decreased their basal firing rate, attenuated bursting, and abolished responsivity to NMDA compared to wild-type animals. After application of carbachol, the vast majority of dopaminergic neurons increased their firing rate. Interestingly, some of the recorded cells, both in control and NR1DATCreERT2 mice, developed slow oscillatory changes in firing rate, which transformed into robust complex bursts of action potentials. These results show that agonists of cholinergic receptors can modulate rate as well as pattern of firing of the midbrain dopaminergic neurons. Furthermore, our observations suggest that activation of cholinergic receptors alone, i.e. without the involvement of NMDA receptors, can switch a subpopulation of dopaminergic neurons to a burst firing mode. Funding: NCN, Poland, PRELUDIUM 2015/19/N/NZ4/00960.

# S3. SYNAPTIC TRANSMISSION AND NEURONAL EXCITABILITY

## S3.1. MECHANISMS RESPONSIBLE FOR 5-HT7 RECEPTOR-MEDIATED EFFECTS IN THE RAT HIPPOCAMPUS

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The 5-HT7 receptor has been implicated in mood regulation, circadian rhythmicity, and sleep, the disturbances

of which are evident in the course of depressive disorders. Research into 5-HT7 receptor signalling in the hippocampus has indicated that activation of the 5-HT7 receptor increases the excitability of pyramidal neurons of the CA1 and CA3 areas. The aim of our study was to investigate ionic mechanisms underlying this effect. We performed whole-cell current clamp recordings from rat CA1 pyramidal cells and tested the effects of 5-HT7 agonists on neuronal excitability and spiking dynamics. Voltage clamp recordings were used to determine changes in voltage-dependent currents following 5-HT7 receptor activation. Finally, we stimulated Schaffer collaterals and recorded evoked AMPA currents to examine whether these newly discovered ionic mechanisms influence synaptic transmission. Administration of 5-HT7 receptor agonists increased the excitability of CA1 pyramidal neurons, in line with previous findings. This was accompanied by a significant decrease in the time needed for the cell to fire the first action potential following a depolarizing current pulse. Voltage clamp recordings confirmed that 5-HT7 receptor activation significantly attenuated the A-type current. Pharmacological block of Kv4.2/4.3 channel subunits prevented the increase in neuronal excitability and spiking latency, as well as the 5-HT7-mediated increase in evoked AMPA current amplitude. In the present study we demonstrate that the 5-HT7 receptor-mediated effects on excitability, spiking latency and synaptic transmission are directly associated with inhibition of the A-type potassium current, which is a mechanism not previously associated with this receptor.

## S3.2. 5-HT7 RECEPTOR INCREASES GABAERGIC TRANSMISSION IN MOUSE BASOLATERAL AMYGDALA

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The amygdala is a part of the limbic system involved in emotional processing, which is highly connected with other areas of the brain. Its basolateral region (BLA) receives many inputs, including those from prefrontal cortex, hippocampus, and thalamus. Moreover, the amygdala receives robust innervation from the raphe nuclei. The last serotonin receptor to be discovered, 5-HT7, is highly expressed in the amygdala, suggesting a possibly strong influence on amygdala function. The 5-HT7 receptor is involved in modulation of many physiological processes, such as learning, pain sensation, and mood regulation. Functions of the 5-HT7 receptor at the cellular and network level have been studied in the hippocampus, dorsal raphe nuclei, and frontal cortex. However, very little is known about the physiological role of 5-HT7 receptors in the amygdala. Our study aimed to elucidate the effect of 5-HT7 receptor activation on synaptic transmission, electrophysiological properties, and excitability of neurons in the BLA. Whole-cell patch-clamp recordings were made primarily from principal neurons in the BLA of mice, using acute brain slices (300 μm). After recording a baseline, 5-CT (250 nM) in the presence of WAY 100635 (2 μM), a 5-HT1A receptor antagonist, was bath-applied. Both inhibitory and excitatory synaptic transmission were measured by recording spontaneous (sIPSC/sEPSC), miniature (mIPSC/mEPSC) or evoked (eEPSC/eIPSC) postsynaptic currents. Moreover, excitability, input resistance, and membrane voltage were measured. Specificity of the observed effects was further investigated using the same experimental protocols with the 5-HT7 antagonist SB269970. Our results show an increase in excitability in fast-spiking interneurons in the amygdala. Regarding inhibitory transmission, 5-HT7 activation increased the amplitude and frequency of spontaneous, but not miniature, IPSC in the principal cells, which suggests that this effect was network-dependent. These effects were abolished in the presence of the 5-HT7 antagonist SB269970. Our data suggest that 5-HT7 activation increases GABAergic synaptic transmission onto BLA principal neurons. This is probably due to increased GABA release from local interneurons, where 5-HT7 receptors may be localized. Together, these results suggest that the 5-HT7 receptor may act as a potent modulator of BLA inhibitory transmission. Supported by National Science Centre, grant 2016/21/B/NZ4/03618.

## S3.3. PRENATAL STRESS IN RATS EFFECTS SYNAPTIC TRANSMISSION IN THE DORSAL RAPHE NUCLEUS OF THEIR ADOLESCENT OFFSPRING

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Prenatal maternal stress (PS) can adversely affect the development of the central nervous system in offspring. The effects of PS become evident later in life and may be involved in the pathogenesis of neurological and mental disorders. The dorsal raphe nucleus (DRN), as a major source of serotonin (5-HT) in the mammalian forebrain, plays a key role in the stress response. The DRN is also involved in the development of stress-related disorders. GABA-ergic and glutamatergic transmission in the DRN are modulated by the 5-HT7 receptor, however, little is known about the effects of PS on the activity of the DRN neuronal network. The aim of this study was to determine the effects of PS by analysing excitatory and inhibitory synaptic transmission, and its modulation by the 5-HT7 receptor, in the DRN of rat adolescent offspring of stressed rat dams. Pregnant

Sprague-Dawley rats were subjected daily to three restraint stress sessions, from the 14th day of pregnancy until birth. During each stress session, rats were placed in plastic cylinders and exposed to bright light for 45 min. Control pregnant females were left undisturbed in their home cages. The effects of PS were studied in slices of the DRN prepared from adolescent male offspring of control and stressed mothers. Whole-cell recordings were carried out from putative 5-HT neurons. Spontaneous excitatory (sEPSCs) and inhibitory (sIPSCs) postsynaptic currents were recorded to assess glutamatergic and GABA-ergic transmission, respectively. 5-CT, in the presence of WAY 100635, was applied to the ACSF to selectively activate the 5-HT7 receptor. In prenatally-stressed rats an increased frequency of sEPSCs and a decreased frequency of sIPSCs were evident, compared to control animals. In slices originating from control rats, activation of the 5-HT7 receptor resulted in a decrease in the mean frequency of sEPSCs and an increase in the mean frequency of sIPSCs. These effects were absent from slices obtained from prenatally-stressed rats. These results suggest that prenatal maternal stress in rats causes an enhancement of glutamatergic transmission and an attenuation of GABA-ergic transmission and affects the function of the 5-HT7 receptor in the DRN of their adolescent offspring. These effects may be related to prenatal stress-induced abnormalities in the functioning of the serotonergic system. Support: This study was supported by grant 2015/17/N/NZ4/02455, National Science Centre Poland.

## S4. NEURONAL PLASTICITY

### S4.1. SYNAPTIC PLASTICITY IN THE CENTRAL AMYGDALA DURING ADDICTIVE AND NATURAL LEARNING

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Drug addiction has been proposed as a form of Hebbian learning, as it creates changes in neural networks by strengthening or weakening some synapses. Similar changes occur during natural reward learning and it is believed that they are stored as encoded information-engrams. It has been suggested that drugs of abuse hijack these engrams to create extremely durable forms of memories. Therefore, in this study, we aimed to test for similarities between initial exposure to an addictive substance and a natural form of learning. As a model of "addictive" learning we chose intraperitoneal (IP) cocaine injections, while sucrose self-administration served to represent a natural form of learning. To distinguish different neuronal populations, transgenic mice with labeled GABAergic neurons were used. All experiments were performed with contradistinction between inhibitory and excitatory neurons. A series of electrophysiology experiments were performed on a specific brain pathway: the connection between posterior basolateral amygdala (pBLA) and the central medial amygdala (CeM). This pathway was recently shown to process positive memories. To ensure pathway specificity, viruses were injected into pBLA allowing for channelorodopsin2 expression in neurons. Synaptic changes were tested by whole-cell patch clamp electrophysiological recordings with the use of optogenetic stimulation. Results from electrophysiological recordings were confirmed by confocal microscopy. For this purpose brain slices were immunolabeled with an antibody against c-Fos protein, which is a marker of neural plasticity. Our results indicated that indeed both cocaine IP injection and sugar administration changed the pBLA-to-CeM pathway in the same manner. In these structures we observed generation of silent synapses—immature synaptic contacts. Silent synapses contain mostly NMDA receptors (and not AMPA receptors) and may function as substrates for increased learning. Expression of c Fos protein also indicated that both sugar and cocaine contributed to structural changes in neurons in CeM. Additionally, these changes were independent of cell-type (inhibitory or excitatory). Thus, drug exposure affects a pathway that processes positive memories and engages similar neurons that natural learning does. Our results shed light on the debate surrounding of addiction as a form of simple, appetitive learning.

### S4.2. LONG-TERM GABAERGIC SYNAPTIC PLASTICITY IN HIPPOCAMPUS STRONGLY DEPENDS ON THE ACTIVITY OF MATRIX METALLOPROTEINASE-3

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It is well established that matrix metalloproteinases (MMPs) play an important role in mechanisms of excitatory plasticity, learning, and memory, especially those dependent on hippocampus. Recently, we have demonstrated that MMP-9, but not MMP-3, is involved in spike timing-dependent plasticity in mouse barrel cortex, and that MMP-3 supports NMDA-dependent LTP in the hippocampus. However, the contribution of these enzymes to GABAergic plasticity has not been investigated. To address this issue, we recorded miniature inhibitory postsynaptic currents (mIPSC) in acute hippocampal slices (P18-P21) and induced inhibitory LTP (iLTP) using NMDA treatment (3 min, 20 μM) in control conditions and in the

presence of MMP inhibitors: FN-439 (180 μM), SB3-CT (10 μM) and UK356618 (2 μM). Additionally, we performed immunostaining (against gephyrin and vGAT) of cultured hippocampal neurons and examined the level of MMP-3 using Western blot in hippocampal slice homogenates after iLTP. We have shown that, in control conditions, activation of NMDA receptor significantly potentiated amplitude (122±8%) and prolonged decay kinetics (125±7%) of mIPSC and also increased pro-MMP-3 levels (116±4%). Application of pan-MMP inhibitor (FN-439) prevented induction of iLTP (CTR: 122±8%, n=7; FN-439: 98±6%, n=7; $p<0.05$). Interestingly, MMP-3 inhibitor treatment (UK356618) blocked iLTP, but MMP-9 inhibitor (SB3-CT) had no effect on iLTP (UK356618: 92±3%; n=7, $p<0.05$; SB3-CT: 121±12%, n=6, $p>0.05$; in comparison to CTR: 122±8%, n=7). Thus, our data show that MMP-3, but not gelatinases, supports iLTP. Moreover, in the hippocampal slices from mice lacking the Mmp-3 gene (MMP-3 KO) iLTP is also affected by MMP-3 deficiency (CTR: 122±6%, n=8; MMP-3 KO: 99±4%, n=13; $p<0.05$). Intriguingly, we ascertained that in this model the decay kinetics of mIPSCs were significantly slowed down with respect to control measurements (CTR: 14.81±0.61 ms, n=11; MMP-3 KO: 18.31±0.99 ms, n=12; $p<0.05$). Similarly, iLTP was impaired in the MMP-3 KO group in hippocampal neuronal cultures. In addition, we observed a significant increase in synaptic gephyrin cluster area after iLTP (120±3%), but not after UK356618 treatment (99±3%) in neuronal cultures. Taken together, these data reveal that GABAergic LTP depends on extracellular proteolysis mediated by MMP-3. Supported by Polish National Science Centre grant OPUS/2014/15/B/NZ4/01689 and OPUS/2013/11/B/NZ3/00983.

## S4.3. LEARNING INCREASES INTRINSIC EXCITABILITY OF NEOCORTICAL SOMATOSTATIN-EXPRESSING INTERNEURONS

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Changes in excitability of excitatory neurons, as well as strengthening of excitatory synapses, have been postulated to underlie learning and memory mechanisms. The GABAergic system is also plastic, however, the mechanisms of plasticity in inhibitory systems are poorly understood, especially considering the diverse nature of inhibitory interneurons. There are three main groups of inhibitory interneurons in the neocortex: somatostatin (SOM)-, parvalbumin (PV)-, and vasoactive intestinal polypeptide (VIP)-expressing interneurons. The aim of our study is to analyse the effect of learning on the activity of SOM-expressing interneurons, which have been implicated in state-dependent modulation and experience-dependent plasticity, and with activity regulated by neuromodulators. In our experiments, we used a simple model of sensory learning, where mice were subjected to a conditioning paradigm ingthat consisted of pairing tactile stimulation of whiskers with an electrical tail shock. Previous studies have shown that this paradigm results in an expansion of the cortical representation of stimulated vibrissae and in an increase in GABAergic transmission. Here, using transgenic mice with SOM interneurons genetically tagged with red fluorescent marker, we performed in vitro whole-cell patch-clamp recordings in slices of naïve and trained mice. We analysed basic electrophysiological properties and excitability of SOM cells located in layer IV of the representation of the "trained" whiskers in the barrel cortex. In addition, spontaneous excitatory (sEPSCs) and inhibitory (sIPSCs) postsynaptic currents in SOM cells were recorded. In agreement with the literature, we found two main groups of SOM interneurons in layer 4: low-threshold spiking and irregular spiking. After the learning paradigm, the excitability of low-threshold spiking SOM interneurons increased. There were no differences in either the amplitude or the frequency of sEPSCs (and IPSCs) in SOM cells between groups. These data indicate that sensory training results in a selective and long-lasting enhancement of SOM interneuron activity due to changes in their intrinsic excitability. Hence, this study builds upon a growing body of literature suggesting that increases in inhibition are a common and important mechanism of learning and memory.

# S5. NEURONAL OSCILLATIONS

## S5.1. CELLULAR CORRELATES OF KAINATE-INDUCED POSTERIOR HYPOTHALAMIC THETA RHYTHM IN VITRO

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Hippocampal formation (HPC) theta rhythm is known to be one of the most synchronized EEG patterns in mammals. Theta field potentials in the HPC of rats are high-amplitude, almost sinusoidal, waves in a 3-12 Hz frequency range. It is well-known that the posterior hypothalamic area (PHa including the supramammillary nucleus and posterior hypothalamic nuclei) is an important node in the pathway of HPC theta generation, i.e. the ascending brainstem-hippocampal synchronizing pathway. Furthermore, HPC theta frequency is at least partially modulated by the PHa through the

activity of neurons firing in the frequency of HPC theta, at least during animals' immobility-related behaviors. The PHa is thought to complement the activity of the medial septal area, widely known as the pacemaker of HPC theta rhythm. However, in our previous studies we discovered for the first time that cholinergic theta rhythm can also be recorded locally in deafferented posterior hypothalamic slices. Hence, in the present study we investigated PHa-recorded theta-related single cell activity in relation to local theta rhythm following kainic acid administration. 36 in vitro experiments were performed using brain slices (=72) taken from 36 adult Wistar rats. Each slice was perfused with 0.1 μM kainic acid to induce rhythmic activity and neuronal firing. Both field activity and corresponding cellular activity were recorded extracellularly. The relation of neuronal firing patterns to local field theta rhythm was investigated according to an existing universal classification of HPC theta-related neurons. This study resulted in recording 17 theta-related neurons and 77 neurons classified as non-related to local theta rhythm. A new neuron type (=21 cells) has been identified amongst the non-related group, which we termed timing cell, with a very rhythmic firing pattern in a nearly fixed frequency in the theta band. Kainate-induced neuronal activity, recorded in the posterior hypothalamic area in vitro, resembles cholinergically-induced PHa neuronal activity, as well as well-documented patterns of theta-related cell discharges in the hippocampal formation in vitro and in vivo. Newly discovered PHa timing cells are discussed in light of an HPC theta rhythm frequency control mechanism. Supported by National Science Centre, Poland, No. UMO-2017/25/B/NZ4/01476.

## S5.2. HIPPOCAMPAL THETA RHYTHM AS A BIO-INDICATOR OF THE EFFICIENCY OF VAGAL NERVE STIMULATION

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Vagal nerve stimulation (VNS) refers to any technique that stimulates the vagal nerve, including manual or electrical stimulation. Approved by the FDA at the end of the 20th century, VNS was initially used as an add-on treatment for medically refractory epilepsy. Today, VNS has also been studied as a treatment for mood and cognitive disorders, such as major depression, bipolar disorder, and Alzheimer's disease. Although VNS requires an invasive surgical procedure, this technique is increasingly widespread in medical practice. Recently, we have demonstrated that hippocampal (HPC) theta rhythm can be produced, depending on current intensity, directly during vagal nerve stimulation (VNS) or post-stimulation. This suggests that theta EEG pattern can be used as a bio-indicator of the efficiency of VNS. In the present study, we focused on three specific technical issues related to the stimulation procedure of the vagal nerve: 1) Does the type of electrode used for VNS and the technique of its implantation affect the parameters of the HPC theta rhythm? 2) Does the type of electrode used determine the current intensity threshold of VNS-induced HPC theta? 3) Is the repeatability of the VNS effect determined by the type of electrode used? In rats (male Wistar) with an implanted tungsten microelectrode (0.1–0.9 MΩ) for recording the HPC field activity, VNS was applied using two types of stimulating electrodes: tungsten bipolar fork electrode and platinum-iridium cuff electrode. The first type of stimulating electrode touched a vagal nerve surface only during the stimulation and the second type surrounded the nerve and has contact with it throughout the entire experiment. During experiments the following VNS intensities were tested: 1, 2, 4, 6, 8, and 10 mA. The remaining parameters were constant: pulse duration (1 ms), train duration (10 s), and frequency (10 Hz). A direct (brief) effect of VNS on the HPC field potential was evaluated. We demonstrated that using the cuff electrode for VNS offers a lower current intensity threshold for inducing HPC theta than using a fork electrode. Furthermore, in contrast to a fork electrode, the cuff electrode offers repeatability of the VNS effect on HPC theta activity. The final effect of VNS is determined by many factors including the stimulation protocol and type of stimulating electrode used. This work was supported by RPO grant (RPLD.01.02.02-10-0067/17-00).

## S5.3. THE OLFACTORY BULB GENERATES AND IMPOSES KETAMINE-ASSOCIATED HIGH FREQUENCY OSCILLATIONS IN THE VENTRAL STRIATUM OF RODENTS

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Over the past decade, high frequency oscillations (HFO, 130-180 Hz) recorded in field potentials have been shown to be robustly potentiated by ketamine administration. This rhythm has been recorded in functionally and neuroanatomically diverse cortical and subcortical regions, most notably in the ventral striatum. However, the precise locus of generation remains largely unknown. There is compelling evidence that olfactory regions can drive oscillations in distant areas. Here, we

tested the hypothesis that the olfactory bulb (OB) exerts a top-down role in the generation of ketamine-HFO. We examined the effect of ketamine on electrophysiological activity of the OB and ventral striatum in vivo. Field potential recordings, local inhibition, naris blockade, current source density and unit recordings were used. Ketamine-HFO in the OB was larger and preceded HFO recorded in the ventral striatum. Granger causality analysis was consistent with directional flow from the OB. Unilateral local inhibition of the OB, and naris blockade, attenuated HFO recorded locally and in the ventral striatum. Within the OB, current source density analysis revealed HFO current dipoles close to the mitral layer and unit firing of mitral/tufted cells was phase locked to HFO. Our results demonstrate a hierarchical top-down relationship between ketamine-HFO in the OB and the ventral striatum. The OB plays a primary role in the generation of ketamine-HFO and orchestrates this activity in a distant region. These findings provide a new conceptual understanding on how ketamine influences fundamental brain activity which may have implications for schizophrenia.

## S5.4. RELIABLE ESTIMATION OF CURRENT SOURCES FROM MULTIELECTRODE LFP RECORDINGS WITH KERNEL CSD AND L-CURVE

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Passive propagation of electric fields can induce apparent coherence in local field potentials (LFP) recorded over distances of several millimetres hindering their analysis. This issue can be overcome with current source density analysis (CSD). Mathematically, CSD reconstruction is an ill-posed problem which means that many different possible current source distributions fit the measured LFP and the challenge is to find the most probable one. Furthermore, LFP recordings are always noisy, particularly in data obtained from freely moving animals, which may affect CSD estimation. Previously, we proposed the kernel CSD (kCSD) method for reconstruction of the spatial distribution of sources and sinks in biological tissue from noisy data. Here we show how the method parameters can be estimated quickly and reliably using an L-curve approach. We demonstrated the feasibility of this approach on model data and illustrated its power in the analysis of LFP recordings from linear probes implanted in the olfactory bulb (OB) of freely moving rats. We focused on ketamine-induced high frequency oscillations (HFO, 120-200 Hz) since, to date, the locus of generation of HFO remains unclear. kCSD is a model-based CSD estimation method which assumes a flexible model of CSD and estimates its parameters from data. L-curve is a technique for finding the optimal way of weighting the complexity of the model against the difference between model predictions and the actual set of measurements. The LFPs we analysed were recorded from freely moving rats implanted with a 32-channel linear probe targeted to the OB. Recordings were made at baseline and post injection of 25 mg/kg ketamine (i.p.). To examine the faithfulness of kCSD reconstruction we tested this method on model LFPs from ground truth data. We showed that the L-curve provides reliable and practical estimation of regularization parameters for robust kCSD estimation of sources from noisy LFPs. After validating this method, we estimated the current sources from recordings in the rat OB. We found HFO dipoles close to the mitral layer, whereas above it there was little evidence of any phase reversal. kCSD with L-curve is a robust method for estimation of current sources from noisy data. It facilitates localization of the sources of abnormal HFO activity to a specific layer within olfactory bulb which is consistent with histology.

# S6. NEURONAL SYSTEMS

## S6.1. RETINAL OR CORTICAL? WHICH INPUT TO THE RAT DORSAL LATERAL GENICULATE NUCLEUS IS MORE 'IMPORTANT'?

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The thalamic dorsal lateral geniculate nucleus (dLGN) serves as a gateway for light information transfer en route to the primary visual cortex (V1). Although the nonretinal modulatory input arising from the deep layer of V1 to the dLGN is well characterized, little is known about its influence upon dLGN activity under brain state dependent changes. Urethane anesthesia provides a powerful tool for acute in vivo studies, which allows for the observation of cyclic alternations of REM- and NREM-like stages during electrocorticographic (ECoG) recording, resembling natural sleep. Our study aimed to investigate the nature of spontaneous neuronal activity and stimulus responsiveness of the rat dLGN under alternating sleep-like phases with intact and silenced V1. Extracellular multi-unit activity of the dLGN and cortical ECoG signals were recorded in vivo from 48 adult male Long Evans rats under urethane anaesthesia. All recordings were performed under dark conditions and were combined with white light stimulations and V1 muscimol application. First, we described

different relationships between single-unit dLGN activity and brain state alternations: neurons led by ECoG, neurons whose spike rate preceded ECoG alternations, and neurons correlated and not correlated with ECoG changes. Silencing cortical input altered relationships with ECoG in all groups, however the most prominent changes were observed in cells where firing rate preceded ECoG changes. In terms of light-induced activity, we found that the amplitude of light responses did not change between cortical phases before and after muscimol application. However, the type of response was modulated in 25% of neurons, both by the brain state alternations and cortical muscimol application. We demonstrated that spontaneous activity of rat dLGN cells varies in a state-dependent manner and can be altered by silencing V1. On the other hand, in a majority of cells the amplitude of light-induced responses remained constant, suggesting that retinal input has priority over non-retinal cortical influences. Supported by: 2013/08/W/N23/00700, K/DSC/004648.

## S6.2. THE COMBINATION OF ELECTROPHYSIOLOGY AND OPTOGENETICS FOR THE IN VIVO STUDY OF THE INFLUENCE OF NUCLEUS INCERTUS ON THE ACTIVITY OF MIDBRAIN DOPAMINERGIC NEURONS IN THE RAT

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The in vivo preparation is commonly used to study electrophysiology of brain circuits. Since the whole brain network is preserved, electrical activity of one brain region can be observed while the other region is being manipulated (e.g. stimulated or inhibited) in order to determine and characterise connectivity between these regions. The recently discovered tool of optogenetics is useful for controlling neuronal activity, characterized by high specificity to neurons as well as high temporal resolution. We used this tool to determine if neurons located within the nucleus incertus (NI), a population of brainstem central gray GABAergic neurons involved in stress response, have an impact on the electrical activity of midbrain dopaminergic neurons located within the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). To prepare the tested neuronal circuit for optogenetic manipulations, NIs of Sprague Dawley rats were stereotaxically injected with adenoviral associated vector (AAV2-hSyn-hChR2(H134R)-eYFP) containing genes for Channelrhodopsin-2 (ChR2; a blue light-sensitive cation channel) and enhanced yellow fluorescent protein (eYFP), which are expressed under control of neuron specific promoter (human synapsin 1). Two weeks after the operation, when ChR2 and eYFP are fully expressed, in vivo electrophysiological experiments were performed on urethane anaesthetised animals. Dopaminergic neurons within the VTA and SNc were recorded, while NI was stimulated using blue laser light (473 nm, 10–20 mW) led to the tissue using fibre optics. After each experiment the expression of eYFP in the NI, optic fibre placement, as well as the localisation of recording electrode within the borders of VTA/SNc were histologically verified. The results revealed that most (59%) of the midbrain dopaminergic neurons were strongly inhibited by the optogenetic activation of the NI. NI-induced inhibition was followed by rebound excitation in the majority (69%) of responsive neurons. Additionally, numerous eYFP-positive axons originating from the NI were observed within the VTA and SNc. In conclusion, our results show that NI is a source of strong, presumably direct, inhibitory input to the midbrain dopaminergic system. Optogenetic tools can be used to control the activity of neurons with high temporal and spatial resolution, both in transgenic and wild-type animals.

## S6.3. ELECTROPHYSIOLOGICAL CHARACTERIZATION OF RAT NUCLEUS INCERTUS NEURONS – IN VIVO STUDIES USING MULTI-CHANNEL RECORDING TECHNIQUE

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The nucleus incertus (NI) is a brainstem structure formed of GABAergic projection neurons. It is located in the dorsal tegmental pons, below the fourth ventricle. Axons of NI neurons innervate numerous brain regions, including the septo-hippocampal system. Previous studies have shown that the NI is one of the key elements involved in the induction of hippocampal theta oscillations. More recently, theta oscillations in the local field potential of the NI were described. Nevertheless, the electrophysiological characteristics of NI neurons and the involvement of the NI in the mechanisms of theta rhythm generation are unclear. Therefore, the aim of our research is to determine the comprehensive classification of NI neurons in relation to hippocampal theta oscillations. We have performed in vivo electrophysiology experiments on 12 urethane anaesthetised Sprague Dawley rats. Under this anaesthetic condition one can observe spontaneous cyclical alternations of brain states (activation and slow wave activity; SWA), characterized by the dominance of different EEG waves (theta and delta oscillations, respectively). Neuronal activity was recorded extracellularly using a 32-chan-

nel recording system in combination with acute microelectrode arrays. Theta rhythm and slow wave activity were recorded from stratum lacunosum-moleculare of hippocampal CA1 field. Our results have revealed that electrical activity of NI neurons (n=147) is brain state dependent. Based on the preference to fire in a specific phase of hippocampal theta rhythm, two main groups of NI neurons could be distinguished: theta phase-locked cells (45%, 66/147) and theta phase-independent cells (55%, 81/147). a majority of theta phase-locked NI neurons fired action potentials in bursts occurring at the rising phase of hippocampal theta oscillation (theta bursting neurons; 68%, 45/66). Firing rate of theta phase-locked neurons was higher during brain activation compared to SWA state. Firing of theta phase-independent NI neurons was more heterogeneous and included cells with higher firing rates either during theta oscillations or SWA. Using the multi-channel recording technique, we have shown that the patterns of NI neuronal activity are more complex than previously described. The resulting in-depth electrophysiological characterization of NI neurons help us to better understand the mechanisms underlying the formation and synchronization of theta oscillations. Funding: NSC, Poland UMO-2014/15/B/NZ4/04896.

### S6.4. RETINAL SHEET TRANSPLANTATION IN RATS WITH RETINAL DEGENERATION RESTORES VISUAL CORTICAL RESPONSES

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Age related macular degeneration and retinitis pigmentosa lead to a profound loss of vision in millions of people worldwide. Many of these patients lose both retinal pigment epithelium (RPE) and photoreceptors. Fetal derived retinal progenitor sheets have been successfully transplanted into both rodents (review: Seiler and Aramant, 2012 Prog Retin Eye Res, 31:66187) and humans (Radtke et al, 2008 Am J Ophthalmol, 146:172182). In several models of retinal degeneration (RD), transplants restore rudimentary responses to flashes of light in a region of the superior colliculus (SC) corresponding to the location of the transplant in the host retina; and synaptic connectivity between transplant and RD host retina has been confirmed. However, in order to determine the quality and accuracy of visual information provided by the transplant, here we study visual responsivity at the level of visual cortex where higher visual perception is processed. Specifically, we used the transgenic Rho S334ter3 RD rat, which begins to lose photoreceptors at an early age, becoming blind shortly after one month postnatal. Between 24-40 days of age, RD rats received fetal rat retinal sheet transplants in one eye. Donors were rats expressing human placental alkaline phosphatase in all cells. Three to ten months following surgery, we found several neurons in the region of primary visual cortex (V1) matching the transplanted portion of the retina that were well tuned to stimulus orientation, size, contrast, and spatial and temporal frequency. Each of these response features are considered fundamental properties of V1 neurons that are necessary building blocks for higher level visual processing, such as shape and motion perception. In addition, we find that these response properties are absent in nontransplanted and sham transplanted RD rats, but are on par with normal age matched controls that do not suffer from RD. Moreover, in rats with normal retinas and RD rats with retinal transplants spontaneous firing rates were low, whereas in the RD rats without transplants spontaneous firing rates were often higher, indicating abnormal function in the absence of visual input. In conclusion, our data thus far indicate that fetal rat retinal sheet transplants can restore visual cortical responses in transgenic RhoS334ter3 RD rats. This restoration of 'normal' cortical physiology in a rat model represents a critical step towards developing an effective remedy for the visually impaired human population.

## S7. HUMAN COGNITION

### S7.1. TIME-FREQUENCY ANALYSIS OF AUDITORY WORKING MEMORY

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Working memory (WM) is defined as a cognitive system with a limited capacity that is responsible for temporarily holding task-relevant information (Sreenivasan et al., 2014). It is hypothesized that WM recruits the same brain areas that process sensory information. Huang et al. (2016) found, in a carefully crafted experiment that enabled the separation of activity related to working memory engaged in remembering tones from activity related to other mental processes, that there is an enhanced sustained field type activity during a high load task in sources seeded in the auditory cortex. The aim of the current study was to further analyse MEG data obtained from these auditory WM experiments for possible correlates of high WM load for tones. Specifically, we were interested in

markers of auditory WM in the time-frequency domain on the level of individual MEG sensors, especially those with a strong signal from the auditory cortex. We analysed 2 sec long epochs of signal from the delay period between two sounds under two different WM load conditions. We analysed contrast time-frequency maps with cluster-based extreme value statistics. The methodology and results are presented. References: Sreenivasan KK, Curtis CE, D'Esposito M. Revisiting the role of persistent neural activity during working memory. Trends in Cognitive Sciences. 2014; 18: 82–89. Huang Y, Matysiak A, Heil P, König R, Brosch M. Persistent neural activity in auditory cortex is related to auditory working memory in humans and nonhuman primates. King AJ, ed. eLife. 2016; 5: e15441.

## S7.2. STRATEGIES OF ANTICIPATORY ATTENTION

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Anticipatory attention is a downstream modulation of brain activity, directed towards facilitating the processing of upcoming stimuli. We studied anticipatory attention in a visual task comprised of two types of cues announcing a difficult (visual search of 16-element matrix) attentional task versus an easy motor response. The pattern of behavioural responses revealed existence of two distinct subpopulations of subjects: working under time-pressure (77% "fast-responders") and delaying the response (23% "slow-responders"). As predicted by the speed-accuracy trade-off, fast-responders performed worse than slow ones. A similar poor performance was also observed in a subgroup of fast participants that matched slow ones in the time spent on providing response in attention trials. Thus, the sole motivation to respond as fast as possible changed performance in our task. Both groups were significantly better in trials preceded with longer cues, indicating active preparation during this period. However, the implemented anticipation mechanisms were different in both groups. Fast-responders during the anticipation period exhibited significant top-down modulation of alpha power, which was positively related to their performance. No such relation was observed in the slow-responders. In contrast, slow responders expressed significant top-down modulation of contingent negative variation (CNV) potential, previously described as related to subjective assessment of time flow. The power of CNV was positively correlated with behavioural performance only in the slow-responding group. Different anticipatory mechanisms resulted in different performance in the visual search task, during which slow-responders exhibited larger P300 amplitude. The power of P300 correlated positively with performance only in the slow-responders.

## S7.3. EEG ESTIMATES OF ATTENTIONAL CONTROL DURING MODIFIED MULTI-SOURCE INTERFERENCE TASK (MSIT+)

Dzianok P[1], Antonova I[1], Wojciechowski J[1,2], Rogala J[1,3], Kublik E[1]

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Multi-Source Interference Task (MSIT) is frequently used in fMRI research to robustly activate the cingulo-fronto-parietal attention network (CFP). MSIT contains two stimuli conditions: Easy, spatially congruent with no flanker distractors (EC), and Hard, with multi-source interference, i.e. incongruent locations and flanker distractors (HI). In order to gradually increase MSIT difficulty and possibly observe progressing engagement of CFP network, we expanded it with two intermediate conditions: Easy with Incongruent (EI) digit locations (introduced for the first time); and Hard, with flanker distractors but Congruent digit locations (HC). We used high-density EEG (128 active electrodes) to study the dynamics of brain activity during MSIT+. Pilot EEG and behavioral (reaction times, RT) data were acquired from six participants. Gradual increase of RT was observed between four MSIT+ conditions, with flanker-interference requiring more time than spatial incongruence. Power spectral analysis using Welch method performed on z-scored EEG data revealed significantly larger theta (4-8 Hz) power in both incongruent versus congruent conditions, with the largest theta power observed for the most demanding HI trials. Inverse results were found for alpha power (8-13 Hz), which was stronger in both congruent conditions in contrast to incongruent ones (highest alpha in easiest EC trials). Spectral analyses were repeated for electrode clusters: Frontal, Central, Parietal, Occipital, Posterior Temporal Left and Right, and Anterior Temporal Left and Right. Significant differences were also found for alpha and theta power: the strongest theta power was observed for Frontal cluster followed by Occipital electrodes. For alpha power, the strongest results were found for Occipital and Frontal clusters. Our results are in line with earlier EEG MSIT literature and with established theory of midline frontal theta being a neural signature of anterior cingulate cortex activation during conflict processing with its occurrence and power related to increasing task difficulty. Observed suppression of alpha in the more demanding condi-

tions also corroborates the earlier proposal that alpha power seems to be inversely related to increased attention demand. Observed discrepancies between behavioral and EEG results requires further study. Supported by National Science Centre, Poland, UMO-2016/20/W/NZ4/00354.

## S7.4. AMPLITUDE OF THE RESTING STATE BETA BAND OSCILLATIONS PREDICTS ERP CHARACTERISTICS AND BEHAVIOURAL PERFORMANCE DURING ATTENTION TASKS

Rogala J, Kublik E, Wróbel A

*Nencki Institute of Experimental Biology, Warsaw, Poland*

Growing amounts of data indicate relationships between resting state EEG and the cognitive functions of healthy subjects or symptoms of neurological disorders/dysfunctions in clinical investigations. To investigate these correlations we designed an experiment in which participants were grouped on the basis of their resting state EEG spectral power and tested for differences in event related potentials and behavioural performance during repeated tasks addressing attention processes. 33 healthy adults were tested twice (TEST and RETEST, two months apart) with top-down and bottom-up visual attention tasks. EEG was recorded during tasks and preceding resting state sessions. Analyses included: correlation between reaction times (RTs) and resting state EEG powers in theta, alpha, beta 1, and beta 2 bands, and two-way ANOVA analysis of the RTs and amplitudes of contingent negative variation (CNV) in TEST and RETEST of the two subgroups defined by highest and lowest resting state amplitudes. Only the beta 2 band power correlated with RTs measured in top-down and bottom-up attention tasks. Subjects with the lowest beta 2 resting state amplitudes were characterized by shortening of RTs and increasing amplitudes of the CNV wave in RETEST as compared to TEST. These findings posit a link between individual resting state brain activity in the beta 2 range and susceptibility to long-term changes in the functional processing of visual stimuli. Supported by the National Centre for Research and Development grant POIR-01.01.01-00-178/15 and Polish National Science Centre grant UMO-2016/20/W/NZ4/003554.

## S7.5. UNCONSCIOUS DETECTION OF ONE'S OWN IMAGE

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The question of whether unconscious processing is involved in the detection of one's own image has yet to be answered. In recent studies, an automatic shift of attention toward this stimuli was shown. Here, based on a theoretical framework of bottom up visual selection, we predicted the emergence of N2pc component (neural marker of attentional shifts) in conditions where conscious identification of one's own face was precluded. This hypothesis was tested in a dot-probe paradigm with masked and unmasked pairs of faces (other and self) coupled with electrophysiological (EEG) recording. The validity of the masking procedure was verified by a sensitivity measure (d'), $t(17)=1.57$, $p=0.135$, 95% CI=[-0.07, 0.5], BF=0.68. A clear N2pc was found in both masked ($t(17)=-2.34$, $p=0.031$, 95% CI=[-0.48, -0.03], d=-0.55, BF=4.07) and unmasked ($t(17)=2.91$, $p=0.01$, 95% CI=[-0.87, -0.14], d=-0.67, BF=10.71) tasks, which indicates automatic allocation of attention towards self-face in both unconscious and conscious conditions. This supports the notion that the self-recognition process has a strong unconscious component and sheds new light on the ongoing debate regarding the dissociative nature of attention and consciousness.

# S8. EEG ANALYSIS AND PRACTICAL APPLICATION

## S8.1. PROPERTIES AND EFFICACY OF A NOVEL METHOD FOR ASSESSING PHASE TO AMPLITUDE CROSS-FREQUENCY COUPLING

Bernatowicz G, Żygierewicz J

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Recent studies indicate that coupling between low- and high-frequency (e.g. theta and gamma) brain rhythms provides valuable information on cognitive processing in humans. The purpose of this study was to examine the properties and efficacy of a novel method of assessment of phase to amplitude cross-frequency coupling. The proposed method is based on analysis of time-frequency representation of signals aligned to a given phase in the low-frequency band. Low frequency wave is obtained with Matching Pursuit algorithm by selecting waveforms of interest. The time-frequency representation of a signal's energy density is derived from the continuous wavelet transform, and normalized at each frequency relative to its average value in the baseline period. Next, the representation is thresholded at values obtained from surrogate data. The resulting maps are used to compute comodulograms. The effects presented in the comodulograms are validated with extreme values statistics. The method was tested on synthetic signals. The first signal represents

proper phase to amplitude cross-frequency coupling. It consists of a low-frequency sine (in the range of theta rhythm frequencies) with superimposed spindles of high-frequency (from the gamma band range) and white noise. The second and third signals display epiphenomenal cross-frequency coupling, which originates from their time course. We found that the proposed method is robust for high noise levels, which suggests that it has sufficient sensitivity to detect the theta-gamma coupling as measured by high quality EEG or ECoG. Nonetheless, it is not immune to epiphenomenal cross-frequency coupling, which warns us against drawing conclusions from positive output.

## S8.2. EEG SPECTRAL FINGERPRINTS AS A METHOD FOR CLASSIFYING DIFFERENT REGIONS OF THE HUMAN BRAIN – INITIAL RESULTS

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During rest different brain structures are connected into distinguishable networks. Each region of interest (ROI) tends to preserve its own natural frequency. A long term goal of our team is to develop a reliable method which allows us to access milliseconds-level dynamic features of brain networks from EEG signal, so called EEG brain fingerprint. In 2016, Dr. Anne Keitel and Professor Joachim Gross proposed a method of clustering each ROI MEG source-reconstructed activity. 116 ROIs were selected according to the Automated Anatomic Labeling Atlas. As a result, they obtained a group-level spectral representation (referred to as "spectral fingerprints") of dynamic behavior of the human cortex and deep sources. It turned out that spectral fingerprint representation enables accurate ROI classifications. Moreover, using only functional data, the algorithm linked ROIs to clusters that correspond well to large-scale anatomical parcellations of the cortex. We have recreated and adapted this method to analyse resting-state EEG signal. We tested this approach on resting-state EEG data and compared with original results obtained on MEG data. Results show that, despite the difficulty of differentiating between ROIs using EEG data, performance was well above the chance level. Each ROI EEG activity was characterized by fewer numbers of clusters than ROI MEG activity and ROI similarities formed less clear images of brain networks. Nevertheless, the method is still promising and further development could lead us to identify new and reliable tools useful in scientific and clinical practice.

## S8.3. RECENT ADVANCES IN BRAIN-COMPUTER INTERFACES FROM BRAINTECH.PL

Chabuda A[1,2]*, Dovgialo M[1,2], Różański P[1,3], Wieteska M[1,4], Pawlisz M[1], Duszyk A[2,5], Żygierewicz J[2], Rudkiewicz T[6], Durka P[1,2]

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Brain Computer Interface (BCI) is a system that allows communication without the mediation of muscles, using only brain waves. This technology passed from science-fiction to the laboratory decades ago, but real world applications, in fields from gaming and military to assistive technologies and consciousness assessment, are still operating at the proof of concept level. To change this landscape, building on a strong academic background, BrainTech Ltd. (http://braintech.pl) is pursuing a project to create stable, robust, and usable BCI technologies, ready for the above mentioned real world applications. Software includes stable implementations of the major paradigms: P300 evoked potentials (visual and auditory), steady-state visual evoked potentials (SSVEP), and motor imagery. Features aimed at increasing productivity in both academic and practical applications include the "BCI Control Panel"(*), which helps either the experimenter or caregiver in the setup and online control of the BCI session by displaying, for example, electrode impedances and online performance. Several indicators like accuracy or information transfer rate can be stored together with the signal, facilitating offline scientific analysis. Hardware systems include: (1) comfortable headcap with water-based electrodes, offering high quality signal without application of conducting gel, which normally requires washing hair after each EEG session, (2) 8-channel 24-bit wireless EEG amplifier, offering online monitoring of electrode contacts and Gigaohm input impedance, either integrated into the headcap or offering connectors for standard EEG electrodes, (3) next generation of the "BCI Appliance"* – a dedicated hardware renderer for flexible stimuli for high-frequency SSVEP, first presented at CeBIT in 2012 as a base for the fastest BCI presented at this fair. (*"BCI Control Panel" and "BCI Appliance" are trademarks filed for protection to the Polish Patent Office). Demo of the discussed systems will be available in the presentation accompanying the Conference. The lecture will briefly discuss new research possibilities opened by the "BCI Appliance", comfort of application of the novel EEG headcap, and facilitation of both real world BCI applications and scientific research brought about by the presented software.

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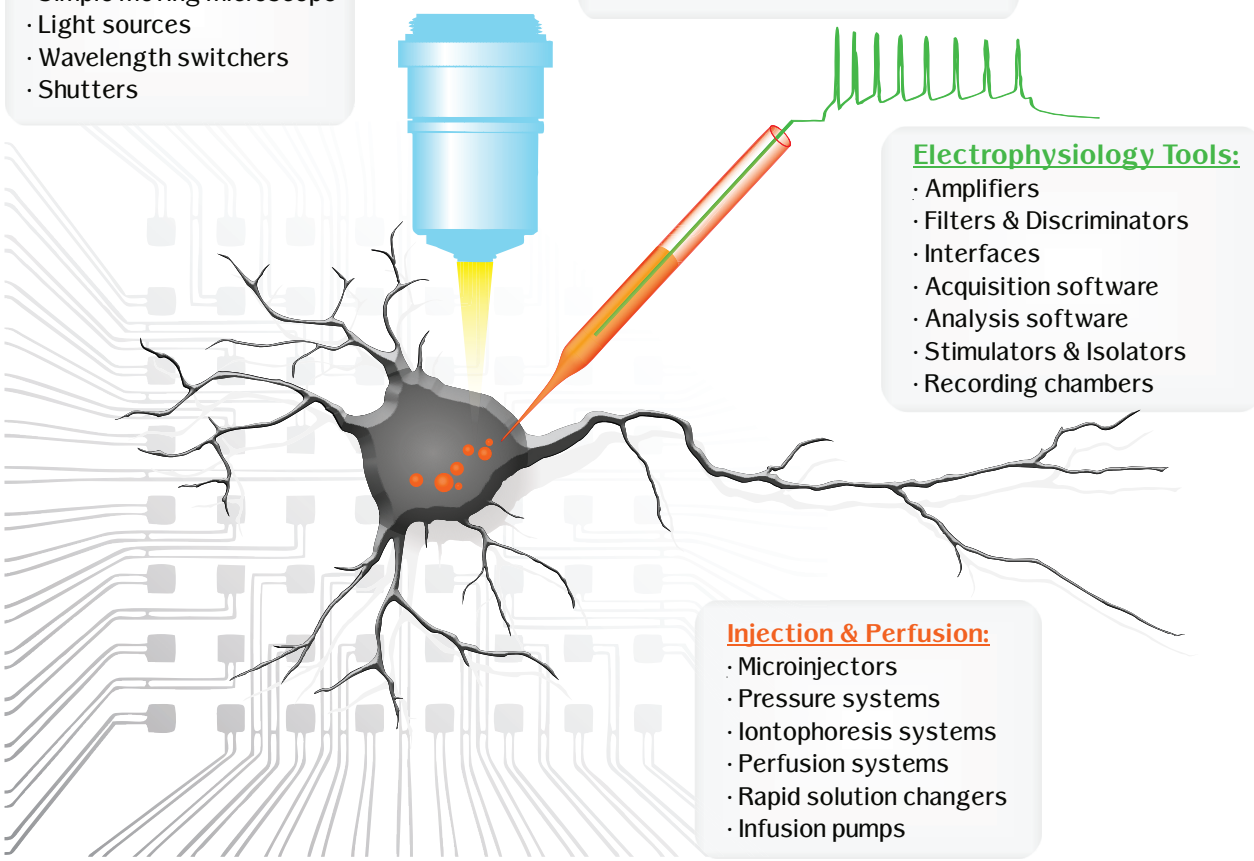

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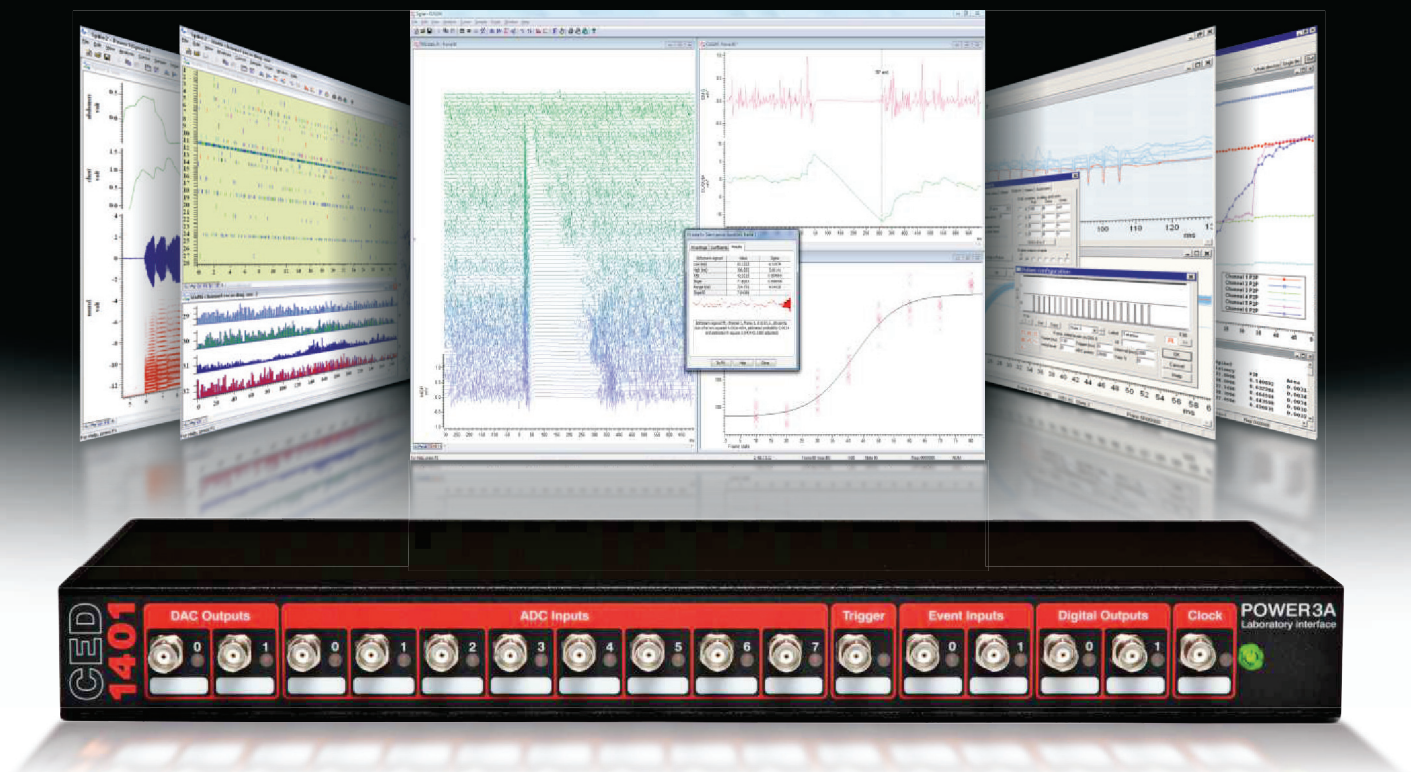


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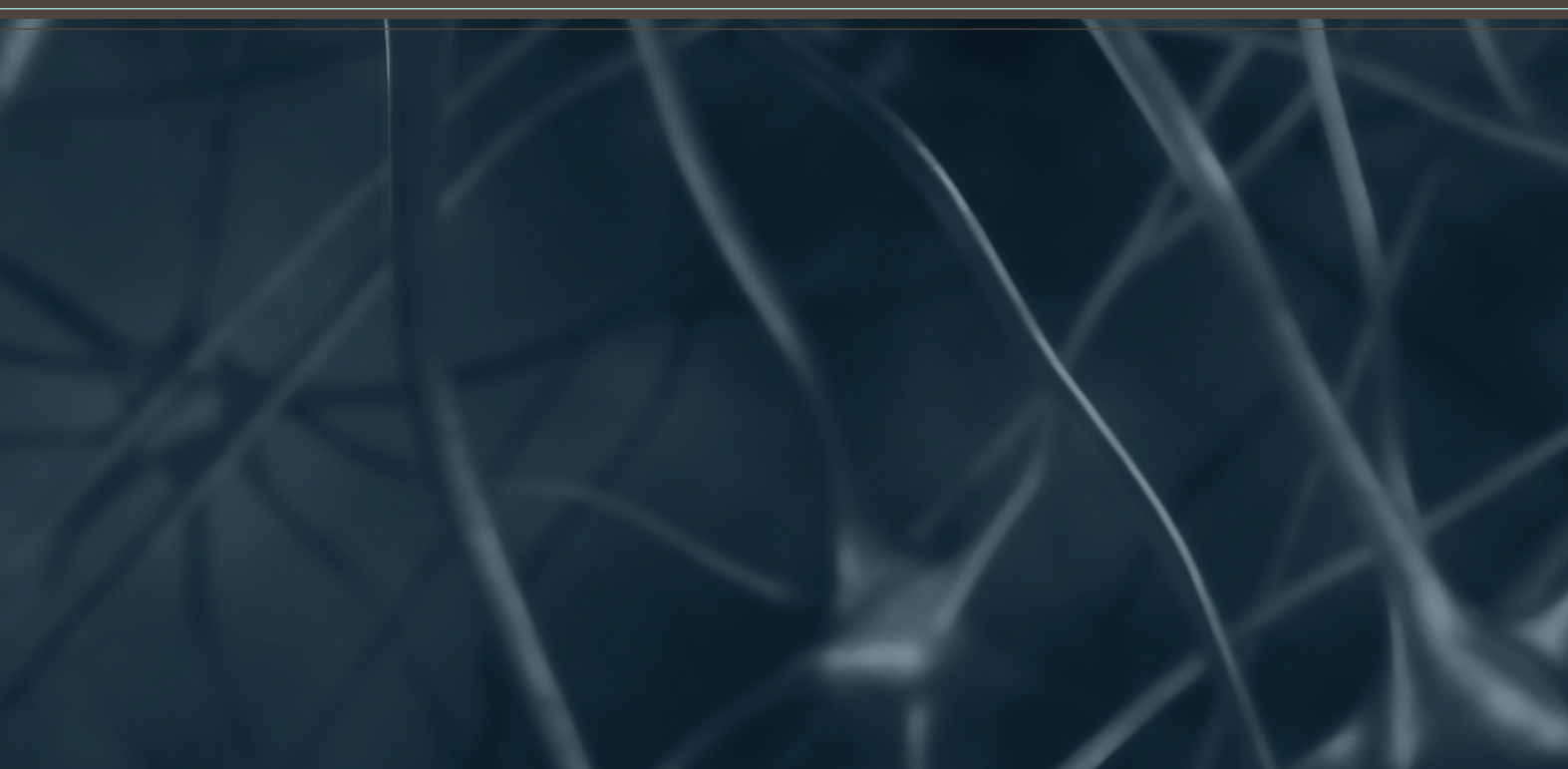

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