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POSTERS ABSTRACTS

A SINGLE EPISODE OF SEIZURES INDUCED BY PENTYLENETETRAZOLE IS FOLLOWED BY A COGNITIVE DECLINE

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Summary. Single episode of generalized tonic-clonic seizures induced by pentylenetetrazole led to slowly developing memory impairments in rats, accompanied by elimination of excessive newly generated young cells which were born in the hippocampus soon after the seizures and transient activation of microglial cells in this neurogenic niche.

Key words. Pentylenetetrazole, seizures, neurogenesis, neuroinflammation.

INTRODUCTION

According to different studies, between 5% and 10% of people suffer a single isolated seizure at some time in their life. However, little is known about effects of a single seizure on the cognitive function, and clinical investigations of this issue are not easy to perform. The aim of our study was to follow the time course of delayed effects of generalized clonic-tonic convulsions on learning and memory functions in rats.

MEMORY TESTS

We have injected rats with a submaximal dose of a hemoconvulsant pentylenetetrazole (PTZ, 70 mg/kg) and measured their memory performance during three subsequent months using novel object recognition test and social recognition test (Fig.1). In both tests, PTZ-induced generalized tonic-clonic seizures were accompanied by a slowly developing decline in short-term non-spatial memory.

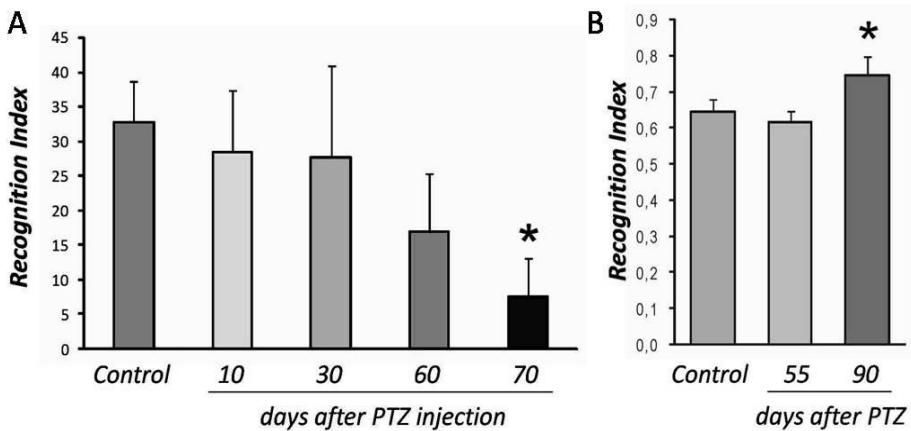


Fig. 1. Performance of short-term memory in novel object recognition test (A) and social recognition test (B) after PTZ-induced seizures in rats.

IMMUNOHISTOCHEMICAL STUDY

The temporal profile of progression of memory impairments along with the absence of profound neuronal damage (as assessed by vanadium acidic fuchsine stain) after PTZ-induced convulsions allowed us to suggest the involvement of aberrant seizure-induced adult neurogenesis in the pathogenesis of observed memory dysfunction. To verify this hypothesis, we injected the rats with S-phase marker 5-bromo-2-deoxyuridine (BrdU) and counted the number of labeled cells in the dentate gyrus (DG) at different time points after the seizures (Fig. 2). We have found that simultaneously with development of memory impairments, an elimination of excessive young cells occurs in the germinative area of the hippocampus.

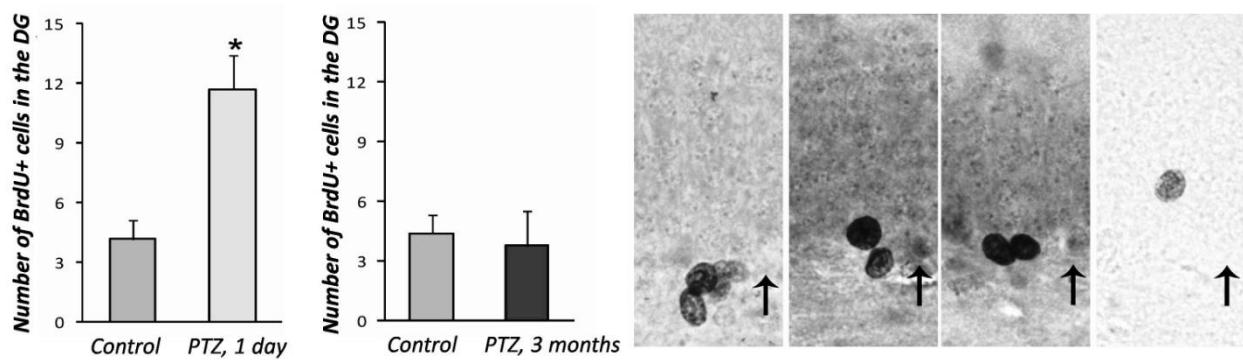
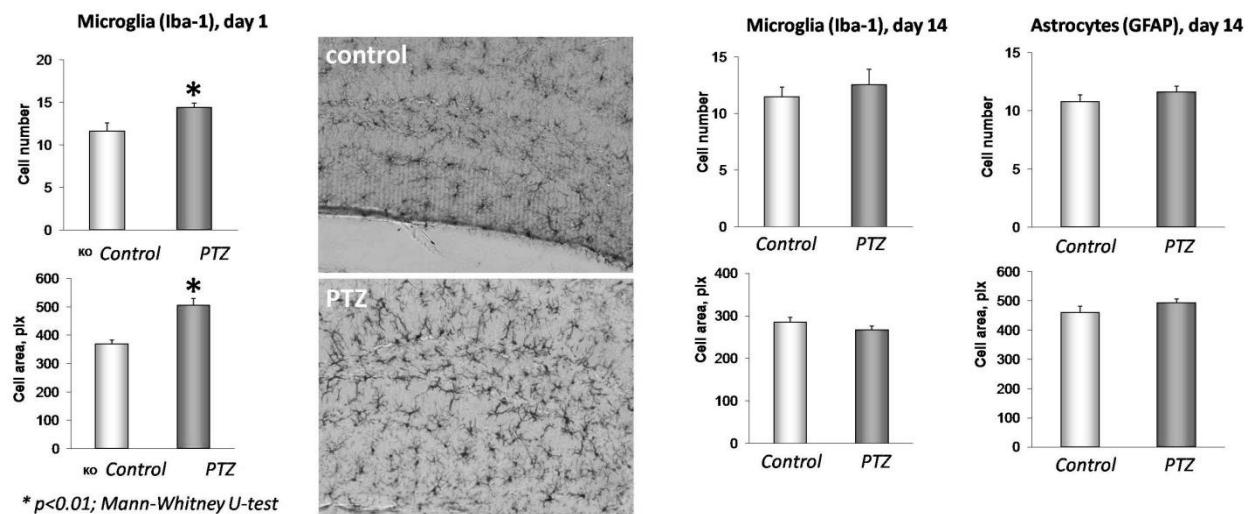


Fig. 2. The number of BrdU+ cells after PTZ-induced seizures and their integration into DG.

The possible mechanism of aberrant maturation of the newly generated cells in the absence of their visible structural abnormality can be launched by the neuroinflammatory alteration of the local microenvironment within the neurogenic

niche. To check this supposition we stained rat brain slices for glial markers Iba-1 (microglial cells) and GFAP (astrocytes) and analyzed their expression at different time points after the seizures (Fig. 3). On the next day after the seizures an activation of microglial cells occurred in the DG. Two weeks later, no signs of microglial activation were present; moreover, astrocytic glia was also not increased in number and size as assessed by GFAP immunostaining suggesting no chronic neuroinflammation after single PTZ-induced convulsion.



CONCLUSIONS

Single episode of generalized tonic-clonic seizures induced by PTZ led to slowly developing memory impairments in rats, accompanied by elimination of excessive newly generated young cells and transient activation of microglial cells in this neurogenic niche.

The study was partially supported by RFH grant # 13-36-01277 and RFBR grant # 14-04-31523.

The influence of Brain-derived neurotrophic factor (BDNF) on functional activity of the culture hippocampus during hypoxia (in vitro modelling).

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Oxygen deficiency is the major cause of cell death at a large range of pathologies. The neurons are among body cells, which are the most sensitive to lack of oxygen, concerning the problem of brain hypoxia retains emergency medical and biological significance.

The purpose of research is studying the impact of brain-derived neurotrophic factor (BDNF) on the functional activity of dissociated cultures of hippocampus in modeling normobaric hypoxia.

In the in-vitro study we used dissociated hippocampal cell cultures derived from CBA mice 18 day embryos. On the 14th day of the cultivation, the cells exposed to hypoxia. 1ng / ml BDNF was preemptively added in the examined cultures. To measure the functional activity of the hippocampal cultures RNA detection probe SmartFlare was used.

To assess the changes in the functional activity of the 1st day after the simulation hypoxia detection of mRNA BDNF was carried out. For detection we used RNA probe SmartFlare, whose fluorescence was determined as helium-neon laser with $\lambda = 543$.

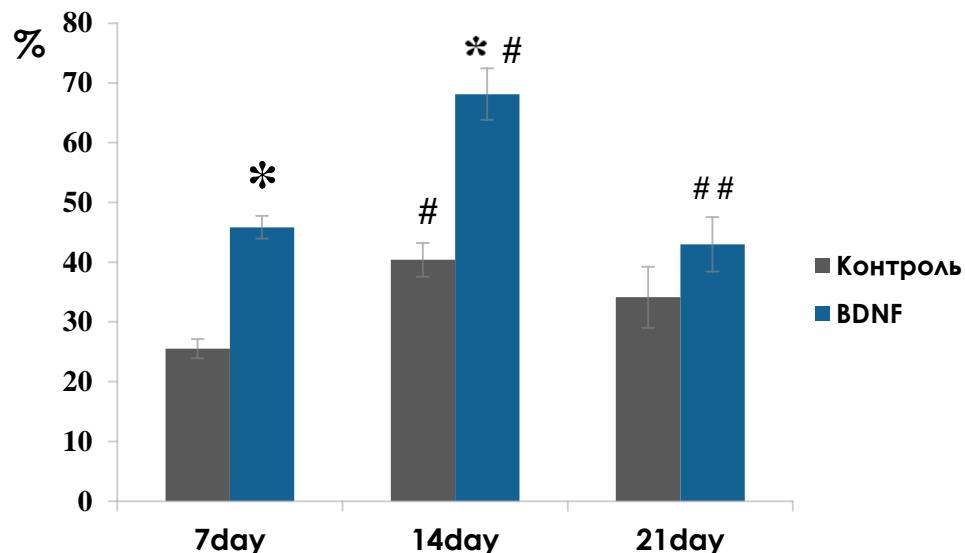


Figure 1. Percentage of mRNA BDNF - positive cells in primary cultures of hippocampal dissociated at different stages of development ($p \leq 0.05$)

During examination of the percentage BDNF mRNA-positive cells in primary cultures of dissociated hippocampal cultures between 7 and 14 days of development (Fig. 1) we found a significant increase of BDNF mRNA-positive cell group which preventively got BDNF, relatively to the control group. There also a slight increase in mRNA BDNF-positive cells relatively to controls at 21 days of development, but no significant differences were found.

During analyzing the changes in mRNA BDNF-positive cells in the temporal dynamics, we found that the proportion of BDNF mRNA was significantly increased at the 14th day of development in comparison with 7 days. Next 21 hours of significant drop of the mRNA BDNF. Statistical differences between 7 and 21 days was found. These data suggests that the preventive addition of BDNF percentage to dissociated primary hippocampal cultures on the 14th day of the development affects the synthesis of endogenous BDNF in the most positive way.

Receiving of adenoviral vector for the study functions of synaptopodin, the protein of spine apparatus.

Borisova Ekaterina Vladimirovna, Epifanova Ekaterina Alexeevna, Salina Valentina Alexandrovna, Babaev Alexey Alexandrovich.

Synaptopodin is the founding member of a novel class of proline-rich actin-associated proteins highly expressed in telencephalic dendrites and renal podocytes. That protein expresses in dendrites of mature neurons in telencephalon. Synaptopodin exists in 3 isoforms: neuronal Synpo-short (685 AA), renal Synpo-long (903 AA), and Synpo-T (181 AA). All 3 isoforms specifically interact with alpha-actinin and elongate alpha-actinin-induced actin filaments.

According data from recent studies, we can suggest that dendritic spines containing sinaptopodin greatly differ in structural and functional properties from the neighboring spines that do not contain sinaptopodin. Clusters of synaptopodin in spines colocalize with internal functional flow of calcium. Thus, sinaptopodin plays a role in synaptic plasticity, as well as the investigation of its function may provide information related to the understanding of mechanical functioning of synapses.

Our aim is to develop strategies and to receive an adenoviral vector. To achieve this goal the following have to be done: to choose the template and PCR conditions, to construct a plasmid with the sequence of the protein sinaptopodin, the supersinapsin promoter and the fluorescent protein mKate2 for co-transfection, to receive an adenoviral vector, to define its titer and biological activity.

The main methods of this study were: polymerase chain reaction and plasmid cloning. A commercial kit of reagents «Phusion High-Fidelity PCR Kit» and thermocycler «Applied Biosystems» were used for PCR.

For construction plasmid we used enzymes «New England BioLabs», competent DH5 α cells, the plasmid purification kit «QIAGEN DNA» and the software «VectorNTI». We defined the primers system (Synpo-HindIII-fw, Synpo-Mlul-rev) and the optimum annealing temperature of primers - 65°C. The template was cDNA isolated from mouse cortex. We obtained the sequence of spine apparatus protein

synaptopodin, which was confirmed by electrophoresis. The system of restriction enzymes for insertion of the nucleotide sequence of synaptopodin and supersinapsin

promoter in a plasmid with a fluorescent protein mKate2 was chosen. We purified plasmids after transformation with the product of ligation reaction. In the following we would plan to co-transfect this plasmid, to accumulate an adenovirus vectors in HEK cells, to purify vectors with ultracentrifugation and to define its titer and biological activity.

ANALYSIS, MODIFICATION AND DEVELOPMENT OF THE STATE-OF-THE-ART TWO-PHOTON LASER MICROSCOPE IN VIEW OF THE FEATURES OF ITS USE IN NEUROSCIENCE

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Summary. This article may be regarded as a quick reference to laboratory staff who are wishing to develop their own microscopy system for self-service and modernization of the system and in order to save the lab budget.

Key words. Two-photon microscope, imaging, uncaging, methods, engineering.

INTRODUCTION

Today one of the main areas of application of multiphoton microscopy is biology. This is due to the fact that this technique allows to obtain 3D images of tissues due to laser focus change, that is possible due to substantially greater penetration depth on the main wavelength into biological tissues. However, there are certain peculiarities arising from the specifics of experiments using this technique. In one case, for example, the analysis of the morphological cell data of hippocampal

slices of the brain of rodents, other experiments *in vivo* depending on the design using the full-sphere of the virtual reality or, for example, in the form of a cylindrical hover platform. Specific features are also in the fact that the level of fluorescence-activated objects is low (only a few suites), and the increase of the laser intensity may overheat and burn the sample. Sample scan methods are constantly changing, and require constant modernization. Creation of two-photon system taking into account its specific use in neuroscience is probably the best solution.

THE CONSTRUCTION OF THE MICROSCOPE

The three-dimensional model of future microscope was developed using software Compass (Figure 1). The model depicts the main frame (Figure 1, 1), attached to the vibration free table, as well as optical elements, attached to the main frame. Technically, the operation of differential interference contrast (DIC) is provided with installation of a visible light source (figure 1, 2) and an IR filter, an iris-shutter, a diffuser, a mirror to reflect the beam in the vertical direction and lens (Figure 1, 3). After passing through the lower block and being reflected the light beam reaches the polarizer, and then passes onto a condenser (Figure 1, 4) with Nomarski prism. Then a laser beam achieve the sample, which is fixed on the table (Figure 1, 5). The table, in turn, is fixed to a U-shaped moving platform (Figure 1, 6) for exact positioning of the sample under the lens in the lateral plane. Passing through the sample, the beam achieve the optical components on a moving platform (fixed to the frame of the microscope) (Figure 1, 7), and passes the second Nomarski prism and the analyzer. Here the interference of previously separated rays is performed. After passing the mentioned optical path the light beam falls onto the so-called illuminator of reflected light (Figure 1, 8), that does not contain any filters or mirrors in the DIC mode operation, and then the beam passes onto IR camera (Figure 1, 9) that displays the image on the monitor.

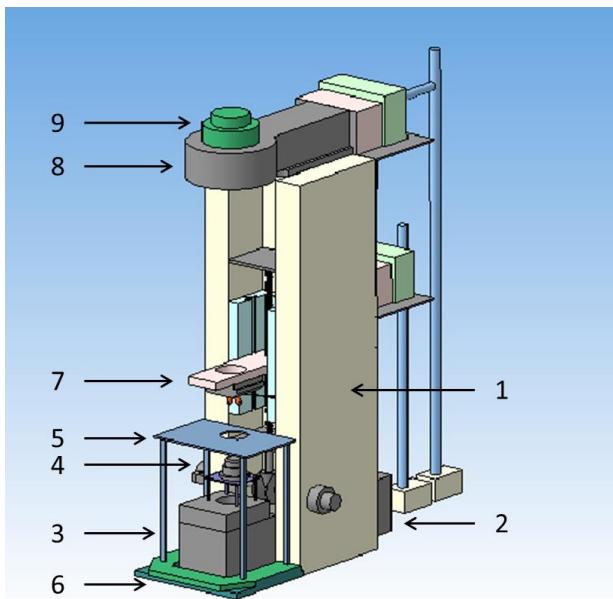


Figure 1.

Principal scheme of the two-photon microscope system is presented at the Figure 2. As the laser source is using femtosecond IR Ti:Sa laser (Figure 2, L1). The range of the wavelength is from 690 to 1080 nm. The laser beam is starting from the laser aperture and passing the optical path including acousto-optic modulator (AOM) to change the radiation intensity and the Galileo type telescope to changing the diameter of the beam. After that the beam enters into the vertical optical bar with a mirror (Figure 2, 14) which reflect the beam to the next mirror (Figure 2, 13) which deliver laser beam to the desired height.

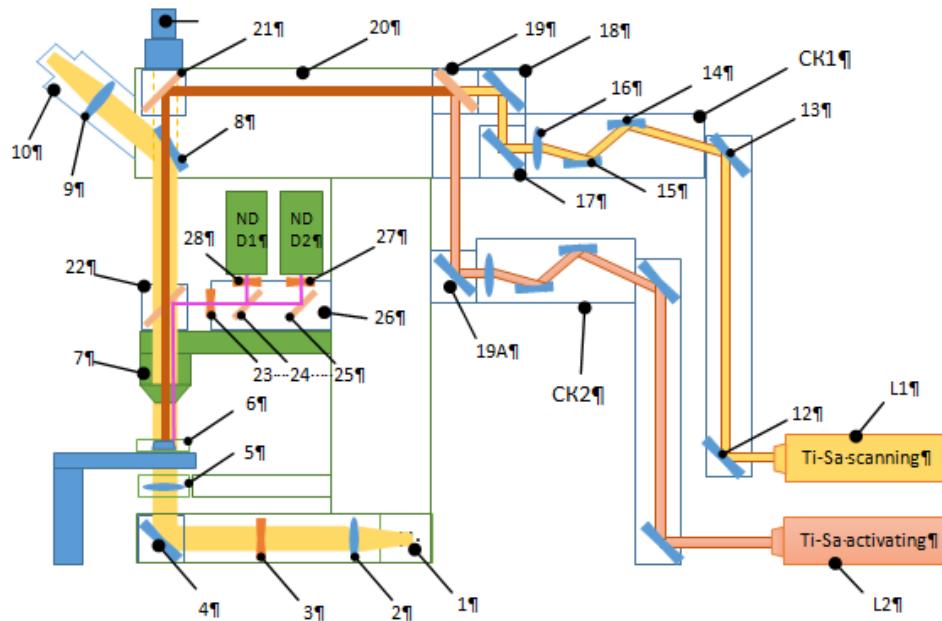


Figure 2.

Further, the beam enters to the resonant scanning unit using a synchronous operation of the mirror and the resonant galvanometer (Figure 2, 14, 15) and the focusing lens (Figure 2, 16) scans the sample. The parameters of the scanner are controlled by the PC. Reflecting by the mirrors (Figure 2, 17, 18) beam achieve dichroic mirror (Figure 2, 19) transmitted the beam falling on it from the scanning laser (Figure 2, L1) and reflects from activating the laser (Figure 2, L2). Then the beam is focusing in the desired plane by the height of objective (Figure 2, 7) and achieve the sample (Figure 2, 6) activating fluorescence at the desired point, and point by point scans the sample. Aperture lens is capturing fluorescent photons (Figure 2, 7) reflected by the dichroic mirror (Figure 2, 22), pass filters (Figure 2, 23, 27, 28) and the falling to the non-descanned detectors (NDD). Distance from the sample to the light detector is about 10 centimeters, which allows to register low level of fluorescence. The optical path of the laser beam from activating laser (Figure 2, L2) is similar to the path from the laser scanning (Figure 2, L1), however in this case using the scanning element including a pair of mirror galvanometers (Figure 2, CK2).

ACKNOLEGMENTS

This work was supported by the Ministry of Education and Science of the Russian Federation, unique identity number of the project is RFMEFI57814X0079.

Regulation of neuronal chloride homeostasis: a new role for extracellular matrix

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Neuronal signaling relies on ion fluxes through membrane-bound channels. Such fluxes are allowed due to the transmembrane ionic gradients created and maintained by membrane transporters. The neuronal gradient for chloride, an anion mediating most of the inhibition in the mature CNS, is the result of activity of two chloride transporters working in opposite directions, NKCC1 moving chloride in and KCC2 moving chloride out of the cell. The KCC2 function may be both up- and down-regulated through a number of mechanisms which allow for the fine-tune adaptation to a varying transporting load which mostly depends on chloride

influx through both synaptic and extrasynaptic inhibitory chloride-permeable ion channels such as GABA_A- and glycine-receptors.

Besides intraneuronal factors affecting KCC2 function and, thus, the chloride gradient, there is a number of extra-neuronal factors suggested to have a great influence over the distribution of chloride across the neuronal membrane. The very recent experimental findings point to the extracellular matrix as an important player in regulation of intracellular chloride. It has been claimed that large anion groups located within the extracellular matrix set the chloride gradient due to the Gibbs-Donnan effect, thus, effectively sidelining KCC2 as a major contributor to the neuronal chloride homeostasis. However, our own experimental findings concerning chloride homeostasis in anterior hypothalamic neurons suggest quite a different mechanism of involvement of the extracellular matrix.

МОЛЧАНИЕ ГЕНОВ MUC1 И IL32 КОРРЕЛИРУЕТ С ПОВЫШЕНИЕМ УРОВНЯ мРНК Fas В ОПУХОЛЯХ МОЛОЧНОЙ ЖЕЛЕЗЫ

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Развитие рака молочной железы связано с изменением экспрессии генов, участвующих в регуляции иммунных реакций. В настоящем исследовании оценивалась взаимосвязь экспрессии генов, принимающих участие в воспалении интерлейкин 32 (IL32), обмена веществ клетки Муцин 1 (MUC1) и Fas зависимого апоптоза (Fas). Исследовали образцы опухолей от 40 пациентов больных раком молочной железы. Анализ уровней мРНК IL32, MUC1 и Fas проводили методом ОТ-ПЦР в реальном времени (qRT-PCR). В качестве эндогенных контролей использовали гены «домашнего хозяйства» клетки: бета-2-микроглобулин (B2M), убиквитин С (UBC), гипоксантин фосфорибозилтрансфераза 1 (HPRT1), тирозин 3 монооксигеназа (YWHAZ). Уровни экспрессии исследуемых генов оценивали методом сравнения

пороговых циклов ($\Delta\Delta Ct$). На первом этапе исследовали стабильность экспрессии контрольных генов в образцах опухолей молочной железы. С использованием компьютерной программы BestKeeper установлено, что для нормализации экспрессии мРНК в образцах опухолей молочной железы человека подходит B2M. Экспрессия мРНК MUC1 была обнаружена в 32 образцах опухоли (80%), мРНК IL32 - в 26 образцах (65%) и мРНК Fas в 38 образцах опухоли (95%). Отмечено, что все образцы опухолей, не содержащие мРНК MUC1, были отрицательны на присутствие мРНК IL-32, что может указывать на общие механизмы регуляции генов. Сравнение уровней экспрессии мРНК MUC1 и IL32 в опухолях молочной железы не показало различий. В опухолях молочной железы также наблюдалась корреляция между повышением уровня мРНК Fas и молчанием генов IL-32 ($r = 0,47, p = 0,004$) и MUC1 ($r = 0,57, p = 0,002$).

Представленные данные могут свидетельствовать о взаимосвязи экспрессии генов, опосредующих воспаление, обмен веществ и апоптоз в

опухолях молочной железы. Мы предполагаем, что существуют общие механизмы регуляции через систему микро-РНК.

CHANGES IN THE ELECTRICAL CONDUCTIVITY OF THE MYOCARDIUM OF ISOLATED RAT HEART UNDER THE INFLUENCE OF VERAPAMIL

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Summary. The study was designed to investigate the effect of verapamil on the speed of propagation of electrical excitation in the myocardium. As a result, it was found that verapamil reduces heart rate, the speed of excitation in the myocardium remains unchanged. The results obtained allow us to estimate the effect of verapamil on the condition of cardiac tissue, based on the change in its conductivity during treatment with the drug.

Key words. Langendorff method, verapamil, conduction velocity, multielectrode mapping.

INTRODUCTION

Calcium antagonists are widely used in the treatment and prevention of heart diseases. Verapamil is a blocker of the slow L-type calcium channel. The action of verapamil in the heart accompanied by negative inotropic and chronotropic effects, since the restriction of entry of calcium ions leads to a quantifiable reduction in the formation of actin-myosin bonds in the working cardiomyocytes and to inhibition of spontaneous diastolic depolarization in the atypical cardiomyocytes of the conduction system [1]. The concentration of calcium ions also affect the speed of propagation of a wave of electrical excitation due to the regulation of connexons, which is placed in the intercalated discs of cardiomyocytes [2]. The aim of the study was to examine the effect of verapamil on the speed of propagation of the excitation wave in the ventricular myocardium of isolated rat heart.

MATERIALS AND METHODS

Isolated hearts of outbred white rats were perfused by the Langendorff method. After 10 minutes of perfusion with Krebs-Henseleit solution (NaCl 118, KCl 4.7, CaCl₂ 2,

MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 20, glucose 10 mkmol/L) in an experimental group perfusion was held with addition of verapamil with a concentration of 7 mol / L. For registration of the electrical activity of the myocardium multi-electrode mapping setting with flexible matrices was used. Heart rate measured by the number of heart beats per minute. The speed of propagation of the wave excitation of the heart was calculated based on the difference between the values of time of occurrence of electrical potentials recorded by the electrodes of the matrix. Comparison of the average values of obtained parameters at different stages of the experiment, as well as a comparison of these values in the control and experimental groups, was performed by Student t-test for dependent samples. Differences were considered significant at a significance level of p <0.05.

RESULTS

As a result of the study it was found that during the perfusion of the isolated rat heart with a solution containing verapamil, heart rate decreased to 1.52 times compare with the control group. The velocity of propagation of excitation between the detection electrode of the matrix was not changed (Fig.1).

CONCLUSIONS

Perfusion with a solution containing verapamil influenced on the calcium dynamics in myocardium of the isolated rat hearts that was expressed in a negative chronotropic effect. But this influence did not cause changes in the speed of propagation of the wave of excitation along the surface of the myocardium, which indicates the existence of specific mechanisms to prevent a change in the conductivity of the myocardium, causing the termination of calcium channels of L-type.

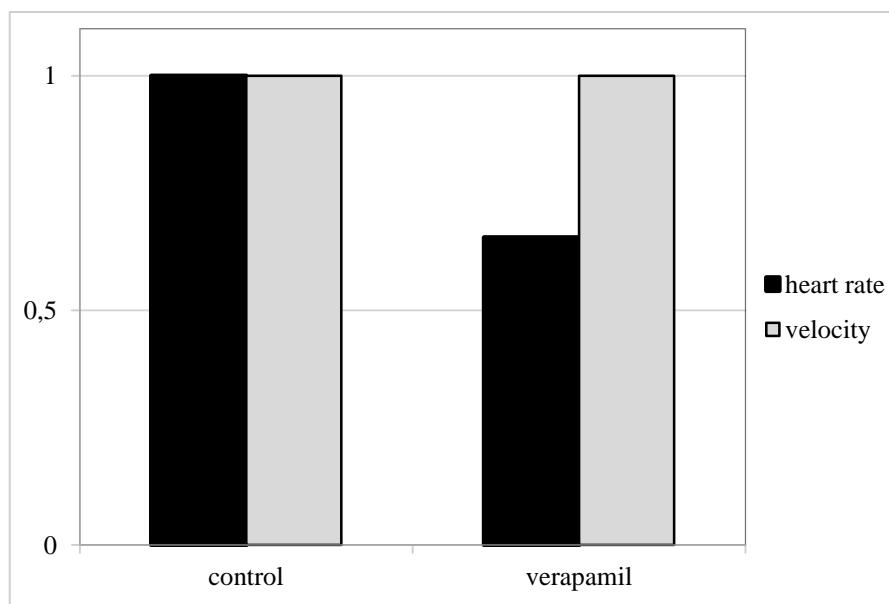


Fig. 1. Changes in heart rate and speed of propagation of the excitation wave in the isolated rat heart under the influence of verapamil

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Summary. In this work we report two successful cases of developing neural networks of spiking neurons for controlling mobile robots. In the first case we use a toy robot, a crocodile, driven by a neural network in-silico. We show that this so-called neuroanimat is capable of detecting internal events of synchronization of network responses to stimuli. In the second example we employ a spiking neural network for building a human-robot interface. Using a bracelet with eight electromyographic sensors we have shown that the interface can faithfully detect myographic signals, classify them according to hand gestures, and send the corresponding commands to the robot. Although the two applications belong to different areas of Neuroscience, they are based on a common approach of neural computations. We note that in both cases besides neural networks there are no additional external algorithms for the decision-making.

Key words. Spiking neuron, neuroanimats, human-machine interface, electromyography, neural calculation.

1 INTRODUCTION

Neurons as a main building block of the brain have enormous computational capacity. Therefore, the development of mathematical models of spiking neurons and neural networks on their basis is a promising approach for applied computations (Paugam-Moisy and Bohte, 2009). However, the number of successful attempts of technical implementations remains very limited. Recent studies have shown that networks of spiking neurons can be used for recognition of patterns of different origin (Bichler et al., 2011; Loiselle et al., 2005; Kasabov et al., 2012).

In this work we report two successful studies of spiking neural networks. In the first case we use a toy robot, a crocodile, driven by a neural network in-silico. We show that this so-called neuroanimat is capable of detecting internal events of synchronization of network responses to stimuli. In the second example we employ a spiking neural network for building a human-robot interface. Using a bracelet with eight electromyographic sensors we classify hand gestures in real time and use them to control a mobile robot.

2 METHODS

2.1 Neuroanimat

We developed a neuro-simulator, called NeuroNet, which models a network of 400 excitatory and 100 inhibitory Izhikevich-type neurons (Izhikevich, 2004). Topologically the neurons are distributed over nodes in a 2D graph whose edges correspond to couplings between cells. Then, the time delay in spike transmission between neurons is proportional to the distance between the corresponding nodes. Each neuron receives about 30 afferent couplings. The coupling probability decreased with the distance between neurons.

The model simulates two types of synaptic plasticity. The short-term plasticity (facilitation and depression) is implemented by varying the transmitter release according to the frequency of presynaptic spikes (Tsodyks et al., 1998). The long-term potentiation is based on spike-timing dependent plasticity (STDP) (Morrison et al., 2008). If a postsynaptic spike follows a presynaptic spike then the coupling strength increases. In the case of inverse spike timings the coupling strength reduces.

An ultrasonic distance sensor placed on the robot head provides sensory information to the neural network. The sensor modulates the frequency of square pulses produced by a virtual generator. The output of this generator is fed to an arbitrary part of the network. Finally, the network output controls the robot movements.

2.2 Human-robot interface

We developed a hardware-software complex, called MyoClass, for real time recording of EMG signals and recognition of hand gestures for controlling a mobile robot. The recording is accomplished by a bracelet MYO™ Thalmic providing simultaneously eight sEMG signals from the sensors (embedded MYO Thalmic gesture recognition was off). We used nine static hand gestures as motor patterns. During an experiment users performed four series of nine gestures each, selected in random order.

For extraction of the discriminating features from sEMG signals we employed the same neuronal model as in the neuroanimat approach. The network output was connected to a multilayer artificial neural network for the feature classification. The standard error backpropagation algorithm was used for learning.

2.3 Robot platforms

Both robot platforms for the animat (a crocodile) and for testing the human-robot interface (a car) were built from a LEGO kit NXT Mindstorms®. Communication between all parts has been implemented through a Bluetooth® interface.

3 RESULTS

3.1 Neuroanimat: basic behaviours

We first checked that the neural network in-silico could exhibit all basic properties of an in-vitro neuronal culture such as bursting activity (Wagenaar et al., 2006) and plasticity provoked by external stimuli (Pimashkin et al., 2013). Adaptive structural changes in the network are related to long-term potentiation of the coupling weights. We found that such changes can lead to new emerging functional properties, i.e. to synchronization of the network firing with external stimuli.

We revealed two criteria working at the low neuron level that allow distinguishing between synchronous and asynchronous network activities:

- a. High frequency ($> 8\text{-}11$ Hz) spiking of neurons;
- b. Stable phase lag (about 60-70 ms) of fired spikes related to the stimulus onset.

To combine both criteria we proposed a neural circuit that includes phase and frequency neuronal filters coupled in series. Then, the filter output passes through a neuron-detector, which fires spikes in case of synchronization of the network activity with the stimulus.

The phase filter employs axonal delays in two inhibitory neurons included between the stimulated part of the network and the neuron-detector. The first neuron receives input through the geometrically shortest path and thus suppresses excitatory spikes in the time range [20-60] ms after the stimulus onset. The second neuron placed at a distance from the detector suppresses the excitation in the range [70-120] ms. Thus, these neurons strongly inhibit all spikes at the detector except those falling into the range [60-70] ms.

The frequency filter relies on the effect of presynaptic facilitation in the framework of short-term synaptic plasticity. The filter parameters have been tuned in such a way that the amount of neurotransmitter release increased for series of presynaptic spikes coming at rates higher than 8 Hz. Thus, the output spikes are generated for high frequency activity of the presynaptic neuron only.

Spontaneous activity in the neural network eventually leads to an arbitrary movement of the robot. Then, in case of the presence of an object in the sensory field of the robot, its sensory system generates an output that innervates the neural network. This in turn may lead to a strong increase of the motor activity. The combination of spontaneous and evoked activities in the neural network may lead to the behaviour of searching for a target. Even in the absence of any object in the immediate neighbourhood, the animat from time to time begins moving and "looking" for objects or walls in the room.

In case of event synchronization we observed "eating" behaviour (Fig. 1). At high frequency synchronization neuronal spikes pass the phase and frequency filters, which leads to activation of moto-neurons driving quick opening and closing of the jaws.

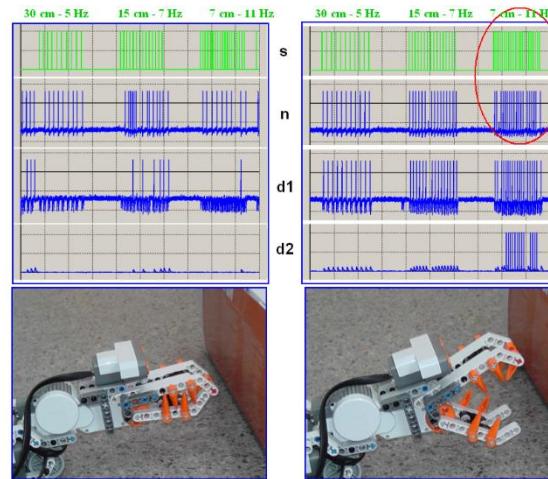


Fig. 1. “Eating” behaviour based on synchronization phenomenon in the animat. Left and right columns correspond to before and after learning, respectively. The output from the ultrasonic sensor (*s*) provokes synchronization (red circled) of neurons in the main network (*n* is a representative neuron). This in turn leads to activation of the phase filter neuron (*d1*) and later of the frequency filter neuron (*d2*) and the animat opens the jaws.

3.2 Spiking neurons in human-robot interface

The myographic bracelet provides simultaneously eight sEMG signals. Then, the purpose of the neural network is to extract the most discriminative features from these signals in such a way that the artificial neural network could easily classify them according to the gestures made by hand.

Spiking neurons, acting as sensory neurons, receive myographic signals from the bracelet and produce some output spikes. We consider the output synaptic signal evaluated in the framework of the Tsodycs-Markram model as continuously changing feature. Then, we can sample this variable at discrete time instants.

Figure 2 shows a representative example of an sEMG signal (top), the transmembrane potential of the spiking sensory neuron (middle) and its output (bottom). During experiments we tuned the parameters of spiking neurons to ensure high accuracy of the classifier, comparable with the use of classic sEMG feature as the root mean square value. For ten subjects (25-56 years old) the classifier accuracy was $92.3 \pm 4.2\%$.

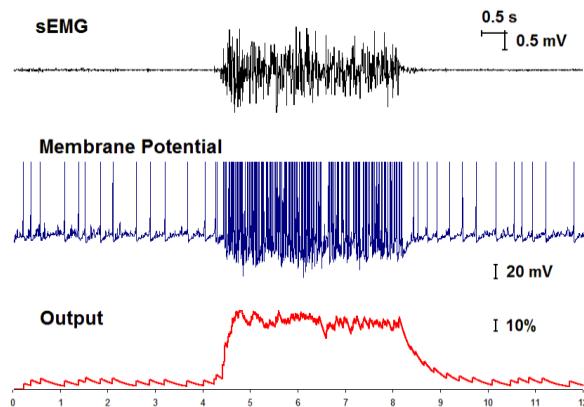


Fig. 2. A representative example of processing of an EMG signal by a spiking sensory neuron.

We then tested the human-robot interface in real time. The user controlled the mobile robot using hand gestures. Every recognized gesture (except “rest”) was associated with an instruction of movement of the robot: “drive”, “reverse”, “forward right”, “forward left”, “reverse right”, “reverse left”, “stop”, and “fire”. Our results show that all users after 3-10 trials managed to control fluently the robot.

4 DISCUSSION

In this work we reported two successful cases of developing neural networks of spiking neurons for controlling mobile robots. In the first case the neural network works autonomously as a “brain” of an animat. We have shown that it is able to learn from the environment and to reproduce basic behaviour of advancing towards an object and “eating”. In the second case the neural network has been used as a processor for human-robot interface. We have shown that the interface can faithfully detect myographic signals, classify them according to hand gestures, and send the corresponding commands to the robot.

Although the two applications belong to different areas of the Control Theory and applied Neuroscience, they are based on a common approach of neural computations. We note that in both cases besides neural networks there are no additional external algorithms for the decision-making.

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ACKNOWLEDGEMENTS

This work was supported by the Russian Science Foundation project 15-12-10018 (Sections 1, 2.1, 3.1 and 4) and project 14-19-01381 (Sections 2.2, 2.3, and 3.2).

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Prenatal development study on phenomenon of delayed implantation

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Summary. Delayed implantation is a very widespread in *Mustelidae* family. It was described more than 100 years ago, but its mechanisms are yet poorly understood. We first made a de novo whole genome sequence of three species of mustelids with delayed implantation stage in prenatal development and find out several mutations in their melatonin pathway genes.

Key words. Delayed implantation, *Mustelidae*, melatonin.

INTRODUCTION

Delayed implantation (DI) is arrest of embryo development at the blastocyst stage, characterized by inhibition of mitotic activity and synthesis of nucleic acids in cells of the inner cell mass of embryo and temporary prevention of it implantation in uterus. The *Mustelidae* family seems to be the most interest for DI research because of extraordinary prevalence of this trait in this family; almost half of mammals with DI are mustelids. Delayed implantation feature is pleomorphic in mustelids. It seems to be inherited from common ancestor. In a process of evolution of this family there were multiple losses of this trait. Furthermore DI length is variable among *Mustelidae* family: from 50-60 days in mink (*Neovison vison*) to 245-275 days in marten (*Martes martes*) or sable (*Martes zibellina*). [Isakova 2004; Thom et al., 2004].

Mechanisms of transduction between active and inactive embryo stages and factors underlying the length of embryonic diapause are not clearly understood to date. It has been suggested that melatonin (pineal gland secret) is the crucial regulator of this processes. Melatonin secretion depends on photoperiod length and regulates synthesis of luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin [Jack et al. 1996; Murphy 2012]. Nonetheless there were no any molecular-genetic studies of DI mechanisms.

Research of DI is of great interest for evolutionary biology and may also be important for reproductive medicine and fur industry. Moreover studies showed that presence/absence of DI in prenatal development are correlated with longevity among mustelids [Thom et al., 2004]. We suggest that presence of DI also may have effects on animal behavior.

We first made whole genome sequence of three mustelids with delayed implantation stage in prenatal development - mink (*Neovison vison*), marten (*Martes martes*) and sable (*Martes zibellina*). Using the data from whole genome sequencing of these animals and assembled genome of ferret (*Mustela putorius furo*) which is closely related specie that does not display diapause we analyzed the genes involved in melatonin pathway.

CONCLUSIONS

We described a set of genetic alteration in genes of melatonin pathway in mink, marten and sable. The data imply the genetic alteration may lead to changes of quantity and regulation level of melatonin in animals with DI stage in prenatal development.

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The role of cannabinoid receptors (type 1 and type 2) in implementation of neuroprotective and antihypoxic effects of N-arachidonoyldopamine in acute hypoxia *in vitro*

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Summary. The aim of the investigation was to study a role of cannabinoid receptors type 1 (CB1) and type 2 (CB2) in implementation of antihypoxic and neuroprotective effects of N-ADA in hypoxia model *in vitro*. The experiments were carried out on primary hippocampal cultures. N-ADA effect on the spontaneous bioelectrical and calcium network activity in dissociated hippocampal cultures in normal and hypoxic conditions as well as the role of CB1 and CB2 in the implementation of these effects were investigated. Registration of extracellular action potentials was conducted by MEA systems (Multichannel Systems, Germany) application. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM (Invitrogen) and a confocal laser scanning microscope (Zeiss LSM510, Germany). Study the expression of mRNA CB1 receptors was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy. Our data demonstrated that N-ADA has strong

antihypoxic and neuroprotective properties associated with activation of cannabinoid receptors type 1.

Key words: neuron-glial networks, endocannabinoid system, N-arachidonoyldopamine, hypoxia, primary hippocampal cultures, neuroprotection

INTRODUCTION

Nowadays ischemic stroke is one of the main causes of death and severe disability of the population in Russia and around the world. Hypoxia considered as a key factor of brain cells damage during ischemic stroke. The endogenous cannabinoid system plays an important role in the modulation of synaptic transmission, plasticity and maintaining the normal functioning of the nervous system. The neuronal activity regulation by cannabinoids receptor's activation in ischemia has shown in a number of studies on different models *in vivo* and *in vitro*.

A recently discovered and synthesized endocannabinoid N-arachidonoidfamin (N-ADA) is a perspective substance for hypoxic damages correction. N-ADA was described as an agonist both cannabinoids (CB1 and less CB2). In our recent studies neuroprotective and antihypoxic effects of N-ADA were shown [1]. However, a question concerning the molecular mechanisms of N-ADA neuroprotective and antihypoxic actions during hypoxia is still open.

The aim of the investigation was to study the role of cannabinoid receptors type 1 and type 2 in antihypoxic and neuroprotective effects of N-ADA in hypoxia model *in vitro*.

MATERIAL AND METHODS

In vitro studies were conducted using hippocampal cells dissociated from 18-day embryonic CBA mice. Hippocampal cells were plated on multielectrode arrays (MEA60, Multichannel Systems) or coverslips. Hypoxia modeling was performed on day 14 of culture development *in vitro* by replacing the normoxic cultural medium with a medium containing low oxygen for 10 min. An application of N-ADA (10 nM) or N-ADA with CB1 or CB2 antagonist was conducted into hypoxic cultural medium. We used SR151716 (SR1) 1 nM (Sanofi) as antagonist of CB1 receptors, SR 141716A (SR2) 1 nM as antagonist of CB2 receptors. The main parameters of spontaneous neural and calcium activity as well as the viability of cells were investigated. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM (Invitrogen) and a confocal laser scanning microscope (Zeiss LSM510, Germany). The viability of dissociated hippocampal cells was evaluated according to the percentage ratio between the number of dead cells stained by propidium iodide (Sigma, Germany) and the total number of cells stained by bisBenzimide

(Invitrogen, USA) for 7 days after hypoxia. Study the expression of mRNA CB1 receptors was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy.

RESULTS

The effect of N-ADA on the neural network activity and cellular viability in primary hippocampal cultures during hypoxia were investigated.

Our experiments showed that 10-minutes acute hypoxia caused the significantly decrease in cellular viability of primary hippocampal cultures (in 4.5 times, $p<0.01$) in the posthypoxic period. N-ADA application maintained the viability of cells at level appropriate normoxic conditions (no significant differences from sham). Blockade of CB1 receptors by SR1 eliminated the N-ADA neuroprotective effect.

Electrophysiological and Ca^{2+} -imaging data demonstrated that hypoxia causes a catastrophic reduction of spontaneous bioelectrical and calcium activity in primary hippocampal cultures and changes in activity patterns. Cannabinoid receptors activation via N-ADA applications during hypoxia partially preserved the spontaneous bioelectrical and calcium activity of neural networks for 7 days after hypoxia modeling. N-ADA neuroprotective effect associated with cannabinoid receptors type 1, as their blockade by using antagonist SR141716A (SR1) exterminate the neuroprotective effects of N-ADA. CB2 blocking by application of antagonist SR144528 (SR2) had no substantially effect.

In this regard, we evaluated the level of CB1 receptors expression in hypoxic conditions. Our data revealed that mRNA CB1 is actively synthesized both neurons and glial cells. N-ADA was significantly ($p <0.05$, ANOVA) reduced the number of mRNA CB1 positive cells in primary hippocampal culture at normoxia. In the control group mRNA CB1 expression was detected in $37.7 \pm 5.41\%$ of cells and in $24.4 \pm 7.26\%$ of cells 48 hours after N-ADA (10 mM) application. Thus, it was shown that exogenous N-ADA application leads to changes the level of mRNA expression of cannabinoid receptor type 1. Hypoxia caused the increase of mRNA CB1 expression especially in astrocytes.

CONCLUSIONS

Therefore, it was shown that N-ADA has strong antihypoxic and neuroprotective properties. The protective N-ADA effect primarily implemented through CB1 receptors.

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Современный взгляд на процесс неквантового выделения медиатора в синаптическом контакте

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Общеизвестно, что регуляция и интеграция клеток в многоклеточном организме, осуществляемая нервной системой, реализуется на уровне межклеточных синаптических контактов. В связи с этим, установление молекулярных механизмов, лежащих в основе функционирования синапса, представляет собой одну из важнейших проблем нейробиологии. Актуальность исследований в этой области продиктована не только стремлением познать фундаментальные аспекты функционирования мозга, но и возможностью понять механизмы развития широкого спектра заболеваний нервной, эндокринной, иммунной и мышечной систем и на основе этих данных разработать эффективные методы терапии.

Согласно общепризнанным представлениям, сигнал с нейрона на эффекторную клетку передается за счет выделения из нервного окончания мультимолекулярных порций (квантов) химического посредника (медиатора) в результате спонтанного или вызванного деполяризацией экзоцитоза синаптических везикул. Однако, уже к началу 80-х годов прошлого столетия были получены данные, свидетельствующие о том, что медиатор способен

выделяться и непорционно, тонически. Данный вид нейросекреции впоследствии был назван «неквантовым выделением» и рассматривался как пассивная утечка сигнальных молекул, не выполняющая какой-то определенной физиологической функции, в связи с чем долгое время был обделен вниманием нейробиологов. К настоящему же моменту установлено, что неквантовое выделение представляет собой активный транспортный процесс, посредством которого в отсутствие нервной импульсации выделяется значительное количество медиатора. В экспериментах на нервно-мышечном синапсе млекопитающих, где в качестве нейромедиатора выступает ацетилхолин, показана специфическая зависимость механизма неквантового выделения от температуры и концентрации внеклеточных ионов, а также возможность регуляции интенсивности этого процесса различными синаптически активными молекулами (глутамат, гамма-аминомасляная кислота, АТФ, перекись водорода и др.). При этом установлено, что блокируется неквантовое выделение ацетилхолина из двигательных нервных окончаний повышением концентрации внеклеточного магния, а также ингибиторами везикулярного транспорта ацетилхолина и системы захвата холина высокого сродства. На примере нервно-мышечного контакта получен ряд данных, демонстрирующих физиологическую роль «неквантово» ацетилхолина в процессах синаптогенеза и регуляции ряда функциональных свойств мышечных волокон.

Относительно недавно были получены доказательства того, что ацетилхолин, выделяется в неквантовой форме не только из двигательных нервных окончаний, но и из терминалей парасимпатических нейронов, иннервирующих как гладкую, так и сердечную мускулатуру, и во всех случаях данный вид нейросекреции также блокируется ингибитором системы захвата холина высокого сродства.

Процесс неквантового выделения медиатора характерен не только для холинергических синапсов. Так, уже к началу нынешнего столетия было установлено, что в синапсах ЦНС такие медиаторы как глутамат, гамма-аминомасляная кислота и глицин также могут выделяться неквантовым образом за счет реверсивной работы транспортных белков системы обратного захвата медиатора.

Таким образом, в настоящее время неквантовое выделение нейромедиатора необходимо рассматривать как самостоятельный вид нейросекреции наряду с процессами спонтанного и вызванного квантового выделения сигнальных молекул и есть все основания полагать, что дальнейшее

изучение феномена неквантовой секреции существенно изменит наше представление о механизмах передачи информации в синапсах химического типа.

Работа поддержана грантами РФФИ и Президента РФ.

GROWING UNIDIRECTIONAL SYNAPTIC ARCHITECTURE IN DISSOCIATED NEURONAL CULTURES USING MICROFLUIDIC METHODS.

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Summary. In this study we developed microfluidic structure with two neuronal cultures grown in separated chambers and connected by microchannels for axon outgrowth. We estimated bursting activity transfer characteristics between chambers in relation to culture development and determine rate of axonal growth in chambers.

Key words. Microfluidic system, microchannels, microelectrode array.

INTRODUCTION

Microfluidics and microelectrode technology recently have been advanced in neuroengineering in fundamental research of the brain and medical applications. Neuronal cells grown in microfluidic structures allow to isolate the cell populations in individual compartments connected by microchannels in which axons and dendrites can grow. The cultivation of neural networks on the microelectrode arrays using multichannel electrophysiology system makes possible to observe development and functional state of connections, their enhancement or suppression.

RESULTS

In this study we designed a microfluidic device combined with microelectrode array which allows to grow two separate cultured neuronal networks with directed

synaptic pathways inbetween. Microchannels' length was 400 μm and 600 μm . We found that 4 days was enough for axons to grow through the whole channel from presynaptic chamber (chamber A) to postsynaptic chamber (chamber B) (Fig. 1).

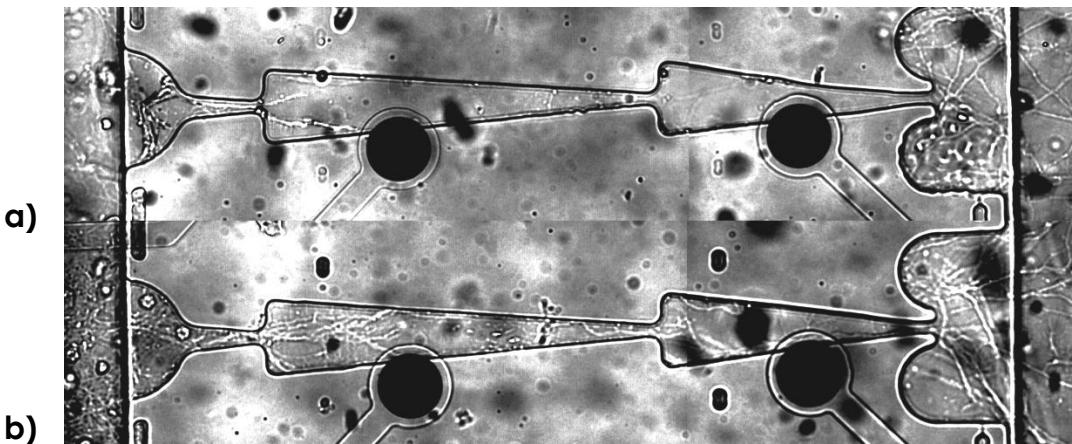


Fig. 1. Axons outgrowth in microchannels on day 2(a) and day 4 (b).

The average velocity of axonal growth in microchannel was found to be 13.8 μm per hour. Next we investigated spontaneous activity propagation through axonal pathways from one chamber to another during 30 days of culture development.

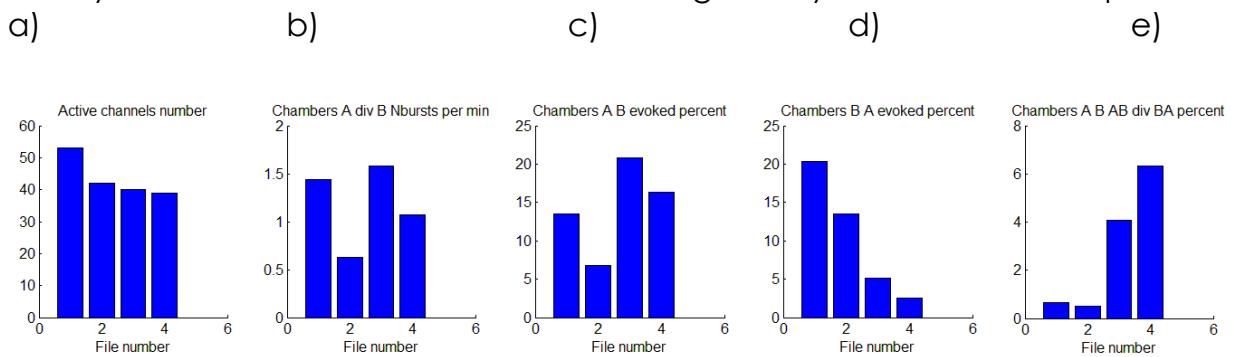


Fig. 2. Change of spontaneous signal propagation during culture development (15, 20, 25, 30 days *in vitro*). The number of active electrodes (a), ratio of spontaneous bursting frequency recorded in two chambers (b), percent of bursts evoked from chamber A to chamber B (c), from chamber B to chamber A (d) and their relation (e).

Specific design of the microchannels defined axon outgrowth. Percent of burst's transmitting in intended direction was changed depending on number of burst evoked in neuronal culture and increased during maturation (Fig. 2, c). Percent of

the burst's propagation in opposite direction decreased during development (Fig. 2, d) and the ratio of directed against backward propagation increased (Fig. 2, e).

We suppose that with culture development strength of synaptic connection increased and the activity propagated primarily in intended direction.

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MICROFLUIDICS APPLICATIONS IN FUNDAMENTAL AND MEDICAL STUDIES IN NEUROSCIENCE

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Summary. Today many fundamental questions in Neuroscience can be addressed with Microfluidics methods which provide unique approaches for cell patterning and control neural branch outgrowth. Such methods can be used to grow two separate subpopulations of dissociated neurons connected with uni-directional connectivity. Combined with microelectrode arrays such approach can be used to simulate and study neurogenesis, learning and information coding in neural networks.

Key words. neuronal cultures, microelectrode arrays, PDMS microchannels, synaptic plasticity.

INTRODUCTION

Today many fundamental questions in Neuroscience such as neuron synaptic coupling in various conditions, synaptic connectivity in the network, morphological structure and cell layers formation and many others require a development of new methods for cell manipulation and observation. Microfluidic chips containing chambers for cell plating can be easily fabricated and used for long-term imaging, and electrophysiological signal registration on MEA (microelectrode arrays).

RESULTS

In this study we designed a microfluidic device combined with microelectrode array which allows to grow two separated neuronal cultured networks with directed synaptic pathways in between into pre- and postsynaptic subpopulations of neurons. We investigated signal propagation through axonal pathways using different shapes of the microchannels in order to find optimal method to control axon outgrowth. After 10 DIV the axons coupled two cultures through 8 microchannels. We found that spontaneous bursts in presynaptic chamber evoked burst in postsynaptic chamber. Also we tested the direction of synaptic pathways in the microchannels by electrical stimuli applied to random electrodes in the pre- and postsynaptic chamber.

Then we designed PDMS chips with two-chambers and three-chambers to investigate different methods of synaptic connectivity modification between neuronal subpopulations. We used high frequency paired pulse stimulation to evoke potentiation of the synapses between neurons in separated chambers. Such direct approach can be used for study of synaptic plasticity on the network level.

Also we used microfluidic device to study progenitor cells differentiation in presence of growth factors expressed by other neurons in order to find optimal conditions for neural tissue regeneration. One separate chamber was used to grow the cells dissociated from embryonic mice (E18) and other chamber for neurospheres (hippocampal progenitor cells E14). We found that progenitor cells differentiated in 7 days and formed synaptic connectivity with mature culture (E18) grown in opposite chamber. Such methods can be used to investigate stem cells and progenitor cell differentiation and functional integration in the brain. The results can be used in the study of neuroprosthetics, neurorehabilitation.

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THE IDENTIFICATION OF THE GENETIC CAUSE OF THE CEREBELLAR HYPOPLASIA DISORDER

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Summary To identified genetic cause of congenital hypoplasia cerebellum in two families with different syndromes high-throughput sequencing analysis was performed. X-linked non-progressive ataxia in first family from Mongolian ancestry was caused by genetic defects in ABCB7 gene and modifying by ATPA7A gene.

Key words Cerebellar hypoplasia/atrophy, congenital ataxia, disequilibrium syndrome, ABCB7, ATPA7A.

INTRODUCTION

Hypoplasia and atrophy of cerebellum form a group of rare development disorders characterized by early childhood onset, gross motor development delay, truncal and limb ataxia, dysarthria, nystagmus and ophthalmoplegia, with or without cognitive decline. Here we present the case of the family from the Republic of Buryatia in Russia that includes the three generations of male patients with non-progressive form of ataxia (Illarioshkin et al., 1996). The characteristic

features of this syndrome are the delay in developmental milestones, inability to sit up to 15 months old and walk without support up to 7 years old. Although motor function was impaired, neither muscle weakness, nor cognitive impairment or memory disorders were revealed and no ionic imbalance was detected.

CONCLUSIONS

Whole-genome sequencing of the patients with non-progressive cerebellar ataxia in large Buryat family revealed the missense mutation in ABC-cassette transporter subfamily B member 7 (ABCB7) and the 41,4 kb deletion in copper transporter gene (ATP7A) with complete loss of phosphoglycerate mutase retrogene (PGAM4) (Protasova et al., 2015). The identified mutation in ABCB7 gene leads to the substitution of high conserved glycine to serine in intra mitochondrial ATPase substrate binding domain (ATM). In addition we found the deletion in ATP7A gene, that truncates first of the six metal binding domain in copper transporter and most likely does not distract the function of protein, however exerts possible modifying effect.

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NEUROPROTECTIVE AND ANTIHYPOXIC EFFECTS OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN HYPOXIA MODELING

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Summary. The aim of the investigation was to assess antihypoxic and neuroprotective properties of the glial cell line-derived neurotrophic factor (GDNF) in hypoxia models *in vitro* and *in vivo*. *In vitro* experiments were carried out on primary hippocampal cultures. Hypoxia modeling was performed on day 14 of culture development *in vitro* (DIV) by replacing the normoxic cultural medium with a medium containing low oxygen for 10 minutes. Registration of extracellular action potentials was conducted by MEA systems (Multichannel Systems, Germany) application. Study the effect of GDNF on synaptic plasticity was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy. *In vivo* experiments were carried out on C57BL/6j male mice. For acute hypobaric hypoxia a vacuum flow-through chamber was used at the ambient temperature of 20–22°C. We have investigated the resistance of animals to hypoxia and their spatial memory retention in Morris water maze test 24 hours after hypoxia. *In vitro* and *in vivo* data demonstrated that GDNF has strong antihypoxic and neuroprotective properties. Preventive GDNF application before hypoxia contributed to the animal survival and spatial memory retention as well as the maintenance of cells viability in primary hippocampal cultures.

Key words: neuron-glial networks, glial cell line-derived neurotrophic factor (GDNF), hypoxia, primary hippocampal cultures, neuroprotection

INTRODUCTION

Nowadays investigations concerning the searching endogenous factors for the nervous cells protection from hypoxic damage is one of the topical issues in modern neuroscience and medicine. Glial cell line-derived neurotrophic factor (GDNF) considered as a possible endogenous substance able to control cellular metabolic rates under low oxygen conditions and promotes neuronal survival. However, a question about the mechanisms of neuroprotective and antihypoxic actions of GDNF during hypoxia is still open.

The aim of the investigation was to study antihypoxic and neuroprotective GDNF actions in hypoxia models *in vitro* and *in vivo*.

MATERIAL AND METHODS

In vitro studies were performed using hippocampal cells dissociated from 18-day embryonic CBA mice. The cells were cultured on multielectrode arrays (Multichannel Systems, Germany).

Hypoxia modeling was performed on day 14 of culture development *in vitro* by replacing the normoxic cultural medium with a medium containing low oxygen for 10 min. The oxygen was displaced from the medium in sealed chamber in which the air was replaced with an inert gas. The neurotrophic factor was added to the

medium 20 min before hypoxia. In the control group hypoxia was induced without additional treatment.

The viability of dissociated hippocampal cells was evaluated according to the percentage ratio between the number of dead cells stained by propidium iodide (Sigma, Germany) and the total number of cells stained by bisBenzimide (Invitrogen, USA) for 7 days after hypoxia.

In vivo experiments were performed on 86 C57BL/6j sexually mature male mice weighing 18–20 g. For modeling of acute hypobaric hypoxia a vacuum flow-through chamber was used at the ambient temperature of 20–22°C. Mice were placed under conditions corresponding altitude 10 000–10 500 m (170–185 mm Hg) with a lifting speed 183 m/s [1]. The long-term memory retention test (Morris water maze re-test) was conducted 24 hours after hypoxia.

RESULTS

In vitro experiments showed that 10-minutes acute hypoxia caused the decreasing of cellular viability in primary hippocampal cultures approximately 4.5 times ($p<0.01$). A preventive GDNF application reduced the number of dead cells in 2 times in comparison with control cultures ($p<0.01$).

Electrophysiological data demonstrated that hypoxia led to spontaneous bioelectrical activity violations and to the destruction of pattern of spontaneous network activity. Preventive application of neurotrophic factor GDNF (1 ng/ml) partially neutralizes the negative hypoxic effects on the spontaneous bioelectrical activity. By the day 7 of the post-hypoxic period in group, which received preventive doses of neurotrophic factor, there was a restoration in the number of small network bursts and in the average number of spikes per burst up to the baseline. At the same time the parameters of spontaneous bioelectrical activity in control cultures, without preventive GDNF treatment, were significantly ($p<0.05$) lower than in experimental groups.

To identify the possible GDNF influence on synaptic plasticity, the level of the expression of mRNA GluR2-subunits of AMPA-receptors in normoxic conditions and after acute oxygen deficiency was evaluated. The received data showed that hypoxia significantly decreased the number mRNA GluR2-positive cells. Preventive GDNF application contributed to the preservation the level of cells, expressing mRNA GluR2-subunits of AMPA-receptors, whether in normal conditions GDNF injection resulted in increased expression of mRNA GluR2.

The following step was to assess the effect of GDNF on animal survival to acute hypobaric hypoxia. It was shown that preventive intranasal application of the neurotrophic factor (4 µg/kg) increased animal resistance to acute hypobaric hypoxia which is manifested as significantly elevated the lifetime on the "height".

CONCLUSIONS

Our data revealed that glial cell line-derived neurotrophic factor has strong antihypoxic and neuroprotective properties. Preventive GDNF application neutralizes the negative effects of oxygen deficiency by increasing the cell viability and maintaining of functional network activity in primary hippocampal cultures at a certain functional level.

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THE ROLE OF THE MEDIAL PREOPTIC AREA GLYCINERGIC SYSTEM IN THE SOCIAL TYPES OF BEHAVIOR REGULATION

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Summary. The medial preoptic nucleus is critically involved in the social type of behavior regulation, such as parental behaviour, social recognition, sexual behaviour, ect.. A big amount of studies are focused on the role of glutamate, GABA, serotonin, and dopamine systems of medial preoptic area (mPOA) in the social types of behaviour regulation. However, the role of glycinergic system in this nucleus has not been investigated.

Key words. medial preoptic nucleus, hypothalamus, glycine, sexual behaviour, social recognition

Medial preoptic area (mPOA) is critically involved in the regulation of male sexual behavior in all vertebrate species in which its role has been studied. Electric stimulation of this area determines consummatory phase of sexual behavior, while mPOA lesions in model experiments inhibit this type of behavior. Furthermore, the studies performed with mPOA slices of male rats showed that fast inhibitory responses in mPOA neurons depend on GABA and glycine. Also mPOA is involved in social recognition regulation. Social recognition supersedes any social type of behavior, particularly sexual behavior. Therefore, after the determining the mPOA glycine role in the male sexual behavior regulation, mPOA glycine role in social recognition regulation was examined.

A microinjection technique and bilateral cannulas implantation into the mPOA were used in both series of experiments. For sexual behavior patterns like session duration, duration of postejaculatory period, number of intromissions and ejaculations were recorded using video registration in freely moving males in the presence of females and then analyzed. For social recognition were used the social recognition test box, divided into three compartments by partitions, and SMART v3.0.01 software for tracking.

Bilateral microinjection of an inhibitory neurotransmitter glycine (1 mM) in male rat mPOA authentically decreases ejaculation latency period, the duration of the postejaculatory period and the number of intromission in session; bilateral microinjection of glycine antagonist strychnine (20 μ M) increases the duration of the postejaculatory period. In this case, presumably, glycine depletes some mPOA inhibitory effect on sexual behavior. These results are consistent with the literature, according to which the concentration of glycine in the mPOA decreases after ejaculation and increases before the next session. An opposite effect of strychnine on this parameter also supports this hypothesis. After bilateral microinjection of strychnine (20 μ M) male rats authentically prefer middle section rather than section A (with resident male rat) or section B (with intruder male rat). After bilateral microinjection of glycine (1 mM) male rats prefer section B (with intruder male rat) rather than section A and middle section. Obtained data allow to suggest that the mPOA glycine stimulation probably makes males more socially active and better contact with the stranger males, whereas strychnine microinjections strongly decreases social activity.

Obtained data show that mPOA glycinergic system plays an important role in social type of behavior regulation and needs further investigations.

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