OM&P

Role of Hypothalamic Glycine Receptors in Regulation of Male Sexual Behavior in Rats

N.A. Titova [1], Z.D. Zhuravleva[1], M. Druzin[1,3], I.V. Mukhina[1,2]*

[1] Neurotechnologies Department, Lobachevsky State University Of Nizhny Novgorod, Russia;
[2] Department Normal Physiology named after N.Y. Belenkov, Central Scientific Research Laboratory, Nizhny Novgorod State Medical Academy, Russia;
[3] Department of integrative Medical Biology, Umeå University, Sweden.
* Presenting e-mail: yanadya.titova@gmail.com

Medial preoptic area (MPOA) in the anterior hypothalamus is one of the main brain structures involved in regulation of sexual behavior in all vertebrate species. As it was previously shown, various manipulations with MPOA have a great impact on sexual performance, especially in males. Thus, electrical or/and pharmacological stimulation of the MPOA neuronal circuits facilitates sexual behavior in males, whereas lesions or pharmacological blockade of the neuronal activity in the MPOA dramatically impairs sexual performance. Although the details concerning the contribution of the MPOA to the appetitive and consummatory stages of sexual behavior are still under discussion, it is generally accepted that normal functioning of the MPOA is required for the integrity of the male sexual behavior.

The vast majority of studies concerning sexual behavior are performed on rats. The MPOA in rats contains several nuclei including the medial preoptic nucleus (MPN) also known as sexually dimorphic nucleus of the preoptic area. Both morphological and functional organization of the MPN significantly differs between the genders underlying gender-specific patterns of sexual and parental behavior in rats.

As the MPN has multiple reciprocal and unidirectional connections with many different brain structures such as accumbens, caudate putamen, ventral pallidum, amygdala and numerous nuclei in hypothalamus, thalamus and brain stem, the MPN neurons are thought to integrate sensory and endocrine information and to constitute the main part of central neuronal circuitry that controls most aspects of sexual behavior.

Most of the published research concerns the involvement of glutamatergic, GABAergic, serotonergic and dopaminergic neurotransmission in regulation of male sexual behavior. However, little is known about the role of glycinergic neurotransmission despite the fact that glycine receptors are widely expressed in the MPN. Glycine receptors are structurally and functionally very similar to the ionotropic GABAA receptors, together they mediate most of inhibition in the CNS as both of them are permeable for chloride, which entry into the neurons causes neuronal hyperpolarization.

Our recent experimental data show an importance of glycine receptors in the MPN for the proper execution of sexual behavior in male rats as pharmacological manipulations of glycine receptor activity led to the changes in duration of copulatory behavior and affected the time of the postejaculatory inhibition of sexual activity. Thus, our findings provide the very first experimental evidence for the involvement of glycine receptors in control of male sexual activity. However, further experiments are required to elucidate the precise mechanisms of glycine receptor-mediated regulation of sexual behavior.

Acknowledgments

The research was supported by Federal Target Program ''Research and development in priority areas of the development of the scientific and technological complex of Russia for 2014 – 2020'' of the Ministry of Education and Science of Russia (Project ID RFMEFI58115X0016)

References

1. M. J.Baum, B. J. Everitt Increased expression of c-fos in the medial preoptic area after mating in male rats: Role of afferent inputs from the medial amygdala and midbrain central tegmental field // Neuroscience. 1992. № 50. P. 627–646
2. E.M. Hull, J.M. Dominguez Getting his act together: Roles of glutamate, nitric oxide, and damine in the medial preoptic area // Brain Research. 2006. C. 66–75.
3. Z.D. Zhuravleva, P.A. Denisov , V.S. Tovpiga, A.V. Lebedeva, A.B. Volnova, A.A. Mironov, I.V. Mukhina, M.Ya Druzin. The Role OF Medial Preoptic Area Glycinergic System In The Social Types Of Behavior Regulation, 2015
4. R.B. Simerly, L.W. Swanson, 1986. The organization of neural inputs to the medial preoptic nucleus of the rat. J. Comp. Neurol. 246, 312–342.
5. S. Maejima , N. Ohishi , S. Yamaguchi , S. Tsukahara. A neural connection between the central part of the medial preoptic nucleus and the bed nucleus of the stria terminalis to regulate sexual behavior in male rats, 2015

Adenoviral Vector Expression in Human Astrocytic Glioma Cells and Primary Astrocytes Monocultures

N.V. Ponomareva, S. A. Tutukova, E.V. Mitroshina, A.V. Lebedeva, E.A. Epifanova, T.A. Mishchenko, M.V. Vedunova, A.A. Babaev*

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: ponomareva@neuro.nnov.ru

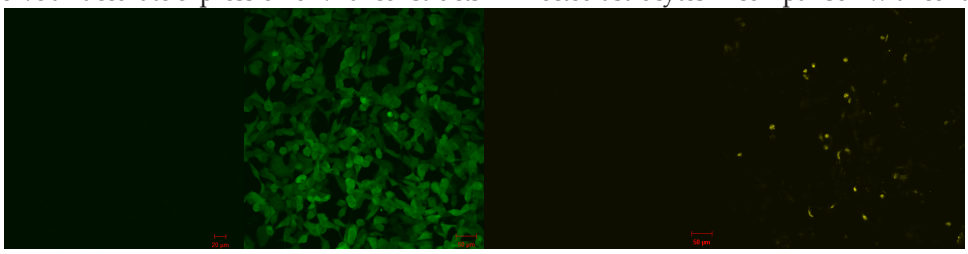
Recombinant viral vectors which able to effectively transport a gene of interest into the cells were previously designed. Adenoviral vectors produce recombinant virus at a high titer with high-level expression of introduced genes. We intend to use adenoviral constructs in optical stimulation experiments for astrocyte cultures with laboratory animals. The aim of this study is to examine the effectiveness of adenoviral vectors application for in vivo expression in neuroglial cells.

To achieve this, on the first stage of our investigation a human astrocytic glioma cell lines - a simple and effective model for testing the obtained samples- were used.

Adenoviral vectors containing promoter of the human glial fibrillary acidic protein GFAP (AVV-GFAP-ChR2-Venus, AVV-GFAP-Case12 and AVV-GFAP-GFP), which were kindly provided by Prof. Sergey Kasparov (School of Medical Sciences, University of Bristol) were used. AVV-GFAP-ChR2-Venus represents a fusion construct consisting of Channel rhodopsin 2 (ChR2) fused to Venus yellow fluorescent protein (Ex/Em 515/528) is necessary to visualize the expression. AVV-GFAP-Case12 contains a genetically encoded fluorescent biosensor for analyzing fluctuations in intracellular calcium ions concentration, and fluorescent GFP protein (Ex/Em 484/507). The amplification of viral vectors in HEK 293FT cell culture was carried out. The viruses were purified and concentrated using Amicon ultra-15 (Merc Millipore) centrifugal filter devices and titrated (AdEasy Viral Titer Kit, Agilent Technologies).

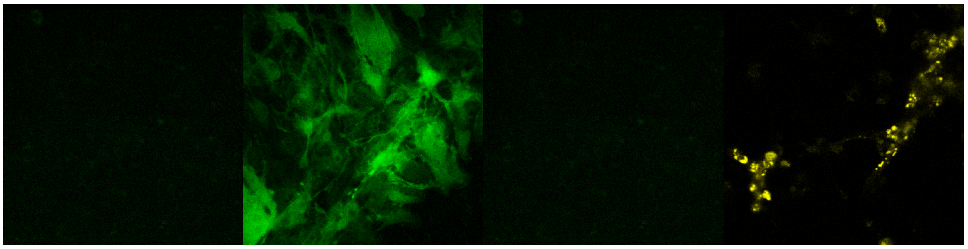
U251 MG cell line of the human astrocytic glioma obtained from the cell collection of the Institute of Cytology (St.-Petersburg) was used to perform a fast and efficient evaluation of in vitro expression. Due to its enhanced growth and ease of cultivation, this cell line is an optimal model for testing the effectiveness of viral vectors. U251 MG cells transfection by the virus was performed at glial cell density of 70-80 per cent per field of vision. Expression of fluorescent proteins within the viral constructs was evaluated by the level of their illumination intensity upon excitation with light of a certain wavelength on the 1st, 2nd and 3rd day using a Zeiss LSM510 confocal laser scanning microscope (Fig.1). It was shown the maximum GFP expression was observed on the first day after the transfection with GFAP-GFP constructs, whereas the expression of Venus fluorescent protein as part of the GFAP-ChR2-Venus construct was low and showed only on the third day after the transfection, which can be explained by the complexity and size of the optogenetic construct.

At the second stage, the efficiency of viral vectors expression in primary astrocytes monocultures we demonstrated. Primary astrocytes monocultures were obtained from the cortex of newborn mice (P0-P2). The cultures were infected on day 10 of culture development in vitro (DIV). The viral vectors expression by using the Karl Zeiss LSM 510 confocal laser-scanning microscope was detected on DIV 5 (Fig. 2). Toxic effects of AVV-GFAP-ChR2-Venus virus at a dose of 1.2×106 TU/ml (cell swelling, the formation of cell conglomerates) were observed. Application of a lower titer of viral preparation allowed to observe an accurate expression of viral constructs in infected astrocytes in comparison with control cultures.



Control ***GFAP-GFP-expression*** *Control* ***GFAP-ChR2-Venus- expression***

Fig.1 *GFAP-GFP and GFAP-ChR2-Venus expressionin U-251 MG human glioma cells*



Control ***AVV-GFAP- Case12-expression*** *Control* ***AVV-GFAP-ChR2-Venus-expression***

Fig.2 *Astrocytes expressing AVV-GFAP-ChR2-Venus and AVV-GFAP-Case12*

This work was supported by the Russian Science. Foundation under project № 16-14- 00201

OM&P

The Influence of Glial Cell Line-Derived Neurotrophic Factor and Modulated Activity of Endocannabinoid System on C3H Mice Sustainability to Ischemic Brain Injury *In Vivo*

*E.V. Mitroshina[1,2] *, B.ZH. Abogessimengane[1], M.D. Urazov[1], I. Khamray[1], T.A. Mishchenko[2,1], T.A. Astrakhanova[1], N.A. Shchelchkova[2,1], R.D. Lapshin[2], I.I. Belousova[2], I.V. Mukhina[2,1], M.V. Vedunova[1,2]*

[1] The Institute of Biology and Biomedicine, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia
[2] Molecular and cell technologies group, Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia
* Presenting e-mail: helenmitroshina@gmail.com

Nowadays, ischemia considered as one of the main causes of death and disability in the population all over the world. Among the consequences of brain ischemia an acute deterioration of memory and neurological status, learning and cognitive functions' impairment should be especially noted. Therefore, development of methods aimed at enhancing the adaptive capacity of the organism to ischemia is extremely urgent scientific issue. The modulation of endogenous systems, contributing to the survival of nervous cells under the influence of stress factors and maintaining their functional activity seems to be promising.

The aim of the investigation was to study the effects of Glial cell line-derived neurotrophic factor (GDNF) and the endogenous cannabinoid system activation by jzl 195 (an inhibitor of endocannabinoid biodegradation enzymes MAGL and FAAH) on the animal resistance to ischemic damage.

Materials and Methods

The study was conducted on 120 adult male C3H mice, weighing 25-40g. Animals were divided into the following groups: 1) Intact (n = 16), 2) Sham - falsely operated mice subjected to tissues incision with subsequent finding the artery without occlusion (n = 16), 3) Control - the animals with bilateral carotid arteries occlusion (n = 40), 4) The animals with bilateral carotid arteries occlusion and a single jzl 195 (10 mg/kg) intraperitoneal injection 45 minutes before surgery (n = 9), 5) The animals with bilateral carotid arteries occlusion and intranasal GDNF (0,08 mg/ml) administration 45 minutes before and during 3 days after the surgery (n = 22), 6) The animals with bilateral carotid arteries occlusion and intranasal GDNF (0,8 ml/ml) injection 45 min before and within 3 days after surgery (n = 17).

In order to develop ischemic lesions in different brain structures, an experimental animal model of bilateral carotid arteries occlusion was performed. The animals were anesthetized with pentobarbital (70 mg/kg) and were placed on an operating table 15 minutes after injection. The left and right carotid artery were allocated and then the simultaneous vessels ligation using non-absorbable ligature strands was carried out. Hereafter, the wound was sutured and sprinkled by the streptocid powder to prevent the inflammation.

To assess the animal's physiological state after ischemic brain damage, the neurological status (standard neurological scale and Garcia scale) was evaluated. To identify the mechanisms of ischemic brain damage, the mitochondrias' functional state by measuring of oxygen consumption rate was conducted. Mitochondrial dysfunction is a key component of the brain cell damages induced by ischemia. Mitochondrias was isolated by a standard differential centrifugation. Brain mitochondrial respiration rate was recorded using a high-resolution respirometer Oroboros Oxygraph-2k (Oroboros Instruments Corp, Austria).

The carried out experiments revealed that the bilateral carotid arteries occlusion causes the animal mortality up to 70-80%. There was no difference in neurological status between intact and falsely operated animals during the observation period. For all experimental groups (except for "Sham") a significant neurological status deterioration in comparison with intact animals 24 hours after surgery was shown. The neurological status of experimental animals treated by jzl 195 (10 mg/kg) and GDNF (0.8 mg/kg) did not differ from intact mice on day 3 after ischemia modeling (Figure 1).
However, there was no difference between the experimental groups on day 7 after ischemia modeling. Thus, it can be assumed that GDNF treatment during 3 days after surgery has a positive effect on the animal sustainability to ischemic brain injury. Further experiments will be focused on the selection of optimal scheme of jzl and GDNF administration to achieve a maximum effect. The mitochondrial oxygen consumption rate was conducted on day 4 after ischemia modeling. Our studies revealed that mitochondrias' oxygen consumption rate in the intact group was 254,8 pmol/(s*ml) whereas in control group this parameter was decreased up to 208,8 pmol/(s*ml) after ischemia modeling. The rate of mitochondrias oxygen consumption in the group treated by GDNF 0.8 mg/kg did not differ from the intact animal (243,5 pmol/(s*ml)), and in animals treated by jzl195 was significantly increased (465.7 pmol/(s*ml)).

Thus, we have shown that GDNF (0.8 mg/kg) application normalizes the parameters of mitochondrial respiration, and activation of the cannabinoid system stimulates the mitochondrias functioning under ischemic conditions. This effect refers to the post-ischemic adaptation and could potentially serve as one of the approaches for ischemic brain injury correction.

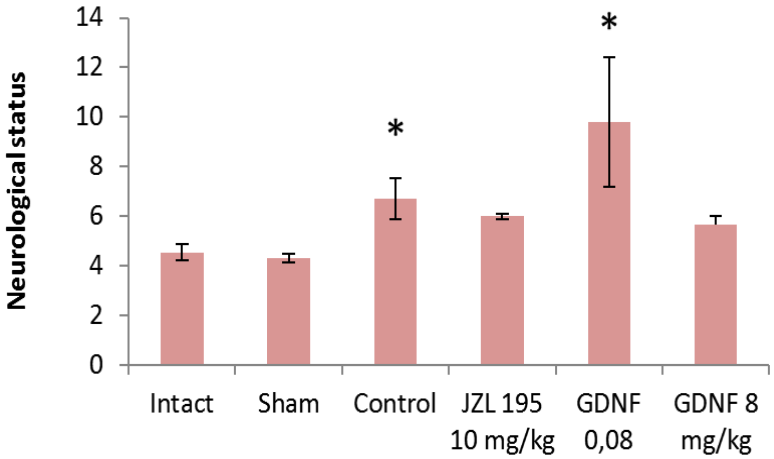


Fig. 1. *Neurological status of experimental animals on day 3 after total cerebral ischemia modeling (standard scale of neurological status); * - statistical significance with intact animals, p<0.05, ANOVA*

Acknowledgements

The research was supported by grants of Russian Foundation for Basic Research № 16-34-00301, №16-04-00245 and prepared as a part of the state project "Provision Scientific Research".

Ephrin Class A Reverse Signaling Guides Callosal Axon Growth Via Efna 4-Ntrk 2 Receptor Complex Downstream of Neurod 2/6

*K. Yan *, O. Grishina, G. Camarero, E. Bessa, U. Günther, R. Wunderlich, I. Bormuth, V. Tarabykin*

Institute of Cell- and Neurobiology, Charité – Medical University Berlin, Germany.
* Presenting e-mail: kuo.yan@charite.de

Abstract. Callosal axon guidance during cerebral cortex development is complicated and far from being fully understood. Previous work in our lab has shown that basic helix loop helix transcription factors Neurod2 and Neurod6 are essential regulators for the fasciculation and guidance of callosal axons. Here we use acallosal Neurod2/6 deficient mice as a model system to selectively study callosal axon pathfinding in vivo. We identify Efna4 as transcriptional target of Neurod2/6 in developing neocortex. In utero electroporation of Efna4 into neocortical pyramidal neurons of Neurod2/6 deficient embryos is sufficient to cell-autonomously rescue callosal axon fasciculation and migration along the normal callosal path towards the midsagittal plane. Mechanistically, Efna4 forms a co-receptor complex with Ntrk2 (TrkB) in reverse signaling, and hence regulate AKT cascades in vitro and in vivo via Ntrk2's SHC-binding tyrosine. Co-electroporation of dominant negative Ntrk2 K571N or Ntrk2 Y515F completely abolishes the ability of Efna4 to rescue callosal axon guidance in Neurod2/6 deficient mice. We also show that the Eph receptors are abundantly expressed in the cortical plate and ventricular zone, but minimally expressed in the intermediated zone (IZ) of the cortex, while ephrinA ligands are largely present on the callosal axons in the IZ. In addition, reverse signaling from extracellular domain of EphA receptors to Efna4 leads to active axonal retraction in vivo. The complementary expression and repulsive interaction of EphA receptors and ephrinA ligands suggest a permissive channel for callosal axon navigation before midline crossing. Thus, ephrinA ligands coordinate fasciculate growth and guidance of callosal axons via interaction with Ntrk2 in cis and with EphA receptors in trans.

Keywords: NeurodD2; NeurodD6; ephrin A ligands; Ntrk2; corpus callosal agenesis.

Sigma-1 Receptor is a Potential Drug Target for Neuropathology Treatment

*A.V. Bolshakova [1] *, V.A. Zhemkov [1, 2], A.N. Gainullina [1], E.O. Kukanova [1], S.A. Korban [1,2], I.B. Bezprozvanny [1,3]*

[1] Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, Russia.
[2] Laboratory of Enzymology, National Research Center «Kurchatov Institute», B.P. Konstantinov Petersburg Nuclear Physics Institute, Gatchina, Russia.
[3] Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA.
* Presenting e-mail: asyab@mail.ru

Sigma-1 receptor (S1R) is a trans-membrane chaperone protein at the endoplasmic reticulum (ER), which stabilizes the calcium signaling between ER and mitochondria. This receptor has a high affinity to bind wide range of chemical compounds of different structural classes with a variety of therapeutic and pharmacological properties [1]. Thereafter S1R has received the most interest in respect to pharmacology application. There are studies that S1R is involved in the formation of many neurological and psychiatric conditions [2-5]. It was proposed that S1R serves as a sensor of normal calcium homeostasis. Recent studies have demonstrated the role of calcium signaling in neuropathology, such as Alzheimer's (AD) and Huntington's diseases (HD). Changes in endoplasmic reticulum calcium homeostasis resulted in the violation of synaptic connections between neurons [6].

We aimed to study S1R role as a potential modulator of neuronal dysfunction, synaptic loss and eventually neurodegeneration on the cellular model of neurodegenerative diseases and using molecular and biochemical techniques. To understand the mechanism underlying the synaptic loss in HD we cultivated mixed cortico-striatal neurons in vitro. YAC128 mice model of HD was used in experiments [7]. Striatal spine loss in YAC128 medium spiny neurons (MSNs) on DIV21 was shown previously [8]. Using immunostaining with DARPP32 antibody of MSN we achieved that exposure of S1R agonist 3PPP leads to spine rescue in YAC128 MSNs. Knockdown of S1R resulted in decreasing of spine density in MSNs both in WT and YAC128 cell cultures.

For better understanding of the structure and functioning of S1R we cloned human SIGMAR1 gene into pMAL-p5x (New England Biolabs) bacterial expression vector which resulted in expression of MBP-S1R fusion protein. The same approach has been utilized by our group for structure determination of a number of difficult proteins [9-11]. In brief, E. coli cells (B834 strain, co-transformed with pRARE2 plasmid) were cultured until they reached OD(600)=1.0 when protein synthesis was induced by addition of IPTG. Cells were cultured at 25° C overnight, the following day cells were harvested and lysed by sonication. Membrane fraction was sedimented by centrifugation and membrane proteins were extracted by DDM: Triton X-100 detergent mixture. MBP-S1R was purified by two chromatography steps: first on the amylose resin followed by size-exclusion chromatography. Protein purity was confirmed by SDS-PAGE and immunoblotting against S1R. In order to confirm protein functionality we used pull-down assay with agarose-immobilized progesterone. Recombinant MBP-S1R can be pulled down by progesterone beads which indicate that the protein produced in bacteria is functionally active in terms of binding selective sigma ligands. We plan to use our expertise in crystallization of structurally difficult proteins for characterization of S1R receptor structure and function.

Thus, the obtained results indicate that S1R has a potential for therapeutic targeting for the development of pharmacological agents for the treatment of Huntington's disease and eventually other types of neuropathology. EphA receptors in trans.

Acknowledgements

This work was supported by the Russian Scientific Fund grant 14-25-00024.

References

1. Su T.P. et al., The sigma-1 receptor chaperone as an inter-organelle signaling modulator. Trends Pharmacol Sci, 2010. 31(12): p. 557-66.
2. Meyer, D.A., et al., Neurosteroids enhance spontaneous glutamate release in hippocampal neurons. Possible role of metabotropic sigma1-like receptors. J Biol Chem, 2002. 277(32): p. 28725-32.
3. Yagasaki, Y., et al., Chronic antidepressants potentiate via sigma-1 receptors the brain-derived neurotrophic factor-induced signaling for glutamate release. J Biol Chem, 2006. 281(18): p. 12941-9.
4. Monnet, F.P. and T. Maurice, The sigma1 protein as a target for the non-genomic effects of neuro(active)steroids: molecular, physiological, and behavioral aspects. J Pharmacol Sci, 2006. 100(2): p. 93-118.
5. Martina, M., et al., The sigma-1 receptor modulates NMDA receptor synaptic transmission and plasticity via SK channels in rat hippocampus. J Physiol, 2007. 578(Pt 1): p. 143-57.
6. Bezprozvanny, I., Calcium signaling and neurodegenerative diseases. Trends Mol Med, 2009. 15(3): p. 89-100.

7. Slow, E.J., et al., Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet, 2003. 12(13): p. 1555-67.
8. Artamonov, D.N., et al., Characterization of Synaptic Dysfunction in an In Vitro Corticostriatal Model System of Huntington's Disease. Biologicheskie Membrany, 2013. 30(4): p. 276-288.
9. Kim, M.W., et al., Secondary structure of Huntingtin amino-terminal region. Structure, 2009. 17(9): p. 1205-12.
10. Kim, M., Beta conformation of polyglutamine track revealed by a crystal structure of Huntingtin N-terminal region with insertion of three histidine residues. Prion, 2013. 7(3): p. 221-8.
11. Zhemkov, V., et al., The 2.2-Angstrom resolution crystal structure of the carboxy-terminal region of ataxin-3. FEBS Open Bio, 2016. 6(3): p. 168-178.

HP1 and Endogenous Retroviruses in the Brain

*Andrew Newman**

Charité Universitätsmedizin Berlin, Germany.
* Presenting e-mail: andrew.newman@charite.de

Heterochromatin Protein 1 (HP1), a structural protein found in the nucleus, is highly conserved across plants and animals and has a mechanism of action that has remained enigmatic since it was first observed 20 years ago. Known for binding to the repressive histone mark H3K9me3 or H3K9me2 (tri- or di-methylated Lysine 9 of Histone 3 respectively), HP1 has naturally emerged to be an essential component in a host of epigenetic systems essential for survival. Briefly, HP1 proteins have been implicated in retrotransposon silencing and heterochromatin spreading, proviral HIV silencing, chromosome stability and mitosis, cell cycle exit, spermatogenesis, DNA methylation, transcription, and embryonic stem cell maintenance. However, whatever the essential interaction is between HP1, H3K9 and respective Lysine methyltransferases remains elusive due to conflicting evidence. Concomitant to this, a growing body of evidence now implicates HP1 proteins as important components of various transcriptionary regulatory systems important in tissue specification during development. Differential interactions have been observed between distinct HP1 proteins and specific members of Nucleosome remodelling histone deacetylase (NuRD) complexes, BRG1 associated factor (BAF) (also called SWI/SNF) complexes, Polycomb (PcG) complexes, as well interacting with Kruppel-associated box (KRAB) containing zinc finger family, including the neuronal specific REST/CoREST complex.

Presented here are two novel findings: 1) Class II endogenous retrovirus (ERVs) are de-repressed in HP1β and HP1γ double knockouts, and 2) this is due to two independent mechanisms that are synergistic. Here we report the mechanisms responsible for Class II ERV repression, the effects of de-repression on behaviour and aging, and test occurrences of retrotransposition due to neuronal stimulation and DNA damage.

Creating of Adenoassotiated Viral Vector for Expressing of Neurotrophic Factor BDNF in Neuronal Cells

*E.A. Epifanova *, E.V. Mitroshina, A.A. Babaev*

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: epifa888@mail.ru

Brain-derived neurotrophic factor (BDNF) is important signaling molecule which takes part in regulation of neurogenesis, growth and survival of neurons in central nervous system. BDNF participates not only in neuronal differentiation and in formation of synaptic contacts during neurogenesis but also can correct the metabolism of mature neurons. According data from recent studies BDNF has strong neuroprotective properties, depresses cell apoptosis, stimulates growth and prevent neuronal death. In rehabilitation period after injuries, ischemic and neurodegenerative diseases it is important to stimulate endogenic reparation of functional neuronal nets. One of the approaches may be therapeutically rising of the BDNF level.

Recombinant adeno-associated virus is one of the most promising delivery vectors for gene therapy due to its nonpathogenic property, nonimmunogenecity to host and broad cell and tissue tropisms.

Aims

The main goal of this research is to create adenoassociated viral vector for expressing of neurotrophic factor BDNF in neuronal cells. For this study we created the adenoassotiated viral vector with fragment of BDNF gene and EGFP and infected transgenic cell line HEK293T and primary neuronal culture to estimate the viral expression.

Methods

We used standard cloning techniques to create adenoassotiated viral vector with fragment of BDNF gene. We extracted mouse total RNA, made PCR with reverse transcription and amplify the cloning fragment of BDNF gene (900 base). Then we cloned the BDNF fragment into AAV-Syn-kid2 plasmid containing strong human synapsin promoter and Woodchuck hepatitis posttranscriptional regulatory element which enhances the synapsin promoter.
Afterwards we contransfected with this plasmid and helper plasmids the transgenic HEK293T cell culture and collected the virus. We had two different AAV packaging system – DJ and pDP5. Next we cleaned the virus using benzonase treatment and additionally cleaned and concentrated the virus on Amicon Ultra columns to achieve necessary purity of virus so far as the purity of viral sample is important for primary neuronal cultures. The last step was the infection of primary neuronal culture and transgenic HEK293T cell culture and estimating the viral expression using confocal microscopy. Primary neuronal culture was received from E18 mouse.

Results

Adenoasstiated virus containing fragment of BDNF gene has strong expression in HEK293T cell culture and primary neuronal culture. The expression of adenoassotiated virus is more efficient in case of using DJ AAV packaging system. Strong expression appeared on 3-4 day in case of HEK293T cell culture infection and on 5-7 day in case of primary neuronal culture infection. The expression was proved by confocal microscopy, immunocytochemistry and PCR methods.

Conclusions

Thereby it was created the adenoassotiated viral vector containing the fragment of BDNF gene and it was tested on primary neurons culture.

Effect of Epileptiform Activity on Hippocampal Astrocytes

A. Lebedeva[1], O. Tyurikova[1,2], A. Plata[1], V. Tovpyga[1], P. Denisov[1], S. Makovkin[1], I. Ivlev[1], A. Semyanov[1]*

[1] Institute of Neuroscience, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;
[2] Department of Clinical and Experimental Epilepsy, University College London, London, UK.
* Presenting e-mail: lebedeva@neuro.nnov.ru

Over decades, epilepsy has been considered a neurogenerative disease mainly manifested by abnormal neuronal firing. However, some recent findings suggest that electrically passive astrocytes strongly contribute to neural function in health and disease, owing to the release of a variety of signalling molecules (such as glutamate, ATP, D-serine) that target receptors in both neuronal and non-neuronal cells. Astrocytes also participate in neuronal signalling trough high-affinity uptake of neurotransmitters and spatial buffering of extracellular K^+. Glutamate transporters deal with up to 80% of the released glutamate, as well as exchanging also Na^+, H^+ and K^+. However, during strong neuronal activity, extracellular K^+ concentration can significantly increase from 2.5 mM to 10-12 mM. In pathological condition, such as epileptiform activity, this concentration can even rise up to 30 mM [Verkhratsky, Butt, 2007]. According to the kinetics of glutamate transporter, increase of extracellular K^+ could interfere with local glutamate uptake. How synaptic activity is affected by epilepsy remains poorly understood. We investigated astrocytic activity in rat slices from control animals and lithium-pilocarpine epilepsy model animals. Ca^{2+} dynamics was monitored in astrocytes loaded with sulforhodamine 101 (200 nM), a specific astrocytic marker, and Oregon Green BAPTA AM (7.95 μM), a Ca^{2+} sensor. Transporter and K^+ currents in astrocytes were measured with patch pipette in response to electric stimulation of Schaffer collaterals in baseline conditions and subsequently after pharmacological blockade of the excitatory amino acid transporters (EAATs) with TBOA (50 μM). The present study demonstrates changes in astrocytes associated with epileptogenesis.

Acknowledgements

This work was supported by the Russian Science Foundation project №15-14-30000.

Identification of Novel Mutations Causing Malformations in Cortical Development by Enu Induced Mutagenesis in the Mouse

*E.V. Borisova *, A.A. Babaev, M.V. Turovskaya, E.A. Turovsky, V.S. Tarabykin*

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: borisovakaterina17@gmail.com

Abstract. The cerebral cortex, is the seat of our higher cognitive capacity that distinguish humans from other species. The development of the cerebral cortex is a complex and highly orchestrated process whose disruption can result in a wide range of developmental disorders that are recognized as malformations of cortical development. Malformations of the cerebral cortex can frequently cause of epilepsy, developmental delay, neurological deficits, and mental retardation in humans. Intellectual disability and epilepsy caused by neurodevelopmental disorders has a high prevalence of about 2% in human population. Consequently identification of novel mouse mutants with malformations of cortical development and identification and characterization of the causal genes will improve our understanding of the genetic regulation of cortical development and pathogenesis of malfunctioning of the brain.

Aims

The main goal of this project is to identify novel genes that control proper establishment of the cortical structure and functions. For this study we generate mouse mutants by N-ethyl-N-nitrosurea (ENU) directed mutagenesis with disrupted structure and function of the cerebral cortex. We will identify mutants with epileptiform activity and then identify respective genes with positional cloning. Also we would like to characterize the behavior of these mutants and underlying molecular mechanisms.

Methods

To screen for recessive mutations we use back cross three generation scheme. Male C3H mice (12 weeks old) are treated with three intraperitoneal injections in a dose of 80, 90, 100, 120 and 150 mg/kg of ENU at weekly intervals. Following injections ENU induces a variable period (10-15 weeks) of sterility during which mutagenized spermatogonial stem cells repopulate the testis. The surviving males after that period were used for mating with the C3H females to produce G1 offspring.

On next step we will take males from G1 and mated them with C57BL6 females fron Satb2-LacZ line, their upperlayer neurons of brain cortex express LacZ as a reporter (Dobreva G. et al., 2006). This makes it easy to visualize changes cytoarchitecture cortex and connections between cells. To detect the presence of LacZ transgene in line Satb2-LacZ mice and genotyping PCR protocol was developed.

Results

The optimal concentration of ENU was determined (100 mg/kg). Also we assume that a fertile period after injections of ENU longer than 12 weeks.

The protocol of genotyping Satb2-LacZ mice was developed. As a samples for PCR we use tail cuts of mice from this line, then lysing performed, we use the phenol-chloroform method for extraction of DNA and after that PCR. Besides brain drugs was optimized staining protocol mice Satb2-LacZ-reporters. Beta-galactosidase (β-gal), encoded by the gene LacZ, hydrolyses beta-galactosides, resulting in the appearance of a blue color. Females, genotyped and carrying the reporter Satb2-LacZ, were further crossed with G1 males.

Conclusions

Thus males after ENU injections were obtained and females with help of the developed genotyping protocol were selected for mating to produce G1 population.

Acknowledgements

Research carried out with the financial support of the grant of the Russian Scientific Foundation (project №15-14-10021)

References

1. G. Dobreva, M. Chahrour, M. Dautzenberg, L. Chirivella, B. Kanzler, I. Fariñas, G. Karsenty and R. Grosschedl, Cell, 2006, 125(5), 971-986.

OM&P

Human-Machine Interface Built on sEMG Toolkit with Artificial Neural Network Feature Classifier

*I.A. Kastalskiy[1] *, V.A. Makarov[1,2] and S.A. Lobov[1]*

[1] Lobachevsky State University of Nizhny Novgorod, Russia;
[2] Universidad Complutense de Madrid, Spain.
* Presenting e-mail: kastalskiy@neuro.nnov.ru

EMG (electromyographic) signal is a superposition of action potentials generated by motor units. Signal recording typically implement via the surface electrodes placed on the skin [1,2]. It is known that sEMG (surface EMG) toolkit is widely used to interpret bioelectric patterns for controlling a variety of devices [3-8]. The aim of this study was to investigate the possibility of using a wearable sEMG bracelet to control electronic devices, including interaction with the personal computer. In order to execute the set of commands needed to control the position of the cursor on the computer screen, we propose an artificial neural network (ANN) driven by the signals recorded on arm. The ANN passed through a supervised learning is able to measure the degree of arm muscles effort during movement and classify gestures. It has been shown that the human-computer interface allows controlling the cursor remotely by hand movements and simulating mouse clicks by clenched fist. The average classification accuracy of six gestures (right, left, up, down, left (single) click, right (double) click) varies around 97%.

We used the bracelet MYO (Thalmic Labs) with eight equispaced sEMG sensors acquiring raw myographic signals which were being sent through a bluetooth interface to a PC. The software allows for recognition of hand gestures and estimating muscle efforts that control the cursor on the screen in a way similar that one can achieve with ordinary computer mouse. We used root mean square (RMS) value calculating to evaluate the EMG signal obtained by each electrode. The RMS data, as a composite feature of the current hand gesture, are fed into an ANN. The network neurons apply weighted sum over inputs and use sigmoidal activation function to generate the output. The learning, i.e., adjustment of the neuron weights, is achieved by the back-propagation algorithm [9].

As a result, the cursor movement direction is defined by gestures, while its speed is controlled by the degree of muscle contraction. This significantly improved the user experience. Experimental data shows that all users were able to move the cursor and simulate left and right mouse clicks.

Acknowledgements

This work was supported by the Russian Science Foundation under project 15-12-10018.

References

1. Bishop MD, Pathare N. Considerations for the use of surface electromyography. Phys Ther Korea. 2004;11(4):61-69.
2. Pullman SL, Goodin DS, Marquinez AI, Tabbal S, Rubin M. Clinical utility of surface EMG Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 2000;55(2):171-177.
3. Chowdhury A, Ramadas R, Karmakar S. Muscle Computer Interface: A Review. In: Chakrabarti A, Prakash RV, eds. ICoRD'13, Lecture Notes in Mechanical Engineering. Springer India 2013; 2013:411-421.
4. Hahne JM, Biessmann F, Jiang N, et al. Linear and nonlinear regression techniques for simultaneous and proportional myoelectric control. IEEE Trans Neural Syst Rehabil Eng. 2014;22(2):269-279.
5. Lorrain T, Jiang N, Farina D. Influence of the training set on the accuracy of surface EMG classification in dynamic contractions for the control of multifunction prostheses. J Neuroeng Rehabil. 2011;8:25.
6. Mironov VI, Lobov SA, Kastalskiy IA, Kazantsev VB. Myoelectric Control System of Lower Limb Exoskeleton for Re-training Motion Deficiencies. Lect Notes Comput Sci. 2015;9492:428-435.
7. Naik GR, Kumar DK, Palaniswami M. Multi run ICA and surface EMG based signal processing system for recognizing hand gestures. In: 8th IEEE International Conference on Computer and Information Technology, 2008. IEEE; 2008:700-705.
8. Peerdeman B, Boere D, Witteveen HJB, et al. Myoelectric forearm prostheses: State of the art from a user-centered perspective. J Rehabil Res Dev. 2011;48(6):719-738.
9. Rumelhart DE, Hinton GE, Williams RJ. Learning Internal Representations by Error Propagation. In: Parallel Distributed Processing. San Diego: La Jolla Institute for Cognitive Science, California University; 1985:318-362.

Metabolic Therapy in Post-Traumatic Period of Craniocerebral Trauma

*A.V. Shumilova[1] *, A. V. Deryugina[1] and G. A. Boyarinov[2]*

[1] Federal State Autonomous Educational Institution of Higher Education «Lobachevsky State University of Nizhny Novgorod», Nizhny Novgorod, Russia.
[2] State budgetary educational institution of higher professional education «Nizhny Novgorod State Medical Academy», Nizhny Novgorod, Russia.
* Presenting e-mail: shumilowanastya@gmail.com

Despite significant advances in medical science and the improvement of the quality of medical care, traumatic brain injury (TBI) remains a health problem with high mortality and disability (Govorova, 2013). The main factor in the formation neurodestructive pathologies is the loss of nerve cellular elements (neurons and glia). The causes of acute neuronal-glial degradation after an initial damaging effect are endogenous changes that lead to the development of oxidative stress (Shanko, 2009). Oxidative stress generates multiple organ failure. Most promising treatment is the use of intensive therapy of metabolic and antioxidant action, capable of influencing the pathogenesis of TBI. Significant value to the perfusion and oxygenation of the brain during the early posttraumatic period has a microrheological blood disorders that determine its fluidity at the level of capillaries and depend on the state of erythrocyte membrane.

Aims

This study was conducted to assess the dynamics of changes in the concentration of MDA, catalase activity and electrophoretic mobility of erythrocytes under the influence of Mexicor and Cytoflavin after TBI.

Methods

We simulated head injury by free fall of a weight of 100 g from a height of 80 cm on the parietal-occipital area of the head rats (Tsymbalyuk, 2008). After TBI the animals intraperitoneally administered Mexicor in a dose of 8.0 mg / kg body (group 1) or Cytoflavin in the dose 02,0 ml / kg body (group 2).The control group consisted of rats with TBI, which were injected with saline. The first drug was injected 1 hour after the injury, and then during 12 days of therapy. The levels of physiological norms of the researched parameters were determined in intact animals. Oxidative changes of red blood cells were investigated by assessing the activity of catalase (Storozhuk, 2003) and concentration of malondialdehyde (MDA) (Krylov, 2011). Surface structural changes of red blood cells (electrophoretic mobility of red blood cells (EPME)) were assessed using micro-electrophoresis (Barinova, 2014).

Results

The results of these studies showed that TBI was accompanied by decrease of electronegativity of erythrocyte membranes, activity of the antioxidant system and increased oxidative processes in red blood cells. The use of Mexicor and Cytoflavin led to a decrease in the concentration of MDA and increased catalase activity. The most pronounced effect in post-traumatic period was manifested in the action of Mexicor. So, the introduction of this drug determined significant decrease of MDA concentrations to values of intact animals by 3 days of the experiment, while the introduction of Cytoflavin a similar effect was noted by day 7 of observation. The change of MDA combined with the work of the antioxidant system. Under the action of Mexicor was observed an increase of catalase activity from the first days, under the action of Cytoflavin with the 3 days of the experiment. Analysis of the results shows that efficacy was shown in various stages of post-traumatic period, that, probably, it is necessary to consider at various intensities of oxidative stress. In the control group the severity of changes in the studied parameters was less significant compared to experience.

Conclusion

The use of Mexicor and Cytoflavin in the treatment of TBI reduces the severity of oxidative stress. 2. The use of Mexicor improves the performance of red blood cells in the initial period post-traumatic period. 3. The use of Cytoflavin after TBI has a positive effect 7 days post-traumatic period.

Recommendations

The use of Cytoflavin and Mexicor in the period of post-traumatic head injury is pathogenetically justified, because they have metabolic and antioxidant effect. These drugs have a corrective action on the microcirculation and the state of pro - and antioxidative processes that lead to the feasibility of their use to prevent secondary brain injury.

References

1. Govorova N., Emerg. Med. Care, 2013, (2), 36-40.
2. Shanko Y.G., Tanin A.L., Naletko A.N., Grieg T.N. and Vasiljevic E.N., ARS MEDICA, 3 (13), 2009, 97-105.
3. Nayak C.D., Nayak D.M., and Raja A, Rao A., Neurol. India, 1(56), 2008, 31-35.
4. Tsymbalyuk V. I. and Cochin O. V., J. Ukrain. Neurosurg., (2), 2008, 10-12.
5. Storozhuk P. G. Bullet. Herald Intens. Ther. – 2003. – No. 3. – P. 8-13.
6. Krylov V. N. and Deryugina A.V., Hematol. Transfus., 2011, (5), 18-21.
7. Barinova O.YU. and Deryugina A.V. Act. Prob. Modern Sci., 2014. 278.

Effort and Reward Based Decision Making in Individuals at High Risk of Depression

Veronika Samborska and Jonathan Roiser*

University College London, London, United Kingdom;
University College London, London, United Kingdom.
* Presenting e-mail: veronika.samborska.13@ucl.ac.uk

Background

In recent years, deficits in motivation to exert effort have been suggested to account for some of the maladaptive behaviours seen in depression and have been further found to be associated with symptoms of depression such as anhedonia and apathy. However, little is known about how motivation to exert effort is processed in unaffected first-degree relatives of patients with major depression. The present study aimed to examine whether deficits in motivated effort are linked to high risk of depression onset and depressive symptoms such as anhedonia and apathy.

Methods

Twelve first-degree relatives of patients who have never been depressed themselves and eighteen controls with no family history of depression were recruited. The motivation to exert both physical and cognitive effort was measured by means of two behavioural tasks. In each task participants were presented with 80 offers, which contained information about the possible reward they could attain, and the amount of physical or cognitive effort that they would need to exert in order to achieve it. Participants could choose to accept or reject an offer. Additionally, self-report questionnaires were administered to assess the severity of anhedonia and apathy.

Results

There was a significant effect of the amount of effort and reward at stake on participants' willingness to engage in an effortful action in both tasks. No significant differences between individuals with a depressed relative and those without were found in their motivation to exert both physical and cognitive effort. No association between anhedonia and apathy symptoms and motivational processing were found in the overall sample.

Limitations

The sample size was very small and thus limited the power to detect an effect. The task introduced probabilistic discounting at the hardest effort level because participants failed to achieve the correct response at the hardest effort level, possibly confounding.

Use of Optogenetic Technology in Cell Culture Models, Implantable Device to Works in Slices and Live Animals

*A.I. Erofeev[1,2], O.A. Zakharova[1,2], M.V. Matveev[2], S.G. Terekhin[1,2], I.B. Bezprozvanny[1,3],O.L. Vlasova[1,2] **

[1] Molecular Neurodegeneration Lab, Peter the Great Polytechnic University, Saint-Petersburg,195251, Russia.
[2] Department of Medical Physics, Peter the Great St. Petersburg Polytechnic University, Saint-Petersburg, 195251, Russia.
[3] Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA.
* Presenting e-mail: olvlasova@yandex.ru

Abstract. Optogenetic is a powerful method that allows to modulate cellular physiological properties. In our article we demonstrate changes of electrical properties of cellular membranes on HEK-293T and hippocampal neurons transfected with channelrhodopsins and halorhodopsins induced by blue and orange light stimulation. In recent years, a method of developmental research has proved its effectiveness in the nerve cell stimulation tasks. In our article we demonstrate an implanted device for the stimulation of neurons in slices and live animals.

Introduction

Brain is one of the most complicated and poorly understood parts of the human body. There is a large group of disorders related to abnormalities in brain activity called neurodegenerative diseases. At the moment etiology and pathological basis of these diseases are unknown. That's why the fundamental assays in neurobiological field become more and more important. New technology that allows scientists to solve these biomolecular problems is called optogenetics. Optogenetics is a modern approach to modulate physiological status of excitable cells, including neurons. This modulation is achieved by combining the techniques of genetic engineering and photonics [1, 2, 3].

Optogenetic approach in HEK cells

Cells of human embrionic kidney (line HEK-293T) are easily transfected so they are often used as an object of study[4]. In our preliminary experiments HEK-293T cells were transfected with Channelrhodopsin and Halorhodopsin constructs. The responses were recorded using the patch-clamp technique in voltage clamp mode using blue (depolarisation, Fig. 1,A) and orange (hyperpolarisation, Fig. 1, B) light stimulation.

Optogenetic approach in hippocampal neurons Optical-electrode interface

After successful approbation on HEK cells, optogenetic experiments were conducted in mouse primary hippocampal neuron cultures. Neurons were transfected with ChR2-GFP plasmidand stimulated by blue (470 nm) light. Traces of neurons activity were recorded in voltage and current clamp modes.

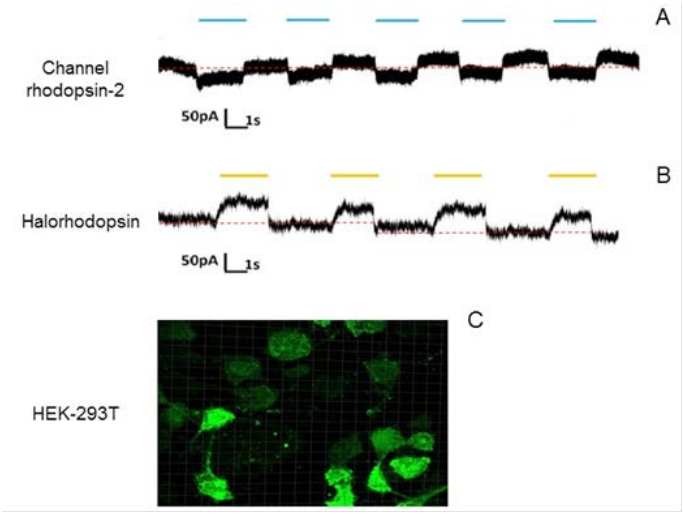


Fig. 1. (A, B, C). (A) Depolarization of the cell membrane during the optogenetic stimulation of transfected by ChR2-GFP plasmids cells linesHEK-293(λex = 470 nm); (B) Hyperpolarization of the cell membrane during the optogeneticstimulation of transfected by HR-GFP plasmids cells linesHEK-293 (λex = 590 nm); (C) confocal microscopy of transfected cell linesHEK-293T.

Optical-electrode interface

Brain functions studying requires neuron interface that could record parameters and stimulate brain with high time-space accuracy. Most researchers who use optogenetic method in laboratory conditions on in-vivo animals now use optical fiber that is sent through the implantable cannula [5].

Parameters of the pulses sequence and their generation is controlled by computer graphical interface or manual switch of modes.

Programmed LED control drivers ensure the setting of DC values for one or several separate LEDs or a cluster that consists of several diodes united in one output fiber. Each channel is controlled autonomously (manually in modes of CW, external TTL or analog modulation types) or by software installed on computer.

In department of Medical Physics in our Molecular Neurodegeneration Laboratory we are working on the development and testing of implantable device for monitoring of brain neurons physiological parameters (action potential).

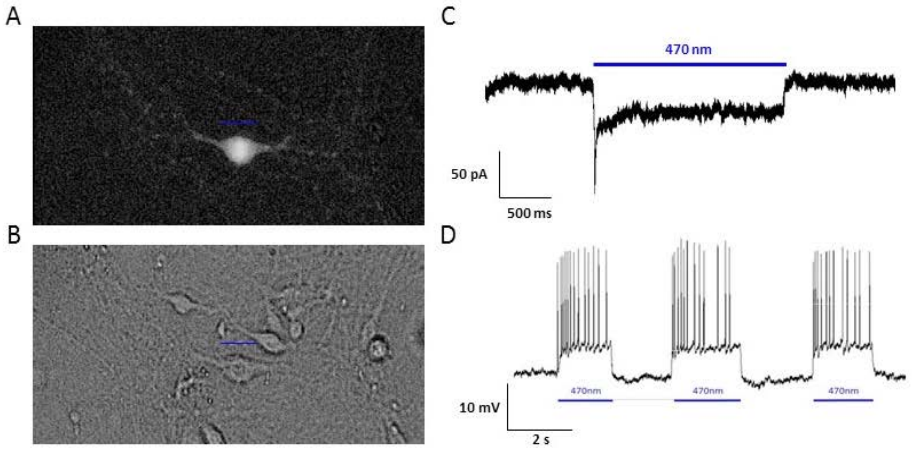


Fig.2. *(A, B, C, D). (A) Hippocampal neurons expressing ChR2-GFP (scale bar 20 μm); (B) Hippocampal neuron culture (DIV15); (C) Inward current in voltage-clamped neuron evoked by 470 nmlight (indicated by blue bar); (D) Voltage traces showing response to light stimulation.*

Together with "Nano and Microsystem Technology" research laboratory we are developing a combined optical-electrode device that allows you to carry out combined research with the use of intravital microelectrode stimulation and optogenetic activation of genetically differentiated neurons (Fig. 3).

Optrode allows to record electrical activity during optogenetic experiments. This combination of several microelectrodes allows you to record the activity of several neurons in light affected areas. It minimizes the effects of light diffusion inside the tissue and the mismatch of positions of the light source and the detector that records neurons excitation / inhibition parameters. [6] An implant consists of the coaxial conical optical wave guide (optrode) integrated inside the implantable electrode array (multi-electrode array-MEA) for recording the experimental data.

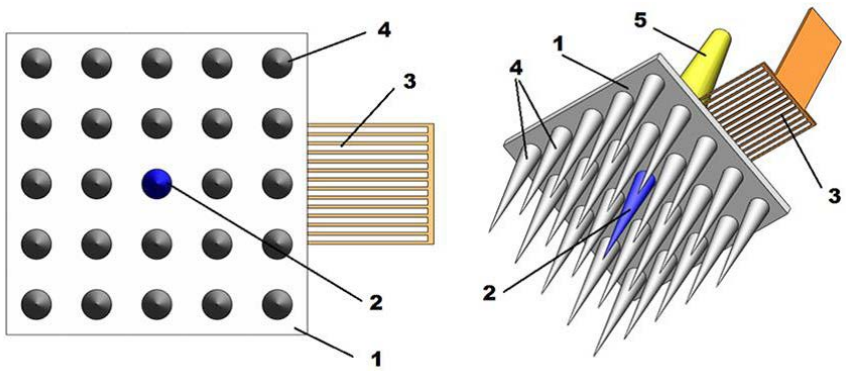


Fig.3. *Schematic representation of an implantable optical-electronic array: 1 - the body of the array; 2 -integrated optrode; 3 - multichannel electrical data recording; 4 – electrode needle; 5 - optogenetic control signal.*

Future plans and conclusions

Alzheimer's disease (AD) and aging are resulting in impaired ability to store memories, but the mechanisms responsible for these defects are poorly understood. It is known that electrophysiological response of mutant mouse neuron cultures in case of electrical stimulation results in significant decrease in frequency of action potentials [7]. Our future plans include use of optogenetics in slices and live animals from AD models. For these purposes we want to use the prototype of our optrode. Our future plans also include using the device in long-term experiments on the spinal cord motor neurons stimulation using optogenetic techniques.

Acknowledgments

This work was supported by the state grant 17.1360.2014/K, by the Russian Scientific Fund grant 14-25-00024: The financial support was divided in the following way: experiments depicted on Figure 1 was supported by the state grant 17.1360.2014/K, experiments depicted on Figure 2 was supported by the Russian Scientific Fund grant.

References

1. Deisseroth K 2010Sci.Am.303(5)48
2. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K 2005 Nat. Neurosci.8(9) 1263
3. Windhorst U, Johansson H1999Springer 190
4. He B, Soderlund D M 2010 Neuroscience Letters469(2) 268
5. Sparta D.R., Stamatakis A.M., Phillips J.L., et al., 2012, 7(1) 12.
6. Anikeeva P., Andalman A.S., Witten I., et al., 2011, Nature Neuroscience 15(1) 163.
7. Zhang H, Jie Liu J, Sun S, Pchitskaya E, Popugaeva E and Bezprozvanny I2015Journal of Alzheimer's Disease (45) 561

Complex Behaviour in Cyclic Competition Bimatrix Games

*Cezary Olszowiec**

Departament de Fisica, Universitat Politecnica de Catalunya, Terrassa, Barcelona, Spain.
* Presenting e-mail: c.olszowiec14@imperial.ac.uk

We consider an example of cyclic competition bimatrix game which is a Rock-Scissors-Paper game with perfect memory of the playing agents.

At first we investigate the dynamics in the neighbourhood of the Nash equilibrium as well as the dynamics on the boundary of codimension 1 - that is when one of the strategies is not played by any agent.

For the analysis of asymptotic behaviour close to the boundary, we provide the description of naturally appearing heteroclinic network, with discretized dynamics in its neighbourhood.

Quotient network is investigated as well and compared to the invalid models already considered in the literature.

It turns out that certain types of behaviour are never possible or appear in the system only for some parameter values.

Moreover, parameter space is divided into four regions where we observe either chaos, or preference to follow itinerary consisting of strategies for which one or the other agent do not lose, or they alternate in winning.

These regions are separated by two analytical curves and lines where game is either symmetric (system is not C^1 linearisable) or is zero-sum (so the system is Hamiltonian).

On each of these curves we observe different bifurcation scenarions: e.g. transition from order to chaos, or from one kind of stability to other one, or just loss of one dimension of the local stable manifold of the subcycle.

Stimulus Induced Plasticity in Dissociated Neuronal Network with Direct Connectivity

*V.N.Kolpakov[1,3] *, Y.I. Pigareva[1], A.A. Gladkov[1,3], E.I. Malishev[2], A.Y. Bukatin[2], A.S. Pimashkin[1], V.B. Kazantsev[1], I.V. Mukhina[1,3]*

[1] Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;
[2] Saint-Petersburg Academic University - Nanotechnology Research and Education Centre of the RAS, Saint-Petersburg, Russia;
[3] Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia.
* Presenting e-mail: kolpakov@neuro.nnov.ru

Aims

In this study we used hippocampal neuronal cultures grown on microfludic device which consisted of two chambers with two cultures. The cultures were synaptically coupled by axons grown through microchannels. We used electrical stimulation applied to these pathways to induce synaptic plasticity of the neuronal culture. This method allows to explore a fundamental mechanisms of synaptic plasticity at the network level, and the interaction of cells in the brain.

Methods

Neuronal hippocampal cultures (E18) grown on microstructures (chips) made of PDMS (polydimethylsiloxane) allow to control the growth of neuronal branches with a given direction through the microchannels. The separation of cellular populations allow to determinethe presynaptic and postsynaptic neurons, which is necessary for effective application of electrical stimulation protocols, causing potentiation or depression. For study of synaptic plasticity we observed changes in efficiency of electrical activity propagation between two separate neuronal cultures, connected by axonal branches. To register the electrical activity of the neuronal cultures we used multielectrode arrays (Multichannel systems, Germany) with 59 independent electrodes.

Results

To induce potentiation and depression we used STDP electrical stimulation protocol. The protocol consisted of a series of stimuli at 50 Hz and it was applied to two groups of electrodes in the pre- and postsynaptic neurons. The delay between the stimuli of different groups of the electrodes in stimulation was 50 ms. First we conducted low frequency stimulation (60 pulses at each of seven selected electrodes - 3 presynaptic area, 3 postsynaptic area, one in the microchannel). Then conducted high frequency stimulation (± 800mV, 260 ms per phase 20 stimuli with 100 ms intervals, 150 trials with 6 sec. intervals.

Conclusions

Preliminary results show that network plasticity can be induced in cultured network of by electrical applied to the neurons in the microchannels. Probability of the burst propagation between chambers significantly changed after high frequency stimulation.

Acknowledgements

This research was supported by Russian Science Foundation (№ 14-19-01381).

References

1. Jimbo Y., Tateno T., Robinson H.P. Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons. // Biophys. J. 1999. 76. № 2. P. 670–8
2. Malenka R. C. and Bear M. F. LTP and LTD: an embarrassment of riches. Neuron 2004. 44,P. 5-21.
3. Pelt J., Vajda I., Wolters P. S., Corner M. A. and Ramakers G. J. Dynamics and plasticity in developing neuronal networks in vitro. Prog. Brain Res. 2005. 147, P. 173 -188.
4. Ruthazer E. S. You're perfect, now change – redefining the role of developmental plasticity. Neuron 2005. 45, P. 825 -828.
5. Wagenaar D., Pine J., Potter S.M. Searching for plasticity in dissociated cortical cultures on multi-electrode arrays. J. Negat. Results Biomed. 2006. 5. 16.

Unidirectional Axon Growth in Microchannels of Various Shapes

*Y.I. Pigareva[1] *, A.A. Gladkov[1,2], V.N. Kolpakov[1], A.S. Pimashkin[1], A.Y. Bukatin[3], E.I. Malishev[3], I.V. Mukhina[1,2] and V.B. Kazantsev[1]*

[1] Department of Neuroengineering, Center of Translational Technologies, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;
[2] Central Research Laboratory, Cell Technology Department, Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia;
[3] Saint-Petersburg National Research Academic University of the Russian Academy of Sciences, Saint-Petersburg, Russia.
* Presenting e-mail: risssum@gmail.com

Aims

Neuronal networks cultured in microfluidic chips are widely used for investigation of functional connectivity of neural culture with defined morphology [1, 2]. In this study we used hippocampal cultures plated in two chambers of microfluidic device which were connected by microchannels. We investigated various shapes of microchannels which guided axon growth in one direction between two cultures.

Methods

Microfluidic devices consist of two chambers, providing unidirectional axon growth between Source and Target neural networks (Fig. 1A). Microchannels' design was based on a sequence of various triangular segments: each segment has a "bottleneck" for reducible backward axons growth probability and convergent walls facilitated axons growing to Target chamber [2].
PDMS microfluidic chips were fabricated by two layer lithography and PDMS molding techniques. Mold design contained: first 5 μm-thick layer, which formed microchannel structure and second 50 μm-thick layer, which formed chambers. For mold fabrication, Silicon wafers and negative photoresists SU8 (MicroChem, USA) were used. Microchannels' structure was based on several types of segments (Fig. 1B) and their length was 600 μm (2 - 7 segments) which is enough to provide a growth only for axons. Dissociated hippocampal neurons were plated into separate chambers.

Results

In order to find optimal design for unidirectional connectivity between neuronal sub-populations we studied axon growth dynamics in various microchannels (Fig. 1B). We proposed three types of segment shapes and each type was consist of three segment lengths of 67 μm, 100 μm and 200 μm. The angle of segment's corner turned to "Target" was 45°. The diameter of "bottleneck" was 5 μm and it could be pulled or not. Each day after plating we analyzed individual neural branches in microchannels using automated microscope system Cell-IQ.

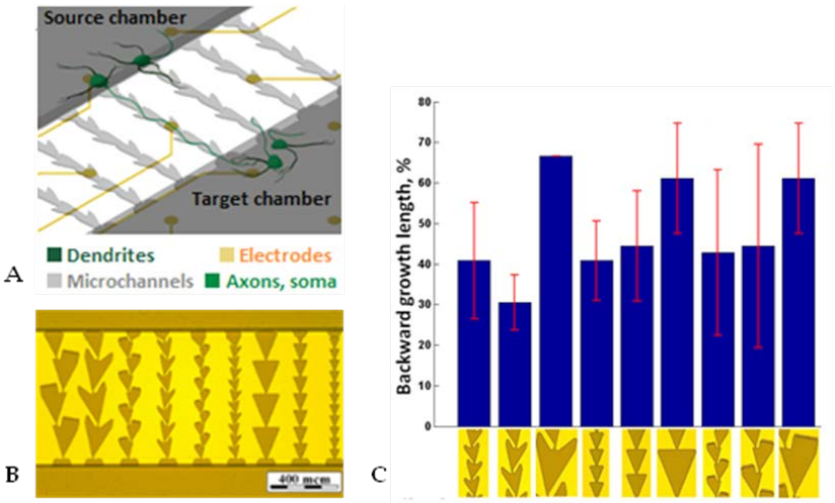


Fig.1 *(A) Design of microfluidic chip. Axons grow in microchannels from Source to Target chamber. (B) Various types of segment shapes. (C) Average distance of "backward" axons growth in relation to length of microchannel.*

For forward grow we found that in microchannels with pulled "bottleneck" axons growth in the "bottleneck" direction over some distance while in microchannels with ordinary "bottleneck" the axon can turn after entry in the segment. In the smaller segments axons pass through microchannel faster than in medium and big segments. For backward growing we found that if axons passed the "bottleneck" most probably they will grew alongside the sidewalls of the segments. For estimate an efficacy of microchannel we investigate axons growth in Target – Source direction during first five days. We measured the average number of segments that axons passed in "backward" direction. We found that "zig-zag" shaped segments with 100 μm length segments were the most effective. In general, 67 and 100 μm segments of all types showed similar results, while large and wide segments were least effective (Fig. 1C).

Conclusions

In this study we investigate microfluidic chips consists of two chambers with neuronal populations coupled by various microchannels wherein axons can grow. We found specific features of axon dynamics during growth in the segments of microchannels and found optimal design among proposed. In further study we plan to collect statistical data for axon growth and measure unidirectional efficacy of each microchannel.

Acknowledgement

The research is supported by the Russian Science Foundation (№ 14-19-01381).

References

1. Habibey R., Golabchi A., Latifi S., Difato F., Blau A. (2015). Microchannel device for selective laser dissection, long-term microelectrode array electrophysiology and imaging of confined axonal projections. Lab Chip, 15(Lmd), 4578–4590.
2. Malishev E., Pimashkin A., Gladkov A., Pigareva Y., Bukatin A., Kazantsev V., Mukhina I., Dubina M. (2015). Microfluidic device for unidirectional axon growth. Journal of Physics: Conference Series, 643, 012025

The Implementation of the Cost-Effective and Adaptive Two-Photon Microscope for Neuroscience

A.V. Popov, M.S. Doronin, Y.V. Dembitskaya, A.V. Semyanov*

Institute of Neuroscience, Lobachevsky State University of Nizhny Novgorod,Nizhny Novgorod, Russia.
* Presenting e-mail: popov@neuro.nnov.ru

Two-photon microscopy plays an important role in studies of the brain functioning. Particularly, it enables to visualize with a high and spatial resolution such crucial processes of neuronal functioning as changes of membrane potential (voltage-sensitive imaging) or alterations of calcium concentration (calcium imaging). Additionally, two-photon microscopy produces a relatively low photodamage to the tissue and allows to conduct relatively long measurements without significantly affecting the cell functioning not only in vitro, but also in vivo. For the last two decades, two-photon microscopy served a key role in the progress of neuroscience. However, commercial versions of microscopes usually are expensive and difficult to adapt for highly specific tasks. The commercially build software that controls the parts on a two-photon system has great limitations for rearrangements and adaptation for a particular experimental tasks as well as the hardware of such systems.

Therefore, our goal was to create a custom two-photon microscope, equipped with two adjustable two-photon femtosecond lasers (680 – 1080 nm) controlled by custom-made software. That is enable us to visualize neurons with high spatial-temporal characteristics and to stimulate locally with the high precision individual synapses (dendritic spines) by glutamate uncaging, e.g. MNI-caged-L-glutamate. Additionally, that provide us with a direct access to the modification and easy access to the program code, that can be quickly adjusted to our specific tasks, such as monitoring of local calcium events in neurons and astrocytes, which requires high sensitivity and efficiency of the system. In order to achieve that, the localization of the photomultiplier was optimized by placing it maximally close to the objective, that greatly reduces the number of lost photons and represents a distinct feature of this microscope. Another a key feature of this microscope is the custom-made software, that is highly adjustable and has been optimized for specific tasks in vitro and in vivo.

As a result , the custom-made two-photon microscope represents a highly efficient, purpose-built and cost-effective system, that is remarkably useful for conducting experimental procedures in vitro and in vivo in order to investigate the brain functioning.

Acknowledgements

This work was supported by the grant Russian Science Foundation (16-14-00201).

Studying on-off Firing Patterns in the Network of Coupled Hodgkin-Huxley Neurons in the Presence of Noise

P.M. Esir, A.Yu. Simonov, S.Yu. Gordleeva*

Lobachevsky State University, Nizhny Novgorod, Russia.
* Presenting e-mail: esir@neuro.nnov.ru

Abstract. Bistable dynamics is observed in various nervous systems [1], and it's considered as one of ways of organization of working memory [2]. Dynamics of single or two unidirectional coupled neurons were studied in previous works [3-4]. In our work we study dynamics of ensemble of recurrently coupled bistable Hodgkin-Huxley neurons, in the presence of noise and coupling delays. The neurons were distributed in a volume and had delays proportional to distance between them. External noise to each on neuron can evoke transitions of individual neurons from oscillatory state and steady state so named on-off firing pattern. Interesting result of our work is that such kind of on-off firing patterns is observed also in mean firing rate of whole network, Fig. 1. We found, that durations of staying in the different stable states for big time scales have exponential statistics, while for small times scales the statistics obey power law function. When the noise was switched off, the system moved to steady state in which partially synchronization between part of neurons is observed. In the case of absence of connection delays this phenomena cannot be observed, mean firing rate of whole network does not demonstrate 2 distinct states. By using Phase Response Curve generalized for 2 stimuli we explain this effect. We conclude that delays play important role in neural systems where on-off firing patterns is observed.

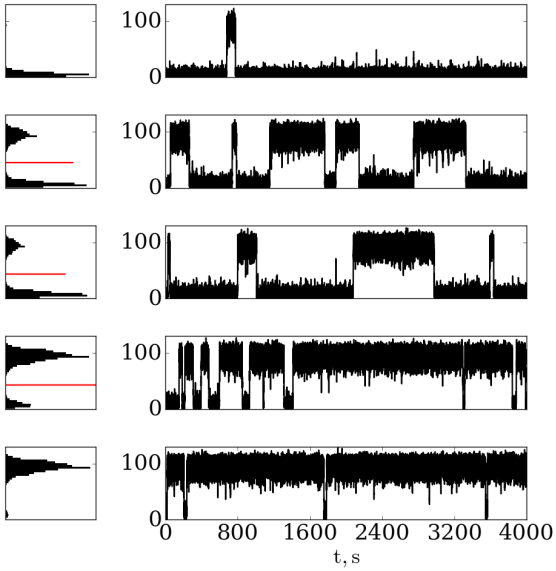


Fig.1. *Firing rate for different noise intensities. From up to down, noise intensity increase.*

Acknowledgements

This work was supported by Russian Science Foundation grants No. 16-12-00077, 14-11-00693.

References

1. Huaguang, G., Zhiguo, Z., Bing, J., & Shenggen, C. (2015). Dynamics of On-Off Neural Firing Patterns and Stochastic Effects near a Sub-Critical Hopf Bifurcation. Plos One, 10(4), e0121028. http://doi.org/10.1371/journal.pone.0121028
2. Durstewitz, D., Seamans, J. K., & Sejnowski, T. J. (2000). Neurocomputational models of working memory. Nature Neuroscience, 3 Suppl(november), 1184–1191. http://doi.org/10.1038/81460
3. Kazantsev, V. B., & Asatryan, S. Y. (2011). Bistability induces episodic spike communication by inhibitory neurons in neuronal networks. Physical Review E Statistical Nonlinear and Soft Matter Physics, 84(3-1), 031913. http://doi.org/10.1103/PhysRevE.84.031913
4. Simonov, A. Y., Gordleeva, S. Y., Pisarchik, A. N., & Kazantsev, V. B. (2014). Synchronization with an arbitrary phase shift in a pair of synaptically coupled neural oscillators. JETP Letters, 98(10), 632–637. http://doi.org/10.1134/S0021364013230136

Two-Theta Neuron Model: Novel Phase Reduced Model Explored in Central Pattern Generators

Aaron Kelley and Andrey Shilnikov*

Georgia State University, 100 Piedmont Ave SE, Atlanta, USA.
* Presenting e-mail: akelley11@student.gsu.edu

We explore dynamical foundations of rhythmogenesis and its stability in neural circuits using a reduced, 2-theta phase model for bursting cells. Of special interest are central pattern generators (CPG) capable of producing multiple [co-existing] bursting patterns/outcomes with specific and robust phase lags that underlie and determine a variety of locomotion functions of animals. Biological applications include models and emulation of swim CPGs of sea slugs such as Tritonia, Melibe, and Dendronotus, as well as lobster pyloric networks.

We show how key features of network dynamics, including robustness, occurrence, and metamorphoses of rhythmic outcomes, can be disclosed and evaluated using 2D return maps for phase-lags that provide the comprehensive network characterization in terms of fixed points, invariant circles and their stability and bifurcations. We show a de-facto proof of the excellent agreement between dynamics of networks comprised of phenomenologically reduced phase models and biologically plausible Hodgkin-Huxley type bursters with chemical and electrical synapses.

The versatility of our approach allows us to extend the model's applicability to larger neural networks, specifically ones with modular organization. The 2-theta neuron model also offers the benefit of greatly reducing computation costs and simplifies constructing large networks with complex intrinsic connectivity.

Development of a Method for the Estimation of Gap-Junctional Parameters

I. Falk[1] and Y. Timofeeva[2]*

[1] Mathematics institute, University of Warwick, Coventry, CV4 7AL, United Kingdom;
[2] Department of Computer Science and Centre for Complexity Science, University of Warwick , Coventry, CV4 7AL, United Kingdom.
* Presenting e-mail: isak.falk@live.se

Gap junctions, also referred to as electrical synapses in neuronal cells, are expressed along the entire central nervous system and are important in mediating various brain rhythms in both normal and pathological states. They are mechanical and electrically conductive links between two adjacent cells that allow the passage of ions and small molecules from the cytosol of one cell to the cytosol of its neighbouring cell. Many instances of gap-junctional coupling are formed between dendritic arbours of individual neuronal cells. Locations and strengths of these distal gap junctions in real neuronal networks are often difficult to measure experimentally. Here we aim to develop an approach for predicting these gap-junctional parameters from a limited number of somatic stimulations. Our approach employs a recently developed method for analytically constructing a response function on gap junction-coupled neuronal networks [1]. It can be applied to simplified neuronal morphologies obtained by a reduction method shown in [1] as well as to more biologically realistic complex structures. Although the focus here is on a neuronal network, the method can be easily modified for a network of astrocytes interconnected through gap junction channels and communicating by intercellular calcium signalling [2, 3].

References

1. L. Yihe and Y. Timofeeva, Biol. Cybern., 2016, pp 1-17, DOI: 10.1007/s00422-016-0681-y.
2. C. Giaume, A. Koulakoff, L. Roux, D. Holcman, and N. Rouach, Nat. Rev. Neurosci., 11, 87-99, 2010.
3. A. Volterra, N. Liaudet, and I. Savtchouk, Nat. Rev. Neurosci., 15, 327-335, 2014.

The Role of Sip1 in the Spine Density of Neocortical Neurons

*V.A. Salina[1] *, E.A. Turovsky[1], M.V. Turovskaya[1], A.A. Babaev[1], V.S. Tarabykin[1], M. Rosario[2]*

[1] Institute of Biology and Biomedicine, Lobachevsky State University of Nizhny Novgorod, Russia;
[2] Institute for Cell and Neurobiology, Center for Anatomy, Charité-Universitätsmedizin Berlin, Germany.
* Presenting e-mail: tina_salina@mail.ru

Abstract. Sip1 (also Zfn1b or ZEB2) is a transcription factor that regulates many processes in embryonic development. The expression of Sip1 is region–specific in developing cerebral cortex. Sip1 begins express starting at E8.5 of development in the neural crest cells and in the developing neural epithelium, then around at E12.5 Sip1 transcripts are present in the cortical plate, ventricular zone of the basal ganglion, midbrain and thalamus pons. In the brain of adult mice Sip1 expression is detected on white matter of the neocortex, dentate gyrus and hippocampus. The transcription factor of Sip1 consists from two clusters of zinc fingers that contributed binding to two spaced sequences of E- box in regulatory regions of target genes, activated Smads - proteins, the chromatin- remodeling corepressor complex NuRD and CtBP-1/2. Also, in human, mutations in one of allele of Sip1 gene is a cause the Mowat - Wilson syndrome that is characterized by epilepsy, intellectual disability, severe mental retardation and craniofacial defects (Srivatsa S. and al., 2015).

The neocortex is the most difficult structure in the brain. The neocortex consists of the white matter, or myelinated axons with unmyelinated fibers, and the grey matter, or neuronal cell bodies.

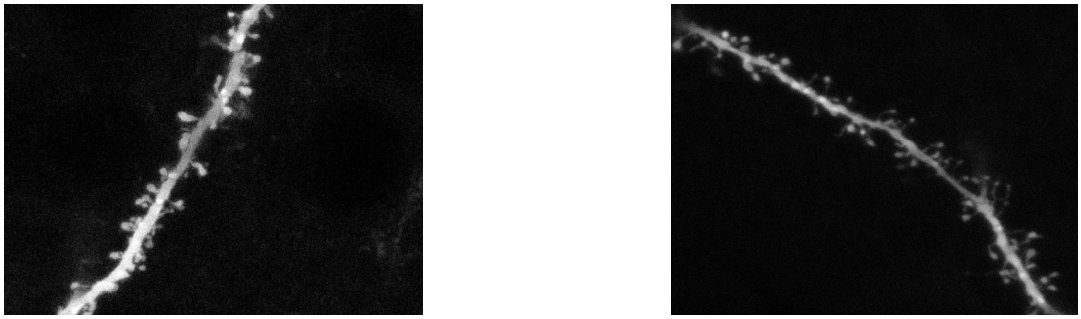
The neocortex plays an important role in memory, sleep and learning processes. Also the neocortex involved in instrumental conditioning, in the neurological processes and in human cognition (Parthasarathy S. and al., 2014).

Goal

To investigate the role of Sip1 in the spine density of neocortical neurons.
Methods: to study the dendritic spine density we did the pictures on confocal microscope Leica TLC SL Microsystems using 60x objective and 6x zoom.

Results

We used 23-old electroporation brains of the Sip1- FNC- line with genotype (fl/wt; cre/cre) and the Sip1- F- line with genotype (fl/fl; cre/wt) or (fl/fl; cre/wt). So the dendritic spine density of mutants are not high , they don't have a large spine head, shot spine neck and mushrooms, stubby, thin compared with wild- type in which is better developed the dendritic spine density (Figure 1).



A B

Fig. 1. *A - The spine density of dendrites in wild-type; B - The spine density of dendrites in mutant.*

Conclusion

The expression of Sip1 regulates many different aspects in the embryonic development and adult stage. Dendritic spines are a small structure where store a synaptic strength and they are also involved in electrical signal transduction between neurons. Also they serve to increase the number of possible contacts between nearby neurons. We suppose that low spine density in Sip1 knock-out mice decreases the number of contacts between cells and significantly reduces transduction of signal.

References

1. Parthasarathy S., Srivatsa S., Nityanandam A., Tarabykin V. Ntf3 acts downstream of Sip1 in cortical postmitotic neurons to control progenitor cell fate through feedback signaling. Development. 2014 Sep;141(17):3324-30. doi: 10.1242/dev.114173. Epub 2014 Aug 1.
2. Srivatsa S., Parthasarathy S., Molnár Z., Tarabykin V. Sip1 Downstream Effector ninein Controls Neocortical Axonal Growth, Ipsilateral Branching, and Microtubule Growth and Stability. Neuron. 2015. Volume 85, Issue 5, Pages 998–1012.

Up- and Down- Regulation of H-Channels Conductance in CA1 Hippocampal Neurons

M.S. Doronin, Yu.V. Dembitskaya, A.V. Semyanov*

Institute of Neuroscience, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: doronin@neuro.nnov.ru

N-methyl-D-aspartate receptors (NMDARs) play an important role in induction of long-term potentiation (LTP). During LTP induction activation of synaptic NMDARs also triggers up-regulation of hyperpolarization-activated (h) channels (Fan et al., 2005). In contrast, prolonged activation of extrasynaptic NMDARs down-regulates h-channels (Gh) (Wu et al., 2012). Thus, Gh plasticity depends on synaptic and extrasynaptic NMDARs activation and balances the overall neuronal excitability increased by LTP. However, it remains unknown if this plasticity can be specific to subcellular regions and if local plasticity can affect the average conductance of the whole neuron.

Therefore, we investigated if the Gh up-regulation in one dendritic branch and down-regulation in another branch might occur simultaneously during induction of LTP and control the overall cell conductance.

We used the two-photon imaging and single-photon glutamate uncaging in order to monitor the state of different subcellular compartments. We performed whole-cell patch-clamp recordings in CA1 pyramidal neurons of C57BL/6 mice (male, P28-35) hippocampal slices. Slices were pre-incubated with a specific inhibitor of vacuolar-type H+-ATPase – bafilomycin A1 (4 μM) to prevent the vesicular release. Neurons were loaded with 50 μM Alexa 594 through the patch pipette that enabled us to visualize dendritic shafts and spines by using the two-photon excitation with the 830 nm wavelength. 400 μM MNI-caged-L-glutamate was applied to the bath and uncaged with 405 nm laser at the vicinity of several (6-9) dendritic spines for 5–10 ms. Uncaging-induced EPSPs (uEPSPs) were recorded in soma. The spike-time dependent plasticity-inducing (STDP) protocol was applied in order to induce local LTP in specific dendritic spines. In these spines ("active") local glutamate uncaging was combined with somatic current injections triggering theta-bursts of action potentials. The amplitude and half-width of uEPSPs in "active" spines, their neighbors on the same dendritic brunch ("neighbor" spines) and spines at different dendritic brunch ("remote" spines) were compared before and after induction of STDP.

We found a significant decrease in the input resistance of the neurons (n=8, p=0.0234), suggesting that plasticity induction in localized dendritic shafts might affect overall cell conductance. However, we did not observe a significant difference in the amplitude and half-width of uEPSP in "active", "neighbor" or "remote" spines. These results might indicate an important mechanism of homeostasis that controls synaptic strength and cell excitability, acts via h-channels and equilibrate overall cell conductance.

Acknowledgements

This study was supported by the research grant of RFBR foundation No. 16-34-00961 мол_а

Analytical and Numerical Study of Finite-Size-Induced Transitions to Synchrony in Oscillator Ensembles with Nonlinear Global Coupling

C. Gong[1] and A. Pikovsky[2]*

[1] University of Potsdam, Potsdam, Germany;
[2] University of Potsdam, Potsdam, Germany.
* Presenting e-mail: cgong@uni-potsdam.de

Abstract. In this study, we consider the Kuramoto model of finite number of phase oscillators coupled via a nonlinear mean field, specifically when it is proportional to the the square of the Kuramoto order parameter (See equation 1). In many experimental setups, a model that allows for couplings which include higher harmonics is needed, such as electrochemical oscillators and φ-Josephson junctions. Moreover, coupling terms can be nonlinear functions of the order parameters. It has also been shown that such a model is microscopically equivalent to a fully connected hypernetwork where interactions are via triplets.

$$\dot{\varphi_k} = \varepsilon R^2 sin(2\Theta - 2\varphi_k) \text{where } Re^{i\Theta} = \frac{1}{N}\sum_{k=1}^{N} e^{i\varphi_k} \quad (1)$$

As observed first by Komarov and Pikovsky [1], oscillators of identical frequency and zero noise under such a model form two asymmetrical clusters, which obeys scaling law of N1/2. See Figure 1.

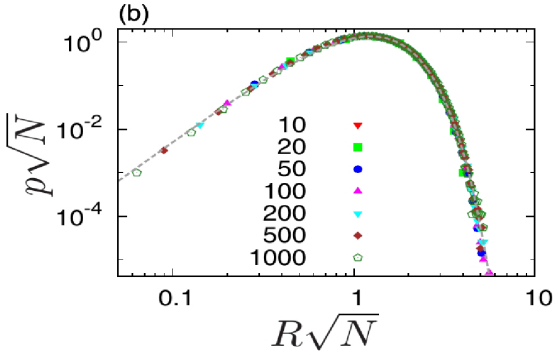


Fig.1. *Histogram of the two-cluster state from disordered initial condition with various ensemble sizes with N1/2 scaling. [1]*

We discuss numerical and statistical approaches, allowing one to establish scaling observed by Komarov and Pikovbsky. Our starting point is to find the breaking point in a chain of phase oscillators when they are brought to synchrony under a conventional Kuramoto mean field. Analytically, we reformulate the Watanabe-Strogatz dynamical equations in complex notations, attempting to find the singularity which corresponds to the "breaking point" or the "unsynchable phase" in the Kuramoto model. Numerically we have been able to locate such a singularity in numerical simulations of all to all coupled oscillators when the unsynchable phase variable actively participates in the mean field. See Figure 2. The location of such a phase via passive flow acertype variable is also being studied.

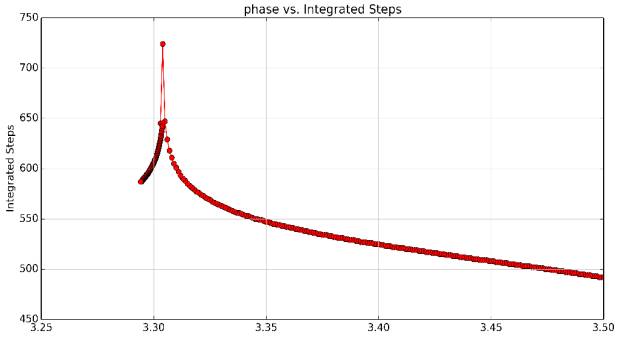


Fig.2. *Numerical simulation of the unsynchable point in a phase chain*

References

1. M. Komarov and A. Pikovsky, Phys. Rev. E, v. 92, 020901(R) 2015

OM&P

Detection of the Eeg-Correlates of Subjective Significance of Visual Stimuli

*I.P. Ganin[1,2] *, E.A. Kosichenko[1] and A.Ya. Kaplan[1,2,3]*

[1] Lomonosov Moscow State University, Moscow, Russian Federation, ipganin@mail.ru
[2] Lobachevsky State University of Nizhni Novgorod, Nizhni Novgorod, Russian Federation
[3] Pirogov National Russian Medical University, Moscow, Russian Federation
* Presenting e-mail: ipganin@mail.ru

Aims of the study

Brain-computer interface (BCI) is a highly developing area of investigation in neurophysiology. BCI provides the users brain with new output pathways, enabling an individual to send messages or control devices without using muscles, directly using their electrical activity (EEG). One of the most popular BCIs, the P300 BCI, was proposed as a variation of the classical visual oddball paradigm, a discrimination task well known for eliciting the P300 wave of the event-related potentials (ERP). In this BCI a command is detected by the maximal P300 amplitude in response to flashes of the attended symbol compared to low P300 amplitude in response to all other unattended symbols. However, the attention response to external stimulus can be obtained not only when the user has to respond to the instructed symbol flash, but even when the user does not have specific instruction and the presented stimuli vary in their significance for the user on the basis of his personal experience or emotional state. We hypothesize that using P300 BCI algorhytms and appropriate experimental design a system for EEG- and ERP-based detection of not overt, but covert, intentions or psycho-emotional states of subject can be built. This can be applied in medicine, marketing research, entertainment etc. For primary evaluation of the classification possibility of ERPs in response to stimuli with covert meaning the primary goal was to study ERPs in response to stimuli with overt emotional meaning. In this study we used images of human faces as such emotional stimuli. Thus the aim of this work was to compare the ERPs in response to presenting emotional stimuli against neutral stimuli under conditions when emotional stimuli are attended or unattended.

Methods

Fourteen healthy volunteers (age 19-20 years, five males) participated in the study. Each participant signed informed consent. The participants sat in an armchair and viewed a 24-inch LCD monitor at approximately 80 cm distance from their eyes. The stimuli were presented in the center of the screen and had an angle size of 7.4 x 5.5°. All the stimuli were emotional or neutral human faces photos from the Park Aging Mind Lab database (UT Dallas). The stimuli presentation was organized in 'stimuli sequences' which means presentation of six different photos consequentially in random order. Each stimulus was presented for 200 ms with 400 ms interval. One run consisted of ten stimuli sequences without pauses between them that corresponded presenting totally 60 stimuli (ten times for each photo). The experiment consisted of three blocks; each of them included ten runs. The participants' task in the first block ('Emotional Unattended') was just to look at the center of the screen, where the photos were presented. There was one emotional photo and five neutral photos in each run. The task in the second and the third blocks was to remember the target photo at the beginning of the run and then silently count the number of times this photo was presented among other photos. But in the one block ('Emotional Attended') the target was always the emotional face among neutral ones, and in the other block ('Neutral Attended') the targets and non-targets were both neutral faces. The order of the last two blocks was randomly alternated in all experiments. EEG was recorded from 24 electrodes with reference on mastoids. We analyzed the ERP waveforms and amplitudes for target and non-target stimuli. We also calculated offline classification accuracy for target stimuli in each block for each participant in P300 BCI-similar fashion. In the first block we analyzed group of emotional photos (as targets) against neutral faces (as non-targets), as there was not any discrimination task.

Results

We compared amplitudes of several peaks for the ERP difference (target minus non-target) in three blocks. The amplitudes of N170 and P200 components were higher for the two blocks with emotional faces compared to neutral faces. The amplitudes of N170, P200, P300 and N400 components were the highest in the 'Emotional Attended' condition. For offline classification we used Fisher's linear discriminant analysis which chose one target from six stimuli in each run (thus the random classification level was 1/6 = 16.7%). The maximal mean accuracy for 'Emotional Attended' and 'Neutral Attended' conditions were 97.1% and 89.3%, respectively. Although in the 'Emotional Unattended' condition participants did not have any instructions except looking at the stimuli, the classification accuracy for emotional faces was 36.4%, which exceeds the random level more than twice.

Conclusions

In our work we made an attempt to find a specific stimuli setup where one stimulus can elicit a prominent ERP response that can be distinguished from ERPs to other stimuli. An emotional face among neutral faces was used as such stimulus under conditions where subjects' task was just to look at all presented stimuli without any other instructions. We found out that such unusual stimulus can not only enhance several ERP components, but also be classified as target stimulus among non-targets as if it was a typical P300 BCI task where the user has to pay attention to the desired stimulus. A particular advantage of using emotional faces as stimuli can be developed from comparing the two conditions where participants had to pay attention to one target stimulus and ignore all the other stimuli: the amplitude of all analyzed ERP components and classification accuracies were higher for 'Emotional Attended' vs 'Neutral Attended' conditions. Our results provide evidence that using stimuli with emotional faces can increase the efficiency of P300 BCI operating and, with adopting presentation paradigm and classification techniques, can be helpful for creating systems for EEG-based emotion detection.

Acknowledgements

This study was partially supported by funding from the Skolkovo Foundation (project #1110034) and from Russian Science Foundation (#15-19-20053).

Multistable Dynamics in the Motif of Inhibitory Coupled Rulkov Neurons

*T. A. Levanova[1] *, A.O. Kazakov[1,2], G. V. Osipov[1]*

[1] Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;
[2] National Research University Higher School of Economics, Nizhny Novgorod, Russia.
* Presenting e-mail: tatiana.levanova@itmm.unn.ru

We study the model of small ensemble of inhibitory-coupled neuron-like elements based on phenomenological Rulkov model of neuron cell [1-4]. We study analytically and numerically possible transitional and stable regimes of neuron-like activity and bifurcation transitions between them. We focus on phenomena of regular and chaotic sequential switching activity. We describe the influence of couplings topology on the dynamics of the ensemble.

Acknowledgements

This study was supported by Russian Science Foundation (Contract N. 14 12 00811) and Russian Foundation for Fundamental Research (Contract N № 16-32-00835)

References

1. N.F. Rulkov, Phys. Rev. E, 2002, 65, 041922.
2. A.L. Shilnikov, N.F. Rulkov, 2003, Int. J. Bif. Chaos, 13(11), 3325.
3. A.L. Shilnikov, N.F. Rulkov, Phys. Lett. A, 2004, 328, 177.
4. N.F. Rulkov, I. Timofeev, M. Bazhenov, J. Comput. Neurosci., 2004, 17, 203.

OM&P

Whether the Motor Cortex Excitability Changes During Control of Phantom Hand Within P300-Based BCI Contour

*N.V.Syrov[1,3] *, D.D.Zhigulskaya[1], D.A.Kirjanov[1,3], S.V.Borisov[1,2], A.Ya.Kaplan[1,2,3]*

[1] Lomonosov Moscow State University, Moscow, Russian Federation;
[2] Pirogov Russian National Research Medical University, Moscow, Russian Federation;
[3] Lobachevsky State University of Nizhni Novgorod, Nizhni Novgorod, Russian Federation.
* Presenting e-mail: kolascoco@gmail.com

Introduction

Motor imagery (MI) activates brain regions participating in motor control, such as primary motor cortex (Brodmann area 4). Transcranial magnetic stimulation (TMS) studies demonstrated increased amplitudes of motor evoked potentials (MEPs) from this area what suggests increasing of cortical excitability during MI. In addition to the MI, an observation of movement also leads to motor cortex excitability increasing and this activation overlaps significantly with activation taking place during actual movement. These facts suggest that MI based brain computer interfaces (BCI) with robotic hand or exoskeleton movement can be proposed as potentially useful tools in rehabilitation from stroke and other brain injuries. However, the ability of a patient to form vivid motor images can be impaired. Even after many training sessions, such patients cannot achieve acceptable accuracy of BCI control.

In such a case, using 'P300 BCI' based on visual evoked potentials and commonly used in BCI spellers could be more promising.

The aim of our experiment was to study the motor cortex excitability changes during control movements of phantom hand fingers within P300-based BCI contour.

Methods

20 healthy right-handed volunteers (mean age, 36 years; age range, 24–68 years; 7 males and 4 females) were included into the study.

The electromyogram was recorded by standard EMG cup electrodes placed on the target muscles of the right hand [extensor digitorum communis (EDC) and flexor digitorum suprficialis (FDS)] and positioned according to the belly–tendon principle. The ground electrode was placed on the left processus styloideus ulnae. We then determined the stimulation intensity and "intermediate" hot-spot i.e. point on the scalp where TMS evokes MEPs of EDC higher than 1 mV and FDC higher than 0,5 mV peak to peak amplitude in 5/10 trials in subjects during rest condition (EDC has the lower threshold).

EEG activity was recorded at the CPz, Pz, Po3, Po4, Po7, Po8, O1, O2 electrode sites placed according to the international10-20 system and referenced to the linked earlobe electrodes. A ground electrode was attached to the forehead. Event related potentials were recorded during oddball visual stimulus presentation. In this study, blinks of light-emitting diodes (LEDs) on fingers of phantom hand were used as visual stimulus. Significant components of ERP were extracted from EEG by linear discriminate analysis (LDA). One finger of phantom hand was flexed when the particular features of ERD were detected. Simultaneously (several ms after start of movement of finger), single TMS pulse was applied to the above mentioned "intermediate" hot-spot. In addition, when participants were fully relaxed and looked at the inactive phantom hand MEPs were also recorded (correspond to baseline). MEPs of all subjects were normalized by baseline and separated corresponding to type of feedback (flexing of target finger, flexing of non target finger and absence of any movements of fingers). Kruskal-Wallis and Mann-Whitney tests were used to test whether MEPs amplitude differs between the conditions. P-values were adjusted by Dunn's test.

Results

Amplitude (peak-to-peak) of MEPs recorded during flexion of non-target finger decreased significantly compared to the other conditions (Fig.1.).

Moreover, post-hoc tests revealed that MEPs amplitude during baseline condition was significantly lower than during the BCI condition (control of finger flexion)(Fig.2). These results suggest the increases in corticospinal excitability during BCI condition, especially during wrong finger movement observation. The latter fact can be related to the most emotional reactions of participants during observation of unexpected finger flexion.

Our results suggest that motor cortex excitability changes may occur within 'P300- BCI' experiments with phantom hand fingers movements as a feedback

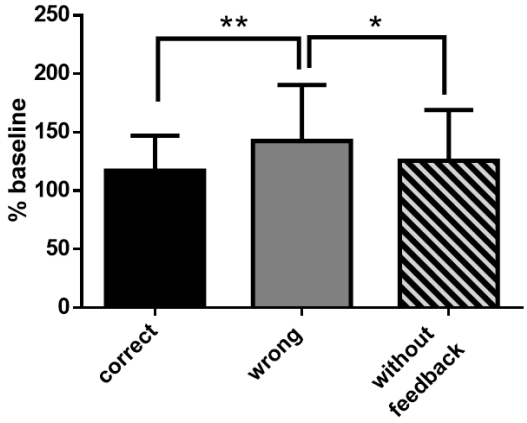


Fig.1. *Motor-evoked potential (MEP) for FDS data of all subjects regarding to different types of feedback. 'Correct' and 'wrong' correspond to MEPs which were recorded during movement of target and non-target finger respectively. "Without feedback" corresponds to the cases when participants watched flashes of LEDs but no any finger flexed . Amplitude of responses is presented as a percentage of the baseline condition mean MEPs . Columns and error bars are median and interquartile range of distribution of each group of MEPs. Note: **p <0,01; *p<0,05*

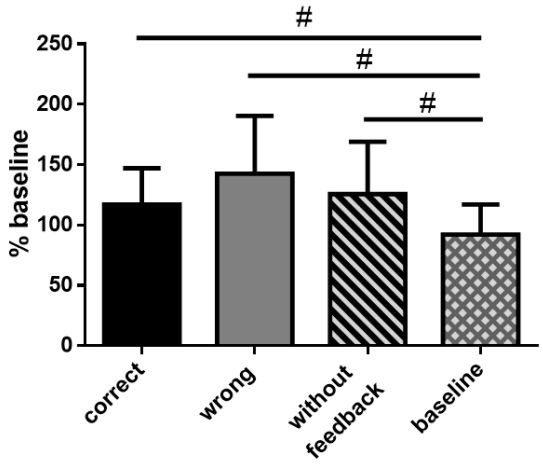


Fig.2. *Motor-evoked potential (MEP) for FDS data of all subjects regarding to to different types of feedback. Amplitude of responses is shown as a percentage of the baseline condition mean MEPs . Columns and error bars are median and interquartile range of distribution of each group of MEPs. Note: #p <0,0001*

Acknowledgements

This study was partially supported by funding from the Skolkovo Foundation (project #1110034) and from Russian Science Foundation (#15-19-20053).

References

1. Williams J. et al. The relationship between corticospinal excitability during motor imagery and motor imagery ability // Behav. Brain Res. Elsevier B.V., 2012. Vol. 226, № 2. P. 369–375.
2. Burns A., Adeli H., Buford J. a. Brain-Computer Interface after Nervous System Injury // Neurosci. 2014. Vol. 20, № September. P. 639–651.

OM&P

Is There a Connection Between BCI Performance and the Neurophysiological Effects of Motor Imagery?

*L.V. Yakovlev[1,2] *,A.N. Vasilyev[1,3], S.P. Liburkina[1,3] and A.Y. Kaplan[1,2,3]*

[1] Lomonosov Moscow State University, Moscow, Russian Federation;
[2] Lobachevsky State University of Nizhni Novgorod, Nizhni Novgorod, Russian Federation;
[3] Pirogov National Russian Medical University, Moscow, Russian Federation.
* Presenting e-mail: leojackovlev@gmail.com

Abstract. Motor imagery (MI) is a mental rehearsal of person's own body parts movement and thiscan be helpful as a training technique for neurorehabilitation [2, 3]. MI is known to promote patterns of the event-related desynchronization (ERD) of mu-rhythm found in EEG over the sensorimotor areas of the human cortex. Brain-computer interfaces (BCI) introduce ways to decode those specific EEG patterns into the control signal for external devices providing direct communication channel between the brain and the outer world [4]. Initially it seems that BCI approach promises additional benefit for the motor imagery practice by adding a feedback and therefore helping the subject to monitor the imagery quality. On the other hand, BCI-training favors subjects with a stronger mu-rhythm response which is not considered to be an indicator of the motor imagery effort quality. Due to the weak or absent EEG response during MI, a substantial portion of the population is characterized as «BCI-illiterate» or «inefficient» indicating poor performance in a brain-computer interface circuit [1], and therefore those people are being eliminated from such activity. The aim of our research is to clarify the connection between user's BCI performance and the neurophysiological effects of motor imagery.

In the present study, we used EEG and TMS (transcranial magnetic stimulation) to quantify and compare two physiological responses during MI in subjects with different levels of BCI performance (ranging from good to poor). The amplitude of TMS-induced motor evoked potential (MEP) in a resting muscle was used as a measurement of the excitability level of M1 cortex (fig. 1A), and 64-channel EEG was used to measure anERD during MI. Both measurements were related to the referential «visual attention» state. All participants underwent at least five sessions of MI-training containing both feedback and non-feedback runs. TMS assessment was conducted once during the last experimental session. Subjects were trained to perform motor imagery of sequential finger flexion and extension. During TMS the electromyogram from two forearm muscles (EDC and FDS) muscles was recorded.

We have found that increased M1 cortex excitability during MI was quantitatively uncorrelated with mu-ERDlevel (Spearman's r=0.23) and BCI performance (Spearman's r=0.28). Our results could be explained by a contribution of two reasons. At first, mu-rhythm power decreases during MI and thereby its modulation range is limited by resting-state power value which varies both in the general population and within subjects on different experimental days. That is why the subjects whose resting mu-rhythm power is low generally demonstrate poor BCI-performance. On the contrary, M1 excitability level increases during motor imagery (fig. 1A,1B) and therefore has greater measurable range.

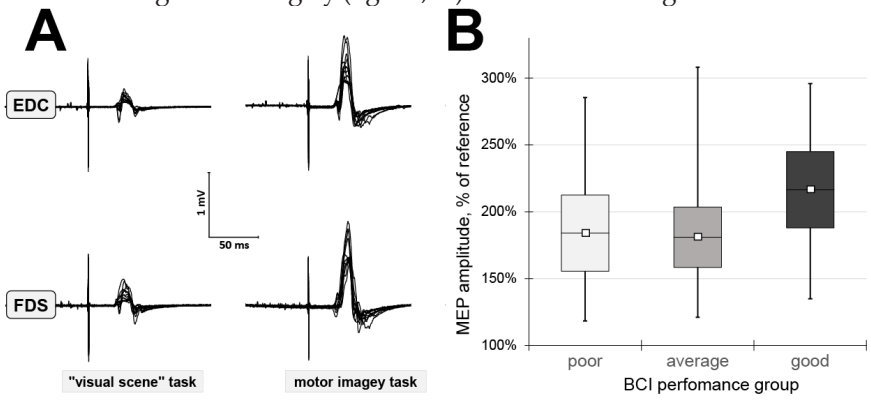


***Fig.1**. A - Example of MEPs from EDC and FDS muscles during MI and "visual task". B -Amplitude increase of MEPs in FDC during finger flexion imagery for subjects of three BCI performance groups.*

Secondly, mu-rhythm appears to be an indicator of the general inhibitory input into the vast cortical areas, whereas M1 excitability reflects the state of the local neuron group corresponding to a discrete muscle [5]. Based on prior knowledge, MI should promote excitability of local cortical pathways involved in imagined movement, but not necessarily alter general inhibitory output of thalamocortical circuits.

Our results suggest that if MI practice is considered to be beneficial in regard of training discrete motor cortex pathways, poor BCI performance should not discourage users from mental exercises. EEG control should be accompanied by other cortex excitability measurements such as TMS to provide a more comprehensive picture.

Acknowledgements

This study was partially supported by funding from the Skolkovo Foundation (project #1110034) and from Russian Science Foundation (#15-19-20053).

References

1. B. Allison andC. Neuper, Brain-computer interfaces, 2010, 35-54.
2. P. Jackson, M. Lafleur, F. Malouin, C. Richards, and J. Doyon, Arch. Phys. Med. Rehabil., 2001,82(8), 1133-1141.
3. S. Page, P. Levine, and A. Leonard, Stroke, 2007, 38(4), 1293-1297.
4. J. Wolpaw, N. Birbaumer, D.Mcfarland, G. Pfurtscheller, and T. Vaughan, Clin. Neurophysiol., 2002, 113(6), 767-791.
5. L. Fadiga, G. Buccino, L. Craighero, L. Fogassi, V.Gallese and G. Pavesi, Neuropsychologia, 1998, 37(2), 147-158.

Computational Model of Neural-Glial-Ecm Interactions

*S.V. Stasenko[1] *, I.A. Lazarevich[1], V.B. Kazantsev[1] and A.E. Dityatev[2]*

[1] Lobachevsky university, Nizhniy Novgorod, Russia;
[2] German Center for Neurodegenerative Diseases, Magdeburg, Germany.
* Presenting e-mail: stasenko@neuro.nnov.ru

Abstract. The study of principles and mechanisms of information processing in the brain is major issue of the modern neuroscience. Recent works have uncovered a participation glial cells (astrocytes) and ECM in information processing [1–4]. Astrocyte influences neural activity by releasing gliatransmitters: glutamate, D-serine and others. It has been discovered that ECM–mediated regulation mechanisms are involved in homeostatic modulation of neuronal activity [1,2]. Besides the well-known interactions, ECM-astrocyte interactions also may exist. Actrocyte acitivity leads to production of ECM molecules [1]. The influence of ECM molecules on astrocytes is associated with the change in the number and the properties of glial cells [5].

We present a model of neuronal activity regulation including ECM-glial-neuronal interactions. The neural network is modeled by a modification of the mean-field Wilson-Cowan-type model. The neural network consist of excitatory and inhibitory populations. The synaptic dynamics and astrocyte dynamics are modeled by using the mean-filed approach. The ECM is described by three activity-dependent variables (ECM molecules, ectoproteases and ECM receptors), which are involved in the following feedback loops: 1) decreasing the neuron excitation threshold due to ECM production; 2) increasing the excitation threshold due to ECM cleavage by ectoproteases; 3) changing effective strength of synaptic inputs due to signaling via the ECM receptors [2]. The ECM provides the regulation of the average firing rate, preventing hypo- or hyper-excitation of neurons due to the ECM-mediated feedbacks.

It was shown that interaction between ECM, astrocytes and the neuronal network leads to spontaneous activity oscillations on extended timescales. The interaction parameters determine the oscillation period (hours to days) and their existence and switching to bistable regimes.

Acknowledgements

The research was supported by Russian Science Foundation (Agreement No. 14-11-00693).

References

1. A. Dityatev and D. A. Rusakov, Curr. Opin. Neurobiol., 2011, 21, 353.
2. V. Kazantsev, S. Gordleeva, S. Stasenko, and A. Dityatev, PLoS One, 2012, 7, e41646.
3. A. Semyanov and D. M. Kullmann, Nat. Neurosci., 2001, 4, 718.
4. M. Min, Z. Melyan, and D. Kullmann, Proc. Natl. Acad. Sci. U. S. A., 1999, 96, 9932.
5. D. M. Kullmann, Neuron, 2001, 32, 561.

Design of Optoelectronic Interface Between Neuron-Like Generator and Living Neuron

M.A. Mishchenko, S.A. Gerasimova, A.V. Lebedeva, V.B. Kazantsev*

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: mischenko@neuro.nnov.ru

Abstract. Design of electronic interface between living neuron and neuron-like oscillator is one of the most intriguing challenges in modern science and engineering. Such systems would permit to develop a neuroprosthesis for biomedicine. Another interesting application is to make a new generation of information processing technologies based on brain computation principles.

Dynamics of electronic neuron oscillator coupled with living neurons via optoelectronic communication channel has been investigated. Such system mimics interaction between synaptically coupled brain neurons where the optical fiber imitates axon. The optoelectronic communication channel consists of light emission diode (LED), optical fiber and photodiode. Electronic neuron modulates the intensity of LED emission into the fiber and the photodiode detects the light and converts optical signal into electrical pulses that stimulates living neurons. We demonstrated experimentally that such connection can provide stimulation of neuronal firing.

Electronic neuron was implemented as pulse signal generator based on the FitzHugh-Nagumo model. This model provides a qualitative description of the main neurons' characteristics including excitable and self-oscillatory dynamics. Different neuron-like signals (single pulse, transients, self-oscillations) can be observed by changing threshold parameter.

Experiment of coupling electronic and living neurons via optoelectronic channel has been carried out. Such unidirectional signal transmission implements the functionality of excitatory synaptic coupling. Different amplitude of stimulation signal was observed by varying the load resistance of amplifier of photodiode signal. Local field potentials from living neurons have been observed by increasing amplitude of stimulation signal.

Acknowledgements

The work was supported by the Federal Target Program "Research and Development in Priority Areas of the Development of the Scientific and Technological Complex of Russia for 2014–2020" of the Ministry of Education and Science of Russia (Project ID RFMEFI57814X0074, Contract no. 14.578.21.0074).

References

1. S. Gerasimova, G. Gelikonov, A. Pisarchick and V. Kazantsev, JCTE, 2015, 60(8), 900-903.

Oscillations in Physiological Adaptation: Limit Cycles, Oscillating Death and Recovery

*A. N. Gorban[1] and T. A. Tyukina[2] **

[1] University of Leicester, Leicester, UK;
[2] University of Leicester, Leicester, UK.
* Presenting e-mail: tt51@le.ac.uk

In 1938, H. Selye [1] introduced the notion of adaptation energy as the universal currency for adaptation. He published "Experimental evidence supporting the conception of adaptation energy": adaptation of an animal to different factors (sequentially) looks like spending of one resource, and the animal dies when this resource is exhausted [2].

We aim to demonstrate that Selye's adaptation energy [1] is the cornerstone of the top-down approach to modelling of non-specific adaptation processes. We analyse Selye's [3] axioms of adaptation energy together with Goldstone's [4] modifications. Goldstone proposed the concept of a constant production or income of AE which may be stored (up to a limit), as a capital reserve of adaptation. It was shown that this concept best explains the clinical and Selye's own laboratory findings.

The models based on Selye's idea of adaptation energy demonstrate that the oscillating remission and oscillating death do not need exogenous reasons. These phenomena have been observed in clinic for a long time (for example, [5]) and now attract attention in mathematical medicine and biology.

We propose a series of models for interpretation of Selye's axioms. The adaptation models introduced and analyzed in this work utilize the most common phenomenological properties of the adaptation process: homeostasis (adaptive regulation) and price for adaptation (adaptation resource).

We started with the simplest model with two phase variables, the available free resource (AE) r_0 and the resource supplied for the stressor neutralization, r [6]. The model comprises only four processes: degradation of the available resource, degradation of the supplied resource, supply of the resource from the storage r_0 to the allocated resource r, and production of the resource for further storage (r_0). Analysis of this model has shown its impracticality as for such simple system immortality is possible under infinite load (stress). To overcome this drawback we introduced the following modification: AE production should decrease for large noncompensated stressors ψ= f - r. The modified system of equations has the form:

$$\begin{aligned} \frac{dr}{dr} &= -k_d r + k\, r_0(f-r)h(f-r); \\ \frac{dr_0}{dt} &= -k_{d0} r_0 - k\, r_0(f-r)h(f-r) + k_{pr}(R_0 - r_0)W(f-r), \end{aligned} \tag{1}$$

where k_d r is the rate of degradation of resource supplied for the stressor neutralization, where k_d is the corresponding rate constant; k_d0 r_0 is the rate of degradation of the stored resource, and k_d0 is the corresponding rate constant, we assume that k_d ≥ k_d0; kr_0 (f - r)h(f - r) is the rate of resource supply for the stressor neutralization, where k is the supply constant, h(f - r) is the Heaviside step function; k_pr (R_0 - r_0) is the resource production rate, where k_pr is the production rate constant; W(·) is the fitness (individual performance) function, given by:

$$W(\psi) = \left(1 - \frac{\psi\, h(\psi)}{\psi_0}\right) h\left(1 - \frac{\psi\, h(\psi)}{\psi_0}\right), \qquad \psi = f - r. \tag{2}$$

The connection between individual performance and 'fitness' is discussed in [6].

Selye, Goldstone and other researchers (for example, Garkavi et al. [7]) have acknowledged that there are different levels of the adaptation energy supply, with lower and higher energy spending. As an extension of the model (1) we introduce two storages of AE: resource r_0 (which is always available if it is not empty) and reserve r_rv (which becomes available when the resource becomes too low). The Boolean variable B_(o /c) describes the state of the reserve storage: if B_(o /c)=0 then the reserve storage is closed and if B_(o /c)=1 then the reserve storage is open. Thus the extended model can be described by the following system of equations:

$$\begin{aligned} \frac{dr}{dr} &= -k_d r + k\, r_0(f-r); \\ \frac{dr_0}{dt} &= -k_{d0} r_0 - k\, r_0(f-r) + k_{rv}\, B_{o/c}\, r_{rv}(R_0 - r_0) + k_{pr}(R_0 - r_0)W; \\ \frac{dr_{rv}}{dt} &= -k_{d1} r_{rv} - k_{rv} B_{o/c}\, r_{rv}(R_0 - r_0) + k_{pr1}(R_{rv} - r_{rv})W, \end{aligned} \tag{3}$$

where $W = 1 - \frac{f-r}{\psi_0}$.

Analyzing this model we found oscillations of the adaptation system near the border of death. Thus, the phenomena of 'oscillating death' and 'oscillating remission' can be predicted on the basis of the dynamical models of adaptation.

References

1. H. Selye, Nature, 1938, 141 (3577), 926.
2. H. Selye , Am. J. Physiol., 1938, 123, 758–765.
3. J.K. Schkade, and S. Schultz , Lippincott Williams & Wilkins, 2003, 181–221.
4. B. Goldstone, S. Afr. Med. J. , 1952, 26, 88–92, 106–109.
5. E. Gudayol-Ferré, J. Guárdia-Olmos, and M. Peró-Cebollero, Psychiatry research, 2015, 226 (1), 103–112.
6. A.N. Gorban, T. A. Tyukina, E.V. Smirnova, and L. I. Pokidysheva, J. of Theor. Biol. 2015, DOI:10.1016/j.jtbi.2015.12.017
7. L.Kh. Garkavi, E.B. Kvakina, and M.A. Ukolova , Rostov University Press, 1979.

OM&P

Effect of Previous Neuronal Activity on Characteristics of Action Potentials

E.N. Vasileva and A.V. Semyanov*

Institute of Neuroscience, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: vasileva@neuro.nnov.ru

Action potential has been being studied for many years, but it still perceived as signal, which operates according to the "all or none" rule, that does not fully reflect the importance of changes in the form of action potential. Therefore, the goal of this work was to study the impact of the previous activity of the neuron on the characteristics of the action potential.

The recordings of electrical activity of CA1 pyramidal neurons in the mice hippocampal slices were obtained in whole-cell current clamp mode. Cell firing in response to current steps of different amplitude, duration, and frequency was recorded. Then threshold, amplitude, half-width, the instantaneous frequency of AP and afterhyperpolarization were analyzed. Threshold, amplitude, and half-width of the first AP don't depend on the current step, and in the next AP's threshold and half-width increases and amplitude decreases with the increasing of steps' amplitude. Previous neuronal activity also increases slow afterhyperpolarization and decreases the instantaneous frequency of AP's.

Because the form of action potential determines presynaptic Ca^{2+} entry activity-dependent changes of AP's characteristics can regulate synaptic transmission by changing the probability of neurotransmitter release.

Acknowledgements

This work was supported by the Russian Science Foundation project № 16-14- 00201

Lactate and Ketone Bodies Regulate Astrocytic Calcium Dynamics in Early Postnatal Development

A. Lebedeva, E. Shishkova, A. Semyanov*

Institute of Neuroscience, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia
* Presenting e-mail: lebedeva@neuro.nnov.ru

Astrocytes may engage in the cellular mechanisms of learning and memory by regulation of energy metabolism. Lactate and pyruvate become energy sources for neurons during intensive work, in early ontogenesis and in some pathological conditions. However, the main energy substrates during early ontogenesis are ketone bodies [Zilberter et. al., 2010]. Since the neural activity depends on the availability of energy substrates supplied by astrocytes, this process can be considered as mechanism regulating neuronal function. Nevertheless, the role of ketone bodies, lactate and pyruvate in regulating astrocyte activity remains unclear. Using laser confocal microscopy, we have investigated how lactate, pyruvate and beta-hydroxybutyrate (thereafter, specific energy substrates), affect Ca^{2+} dynamics in astrocytes in rat hippocampal slices at different stages of postnatal brain development: postnatal day (P) 5, 15 and 30. The astrocytes were stained with Oregon Green BAPTA AM (7.95 μM), Ca^{2+} sensor, and sulforhodamine 101 (200 nM), a specific astrocytic marker. The application of each of specific energy substrate significantly increased the frequency of astrocytic Ca^{2+} events at P5, but not at P15 or P30. However, the specific energy substrates application in the presence of bafilomycin A1 (4 μM), a vesicular release blocker, decreased the frequency of Ca^{2+} events at P5. This suggests that specific energy substrates regulate astrocytic calcium activity at an early development stage though the changes in the vesicular release of neuro- and/or gliotransmitters. Since astrocytes play an important role in the brain development (e.g. the regulation of neuro- and gliogenesis, neuronal pathfinding, synaptogenesis), our results suggest that these processes can be affected by the availability of specific energy substrates.

Acknowledgements

This work was supported by the Russian Science Foundation under project №15-14-30000.

The Effects of Caloric Restriction and Western Diet on Astrocytes

A. Lebedeva, A. Plata, V. Tovpyga, P. Denisov, S. Makovkin, A. Semyanov*

Institute of Neuroscience, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia.
* Presenting e-mail: lebedeva@neuro.nnov.ru

The study of the processes involved in aging has ample importance in neurobiology. Life expectancy has augmented in developed countries with the consequent increase in the incidence of neurodegenerative diseases. Preliminary studies have shown that caloric restriction diet can improve memory in the elderly. Furthermore, high-fatty (Western) diet can contribute to the development of neurodegenerative disorders such as Alzheimer's disease. Most of the recent works have involved different mechanisms of neural networks in aging and neuropathological processes. However, the role of neuroglia has been less frequently studied. In order to elucidate the role of astrocytes in aging, we studied how low caloric and high fatty regimens alter astroglia activity over time. We use male mice C57/BL/6 of different postnatal days (P), slices are taken at day 1-3, 5, 7, 14 and 30 after the beginning of a specific diet. Caloric restriction diet consists of lowering to 70% the standard ration of the animals. Western diet contained 50% more fat than in the standard menu. The control mice receive food ad libitum in compliance with the standards stipulated for laboratory animals. Currents from astrocytes of hippocampal slices were measured by patch clamp technique in physiological stimulation and subsequently under pharmacological blockade of the excitatory amino acid transporters (EAATs) with TBOA (50 μM).

Acknowledgements

This work was supported by the Russian Science Foundation under project № 16-14-00201

GDNF Influence on the Morpho-Structural Integrity of Hippocampal Neural Network in Hypoxia Modeling *In Vitro*

*T.V. Shishkina[1,2] *, T.A. Mishchenko[2,1], E.V. Mitroshina[2,1], I.V. Mukhina[2,1], V.B. Kazantsev[2], M.V. Vedunova[1,2]*

[1] The Institute of Biology and Biomedicine, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;
[2] Molecular and cell technologies group, Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia.
* Presenting e-mail: schischkina.tatiana2012@yandex.ru

The preservation of neural networks' functional integrity under the influence of various stress factors is one of the topical issues in modern neurobiology. Neural networks are regarded as a minimal functional unit of the central nervous system, responsible for the transmission and information storage processes. Stressogenic factors, such as hypoxia, stimulate not only the death of the functionally important neurons, but reorganize the synaptic plasticity processes, which significantly affects the neural network functional structure. Nowadays, a substantial question, concerning the exploration of substances, able to maintain the viability and functional activity of brain cells in stressful conditions, remains open. Particular attention is given to investigation of neurotrophic factors, especially Glial cell line-derived neurotrophic factor (GDNF), which regulate various cellular processes during development and in mature brain. The one of the unique properties, that distinguishes this protein from other neurotrophins, is its ability to affect the synaptic plasticity by influencing on promoter activity of GluR2-subunit of AMPA-receptors.

In this regard, the aim of the investigation is to study the GDNF influence on viability and spontaneous bioelectrical activity of neural networks in primary hippocampal cultures during hypoxia modeling in vitro.

Materials and Methods

Dissociated hippocampal cells were taken from C57Bl6 mice embryos (E18) and cultured during 14 days in vitro according to the previously developed protocol on multielectrode arrays (Alpha Med Science, Japan) or coverslips. Hypoxia modeling was performed on DIV14 by replacing the normoxic cultural medium with a medium containing low oxygen for 10 min. The main parameters of spontaneous bioelectrical activity such as the number of bursts, the number of spikes in a burst, the burst duration were established. In addition, the method of correlation graphs was used. We also conducted the cell viability detection. Moreover, SmartFlareTM RNA Detection Probes (Merck Millipore, SFC-534, France) were applied for intravital detection of mRNA GluR2 in primary hippocampal cultures.

The carried out experiments revealed an acute increase in the number of dead cells (4,5 times) in primary hippocam-

pal cultures and, as a consequence, a complete inhibition of spontaneous bioelectrical activity of neural networks up to day 7 of the posthypoxic period. Preventive GDNF 1ng/ml application eliminates the negative hypoxic effects by increasing cellular viability as well as by maintaining the functional characteristics of neural networks.

Evaluation of the network structural changes by using the method of correlation analysis showed that hypoxia leads to simplification of the internal structure of neural networks in primary hippocampal cultures: there was a significant decrease in the number of active electrodes as well as in the average number of connections per electrodes (before: 4,22 ± 0,48; after 2,69 ± 0,41) on day 3 after hypoxia modeling. Moreover, the time of signal transmission from electrode to electrode was increased in 4,6 times ($p<0,05$, ANOVA).

Changes in the functional structure of network burst towards a simplification could be explained by loss of the part of functionally important neurons. Preventive GDNF application contributes to maintaining a complexity of network architecture: at constant/minor increase of the number of active electrodes an average number of connections on the electrodes is significantly reduced, a large value of electrodes with a low number of connections is detected, the amount of hubbs is decreased whereas the time of signal transmission is significantly higher than in the intact group ($p <0,05$ ANOVA).

To evaluate the possible molecular mechanisms of GDNF neuroprotective action, the GDNF influence on the expression of mRNA GluR2 subunit of AMPA-receptors was investigated. It was shown that hypoxia reduced the expression of mRNA GluR2 in primary hippocampal cultures whereas a preventive GDNF 1ng/ml application negates this effect, contributing to the increase in the number of mRNA GluR2 positive cells.

Therefore, GDNF is able to influence on synaptic plasticity under stress conditions.

Acknowledgements

The research was supported by grants of Russian Foundation for Basic Research № 16-34-00301, №16-04-00245 and prepared as a part of the state project "Provision Scientific Research".

Network Ca^{2+}- Cell Activity Field CA3 Hippocampal Slices of Rat Early and Late Postnatal Development

*Y.I. Mitaeva[1] *, A.M. Mozherov[1], I.V. Mukhina[1,2]*

[1] Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia;
[2] Novgorod State Medical Academy, Nizhny Novgorod, Russia.
* Presenting e-mail: yasya13@mail.ru

Hippocampus - the structure of the central nervous system, which is involved in the mechanisms of emotion and memory consolidation. The hippocampus has a certain topology distribution of cellular elements, which provides the many cellular networks. One of them is the network of neurons in the CA3 field. This network receives inputs from cells of the entorhinal cortex and the dentate gyrus, in addition CA3 pyramidal neurons form the connection between themselves and interneurons, forming a closed network that operates in conditions of acute slice and generates spontaneous Ca^{2+} activity. Neuronal network interacts with the glial network, the main manifestation of activity which is Ca^{2+} oscillations. Therefore, to estimate the age dependence of Ca^{2+} activity in the cells were investigated Ca^{2+} oscillations in neuronal and glial networks and the interactions between them.

In this work, we investigated changes in the characteristics of Ca^{2+} oscillations cells of rat hippocampal CA3 field in early (P5-8, P14-16) and late (P21-25), postnatal development. Also shown the effect of temperature of perfusion solution on cells Ca^{2+} activity of CA3 field hippocampal slices of rats in different postnatal periods. Besides in the study was valued role of network activity in the formation of spontaneous Ca^{2+} oscillations cells of rat hippocampal CA3 field in early and late stages of postnatal development.. Experiments were carried out on acute hippocampal slices from rats. Was used laser scanning confocal microscope Carl Zeiss LSM 510 Duoscan (Germany). Recording fluorescence kinetics were carried out in full frame (field of view of 400x400 mm), with a resolution of 256x256 pixels digital and scanning frequency of 1 Hz. Fluorescence indicators recorded in the range 500-530 nm (Oregon Green 488 BAPTA-1 AM) and 650-710 nm (Sulforhodamine 101). The fluorescence intensity (s.u.) shows the dependence of the concentration of $[Ca^{2+}]i$ in time, indicating the metabolic activity of cells. Method of cross - correlation analysis was used to evaluate synchrony of Ca^{2+} oscillations cells of CA3 field of rat hippocampus. We chose the time interval size in 3 seconds and within this interval were found synchronous Ca^{2+} oscillations in all possible pairs of cells. Further, the number of synchronously occurring Ca^{2+} oscillations were normalized to the minimum number of Ca^{2+} oscillations in one of the cells analyzed pairs.

The studies have shown that the parameters of cell Ca^{2+} oscillations field CA3 of hippocampal slices vary depending on

the period of postnatal rats. Reducing the amount of Ca^{2+} oscillations with age due to the formation and complexity of synaptically connected neural networks, the transition of electrical synapses in the chemical. Transitional period is 14-16 days of postnatal development, and for 21 days - there is a fully formed neural network. Electrically connected network is weakly controlled, excitement is freely distributed over the network, involving work of all cells, resulting in a high Ca^{2+} activity in rat hippocampal cells of younger age group. In mature hippocampal brain slices spontaneous Ca^{2+} activity with low due to lack of active neural network. In this case, the spontaneous Ca^{2+} oscillations are due mainly metabolic activity of cells has been shown in our experiments. This study showed that changes in Ca^{2+} activity in the cells of rat hippocampal CA3 fields occurring during postnatal development directly related to the functioning of the neural networks, and the metabolic state of the cells. Ca^{2+} signaling in mature brain - is a complex multicomponent process involving various receptor systems capable of mutual substitution in violation of the normal functioning of one or more of them.

Acknowledgements

This work was supported by Grant of the President of the Russian Federation for young scientists and graduate students engaged in advanced research and development in priority areas of modernization of the Russian economy for 2015-2017 (СП-1531.2015.4).