Detection of Trace Processes in the Networks of Neurons Cultured on Microelectrode Arrays

A. L. Sukhanova*, O. A. Mineyeva, I. I. Kiselev*, M. S. Burtsev*,**, K. V. Anokhin*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 538-541, May, 2012
Original article submitted March 5, 2011

We compared the effects of electrical stimulation of primary dissociated neuronal cultures cultured on microelectrode arrays in terms on the expression of *c-fos* transcriptional factor that is involved in plastic reorganization in neurons. Dissociated hippocampal neurons cultured on multielectrode arrays were exposed to two stimulation protocols: high-frequency and low-frequency stimulations. Expression of *c-fos* was evaluated using immunofluorescence. Both high-frequency and low-frequency stimulations significantly increased *c-fos* expression in comparison with non-stimulated control. These findings indicate that *c-fos* expression can be induced in neuronal cell culture by different types of electrical stimulations and can be used for studying plasticity processes in microphysiological *in vitro* systems.

Key Words: *neuronal plasticity; microelectrode array; neuronal cultures; gene expression; fluorescent markers*

Microphysiological *in vitro* systems based on primary nerve cells cultured on microelectrode arrays (MEA) are gaining ground in biomedical investigations [9]. They are used for successful trials of toxic and physiological effects of various chemical compounds [11,13], as well as new pharmaceuticals [3,8].

However, currently available microphysiological test-systems are infrequently used in evaluation of the one of the main features of nervous tissue, plasticity. We previously showed that plastic changes in the nervous system *in vivo* are associated with activation of immediate early genes that encode the proteins essential for long-term changes of neuron properties [2]. These genes may serve as cellular markers of plasticity processes in the brain [1], and thousands of studies of brain plasticity under normal and pathological conditions were carried out in the last two decades using this approach [5,6].

*Department of Neuroscience, NBICS Center, National Research Center "Kurchatov Institute"; **Laboratory of Neurobiology of Memory, P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** alsukhanova@gmail.com. A. L. Sukhanova

The objective of this study was to expand this method to detect plasticity processes in neuronal circuits of microphysiological *in vitro* systems. Currently used exposures aimed at neuronal plasticity induction can be ranged into two types: low-frequency [12,14] and high-frequency stimulation [4,7,10]. This study was aimed at evaluation of changes in *c-fos* expression as the measure of nerve cell plasticity *in vitro* in response to low-frequency and high-frequency electrical stimulation, and to compare these two stimulation protocols in terms of capability to induce *c-fos* expression.

MATERIALS AND METHODS

Primary dissociated neuronal cultures were obtained from hippocampi of newborn BALB/c mouse pups (P0-P1). Cells were plated on 64-electrode arrays (MEA, MultichannelSystems) pretreated with 0.05% poly-L-lysine. Cell cultures were incubated at 37°C, 100% humidity and 5% CO_2, in Neurobasal medium containing culture supplement B27 and 0.05 mM glutamine (GIBCO).

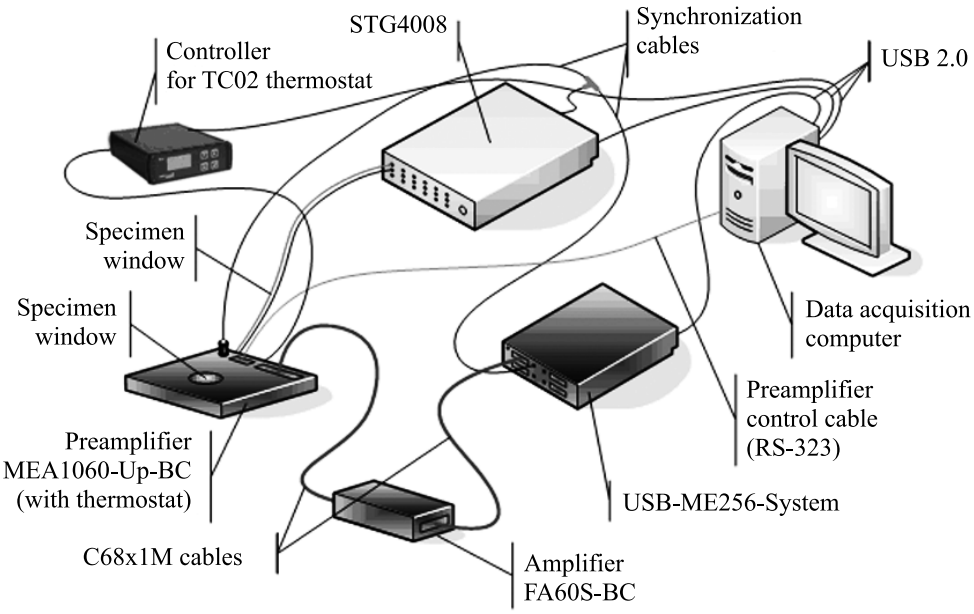

Fig. 1. Apparatus connection scheme.

Neuronal cultures were electricalally stimulated for 10 min using a technology that allowed registration and stimulation of electrical activity on the multielectrode array (Fig. 1) using one of the two protocols (Table 1) reviewed elsewhere [1-5].

Immunofluorescent staining was carried out using polyclonal goat IgG antibodies against c-Fos protein (antic-Fos(4)-Gsc-52-G, SantaCruzBiochem) and subsequent detection using donkey antigoat antibodies labeled with AlexaFluor 488 fluorophore (AlexaFluor-donkeyanti-goat 488; Invitrogen). In addition, cell nuclei were stained with DNA-specific marker Hoechst.

Numerical data were statistically treated using Microsoft Excel, significance of differences was assessed using Student's *t* test.

RESULTS

The experiment employed eight multielectrode arrays with 25-day-old neuronal cultures: 3 arrays were stimulated with high-frequency bursts, 3 arrays were exposed to low-frequency stimulation with single impulses, and 2 arrays were exposed to the same treatment but without electrical stimulation. Neuronal cultures were fixed with paraformaldehyde 2.5 h after electrical stimulation and subsequently stained with immunofluorescent method and visualized under a microscope. Microphotographs (at least 10 view fields 500×300 μ in each array) were analyzed using ImageProPlus 6.0 software (MediaCybernetics). Representative images from each group of samples are presented (Fig. 2) as well as the results of quantitative data processing (Fig. 3).

High-frequency electrical stimulation with bursts and low-frequency stimulation with single pulses induced *c-fos* expression in mouse hippocampal cell cultures that significantly differed from the control (Figs. 2, 3). Hence, this approach can be used for assessment of possibility for *in vitro* model employment in investigations of cellular mechanisms of plasticity as well as

TABLE 1. Main Parameters of Electrical Stimulation Protocols

Parameter	Low-frequency stimulation with single pulses	High-frequency stimulation with bursts
Positive phase	400 μsec, 300 mV	400 μsec, 300 mV
Negative phase	300 μsec, -300 mV	300 μsec, -300 mV
Compensating phase	100 μsec, 80 mV	100 μsec, 80 mV
Interpulse interval	10 sec	10 msec
Interburst interval	–	300 msec
The number of pulses per burst	–	6
Switching pulse	1200 μsec, TTL	1200 μsec, TTL

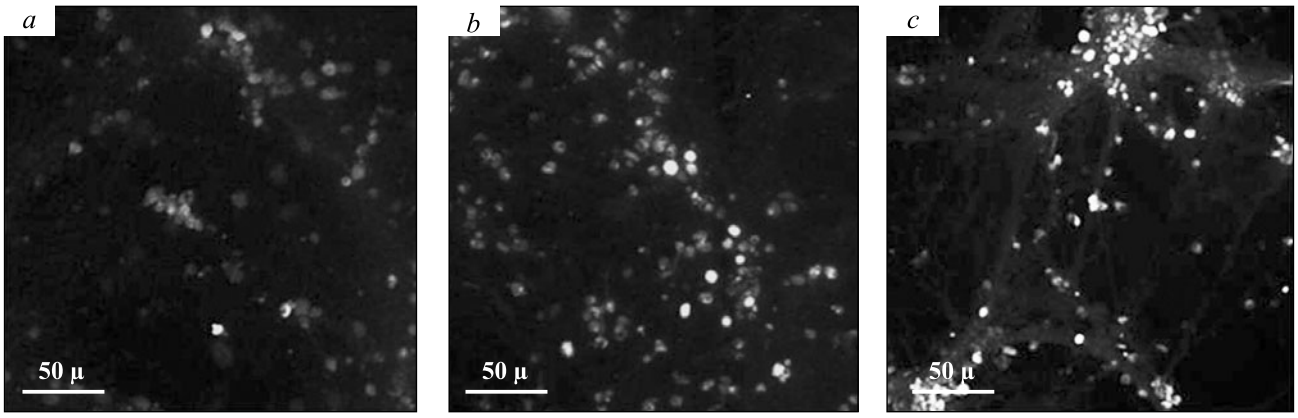

Fig. 2. Immunofluorescent detection of c-Fos protein in dissociated culture of hippocampal cells on day 25 in culture, ×30. *a*) no stimulation, *b*) low-frequency stimulation with single pulses, *c*) high-frequency stimulation with bursts.

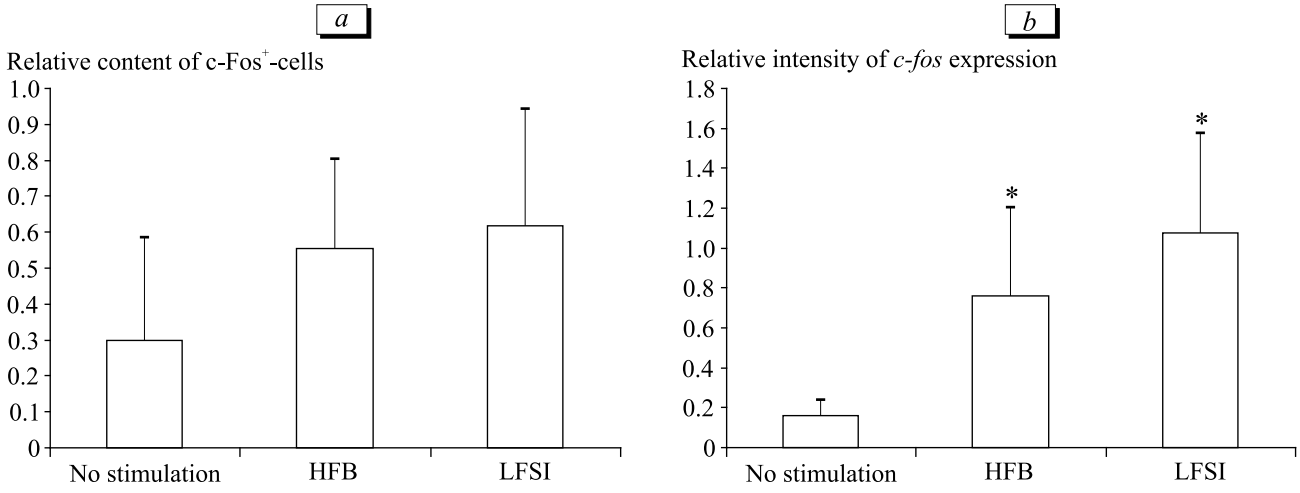

Fig. 3. Quantitative analysis of *c-fos* expression in dissociated cultures of hippocampal cells following different types of electrical stimulation. The number of c-Fos$^+$-cells was normalized per total amount of nuclei, and the mean was calculated for all fields of view (*a*). Total intensity of fluorescence in the regions with cells in green channel (c-Fos) was normalized per intensity of blue channel (nuclei), and the mean was calculated for all fields of view (*b*). HFB: high-frequency stimulation with bursts, LFSI: low-frequency stimulation with single pulses. *$p<0.05$ in comparison with the control (no stimulation).

for development of physiological microsensor systems based on nerve cells cultured on multielectrode arrays with simultaneous detection of gene expression.

No significant differences were detected in the levels of induced c-Fos protein expression between the two types of electrical stimulations (Fig. 3), probably because neuronal cultures were examined only on day 25 *in vitro*. In addition, *c-fos* expression was registered only 2.5 h after the stimulation. The following experiments are planned for further evaluation of expression of molecular markers of plasticity in neuronal cultures: evaluation of changes in expression of molecular markers of plasticity in response to nonspecific influences associated with experimental procedures; evaluation of dynamics of gene-marker expression during cell culture growth and its correlation with spontaneous electrical activity; comparison of different types of electrical stimulation in terms of the dynamics of induced expression of c-Fos and other plasticity markers; asessment of the optimal age of neuronal cell culture for plasticity induction.

The study was supported by Ministry of Education and Science of the Russian Federation within the framework of Federal Target Program "Research and development on priority directions of scientific-technological complex development in Russia for years 2007-2013" (State contract No. 16.512.11.2134).

RERERENCES

1. A. B. Abramova, K. V. Anokhin, O. N. Dolgov, *et al.*, *Vestn. Novykh Med. Tekhnologii*, **1**, 47-53 (1997).
2. K. V. Anokhin, *Zh. Vyssh. Nerv. Deyat.*, **47**, No. 2, 261-279 (1997).

3. T. K. Ahuja, *Drug Develop. Res.*, No. 68, 84-93 (2007).
4. D. J. Bakkum, Z. C. Chao, and S. M. Potter, *J. Neural. Eng.*, **5**, No. 3, 310-323 (2008).
5. D. N. Barry and S. Commins, *Rev. Neurosci.*, **22**, No. 2, 131-142 (2011).
6. J. F. Guzowski, J. A. Timlin, B. Roysam, *et al.*, *Curr. Opin. Neurobiol.*, **15**, No. 5, 599-606 (2005).
7. Y. Jimbo, H. P. C. Robinson, and A. Kawana, *IEEE Trans. Biomed. Eng.*, **45**, No. 11, 1297-1304 (1998).
8. G. Kang, J. H. Lee, C. S. Lee, and Y. Nam, *Lab. Chip.*, **9**, No. 22, 3236-3242 (2009).
9. J. Noraberg, F. R. Poulsen, M. Blaabjerg, *et al.*, *Curr. Drug Targets CNS Neurol. Disorg.*, **4**, No. 4, 435-452 (2005).
10. A. Novellino, P. D'Angelo, L. Cozzi, *et al.*, *Comput. Intell. Neurosci.* (2007). doi: 10.1155/2007/12755.
11. B. L. Robinette, J. A. Harrill, W. R. Mundy, and T. J. Shafer, *Front. Neuroeng.*, **4**, 1 (2011).
12. G. Shahaf and S. Marom, *J. Neurosci.*, **21**, No. 22, 8782-8788 (2001).
13. K. Varghese, P. Molnar, M. Das, *et al.*, *PLoS one*, **5**, No. 1, e8643 (2010).
14. D. A. Wagenaar, J. Pine, and S. M. Potter, *J. Neurosci. Methods*, **138**, Nos. 1-2, 27-37 (2004).