(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 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. excessive young cells occurs in the germinative area of the hippocampus. The possible mechanism of aberrant maturation of the newly generated cells in the absence of their visible structural abnormality can be launched by

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 # 14-04-3152.

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 λ=543. 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

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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 DH5a cells, the plasmid purification kit «QIAGEN DNA» and the software «VectorNTI». We defined the primers system (Synpo-HindIII-fw, Synpo-MluI-rev) and the optimum annealing temperature of primers - 65℃. 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 it 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