DOI 10.18699/SBB-2022-428

Novel approaches for analysis of the functional activity of neural networks *in vitro*

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Key words: primary neuronal cultures, neural networks, network characteristics, spontaneous calcium activity, spontaneous bioelectrical activity, data analysis

Motivation and Aim: According to modern concept, neural networks are the minimal functional unit of the central nervous system responsible for the processing, storage and transmitting information [1, 2]. Currently, the studies on functional neural network activity are mainly implemented using multielectrode arrays (MEAs) and functional calcium imaging. These approaches allow to evaluate the effect of certain stressors and pharmacological agents on the functional reorganization of neural networks and to identify the contribution of such essential elements as astrocytes in the modulation of neural network plasticity. On the other hand, the continuing challenges in the biological signals processing result in the loss of a large amount of information that could have great fundamental and practical relevance.

Methods and Algorithms: Primary hippocampal cultures were obtained from C57Bl/6 murine embryos (E18) and cultured according to the previously developed protocol [3] on multielectrode arrays or coverslips. The initial density of cells was 7000–9000 cells/mm^2. The primary hippocampal cultures were grown under constant conditions of 35.5°C, 5 % CO_2 and a humidified atmosphere in culture incubator for 28 days. Registration of spontaneous bioelectrical activity of primary hippocampal cultures was performed using a USB-MEA120-2-InVBC-System-E-Standard (Multichannel system, Germany) according to the previously developed protocol [2]. Electrophysiological data analysis was performed using original software which allows the detection of the main network characteristics: number of small and large network burst, number of spikes in a burst, duration of the network burst. The algorithm also allows the identification of the functional network structure by the cross-correlation method and graphs and the presentation of the network activation pattern. Registration of spontaneous calcium activity of primary hippocampal cultures was performed using an LSM 800 confocal laser scanning microscope (Carl Zeiss, Germany) and a fluorescent calcium-sensitive dye Oregon Green 488 BAPTA-1 AM (Invitrogen). Analysis of calcium events was based on previously developed algorithm [4]. A pairwise correlation analysis of fluctuations in the intracellular calcium level in the individual cells was then carried out. A functional network is presented as a non-oriented graph with nodes corresponding to the cells, and the edges designating a significant correlation between the levels of calcium activity in cells exceeding the threshold value ($p > 0.3$) [5, 6].

Results: The main regularities of reorganization of neural network activity were reveled using the novel algorithms in a model of acute hypoxia *in vitro* taken here as an example. On the one hand, the algorithms open a possibility to reveal the role of individual cellular elements in the neural network activity; on the other hand, they allow to show general regularities of formation and preservation of functionally active network centers (hubs) under conditions of the network reorganization. Our approach is also resolved the problem of detection of calcium oscillations and allows to reveal the level of correlation between both neighboring and distant cells as well as the signal speed propagation. The use of novel algorithms allows us to study in detail the features of the action of a number of substances with neuroprotective properties, such as neurotrophic factors BDNF, GDNF, HIF prolyl hydroxylase inhibitors, vitamin D3, on the functional architectonics of neural networks in normal state and hypoxic damage. The data obtained undoubtedly expand our knowledge about the CNS functioning as well as can serve as a useful tool for assessing the risk of side effects that contributes to the development of new therapeutic strategies for different pathologies of the CNS.
Conclusion: The use of novel algorithms of analyzing the data of spontaneous bioelectrical and calcium activity of neural networks opens the possibility of identifying the main mechanisms of action of neuroprotectors as well as conducting high-precision screening for selection the most effective methods of neurodegenerative processes correction and prediction the possible side effects of new therapeutic drugs and approaches.
Acknowledgements: The study was supported by Ministry of Science and Higher Education of the Russian Federation, agreement No. 075-15-2020-808.

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