Patterns of Electrical Activity Generated by Biological Neural Network *in vitro*

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***Abstract*—Cultured dissociated neurons forming network *in vitro* is a unique system representing living biological neural network developing in fully artificial conditions. This is a promising model for study of basic mechanisms of the brain functioning that requires special tools for interfacing and investigations. We have developed a set of devices and techniques for culturing of neural network on the surface of microelectrode sensor and registered specific patterns of electrical activity of the living neural network *in vitro*.**

***Keywords*—biological neural network, electrical activity patterns, electrophysiological data processing**

I. Introduction

Human brain can be considered as a highly sophisticated system for semantic processing [1], [2]. The basis of brain functioning is a complex neural network formed by biological cells. An ability to establish communications between neurons by means of electrical pulses and to process information in large neuronal ensembles are unique properties of neural tissue. There are numerous models of semantic processing based on neural networks [3], [4]. One particular class of these models is based on spiking neural networks [5] which closely resemble features of biological neural tissue. In this regard, studies of data processing mechanisms in brain have particular interest for development of intellectual systems design principles.

Mechanisms of brain functioning can be studied at different levels starting from molecular, cellular, network and up to behavioral and psychological. In the context of network level, cultured dissociated neurons forming connections *in vitro* is a unique system representing living biological neural network developing in fully artificial conditions. This is a promising model for study of basic mechanisms of the brain functioning that requires special tools for interfacing. Therefore, development of highly specialized investigation methods is required in order to obtain knowledge about deep mechanisms of neural ensembles functioning. We have developed a set of devices and techniques for culturing of neural network on the surface of microelectrode sensor and registered an electrical activity of the living neural network *in vitro*.

II. Experimental

We have developed the 64-channel microelectrode sensor of neuronal electrical activity suitable for multichannel interfacing with cultured neural networks. The sensor consists of planar glass base with transparent indium-tin-oxide conducting tracks serving as electrodes. The chamber for neuronal culture solution has been developed on the basis of 3D printing techniques as shown at the Fig. 1. For recording of electrical activity of neurons, a specialized 64-channel amplifier has been developed with built-in analog to digital converter and digital serial interface. Basic recording and visualization operations are controlled by the open-source software.

Dissociated neurons of the rat cortex were seeded on surface of a microelectrode array (64 electrodes) in recording chamber and placed in cell incubator. Cells formed dense network of interconnecting neurites after three weeks in culture as shown

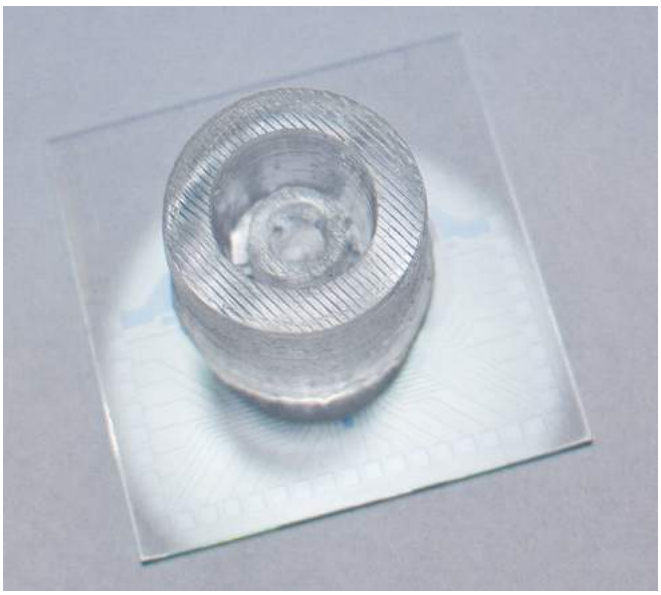


Figure 1. Microelectrode sensor with culture chamber attached.

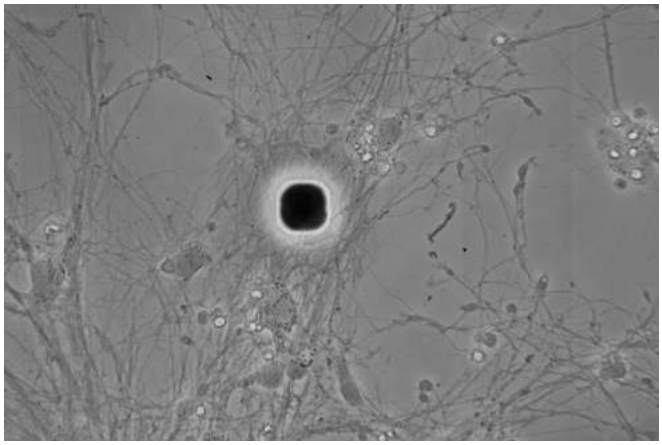


Figure 2. Neurons form dense network of interconnections around one of the working microelectrodes.

at the Fig. 2. Dark spot on the picture is electrode coated with opaque layer of electrodeposited conducting polymer poly(3,4-ethylenedioxythiophene) in order to reduce impedance and recording noise. Amplified extracellular activity of neurons was digitized and recorded to the hard disk of computer during experiments. The rate of data stream is about 10 gigabytes per hour in our case of 64 recording channels and 20 KHz sampling frequency so that specialized high-performance software tools for data processing are required. We used customized open-source module «Tridesclous» [6] for spike detection and classification. Spikes were distinguished from background noise on the basis of threshold crossing and then classified into clusters by principal component analysis.

III. Results

Typical examples of recorded multichannel neuronal electrical activity is shown at the Fig. 3. Each channel corresponds to different electrode of the array. Marks denote spikes divided into distinct clusters on the basis of amplitude and shape so that even different neurons near one electrode can be distinguished. Neuronal activity on the recordings is represented by background single sparse spikes and spike bursts. As shown at the Fig. 3a, a group of neurons at the channel 33 generates intense periodic bursts of spikes. A burst is composed by sum of repeating spikes of nearby neurons. Amplitudes of spikes are different due to different distances from electrode to neurons. This activity remains confined and spreads only partially and locally to channels 42 and 63.

Fig. 3b and Fig. 3c show another type of network behavior. Bursts at the channel 33 are followed by the bursts at the channel 63 and then less intense bursts and single spikes at the other channels. Therefore, activity generated by «pacemaker» group of neurons at the channel 33 propagates via neurites and synaptic connections over the network. Patterns of activation at Fig. 3b and Fig. 3c are similar but number of spikes in individual channels, exact timing of spikes and number of channels involved in network activation are different in sequential network bursts. This variability of the response is due to complex nature of biochemical processes running in living cells and thus constantly changing properties of individual neurons and synapses.

IV. Discussion

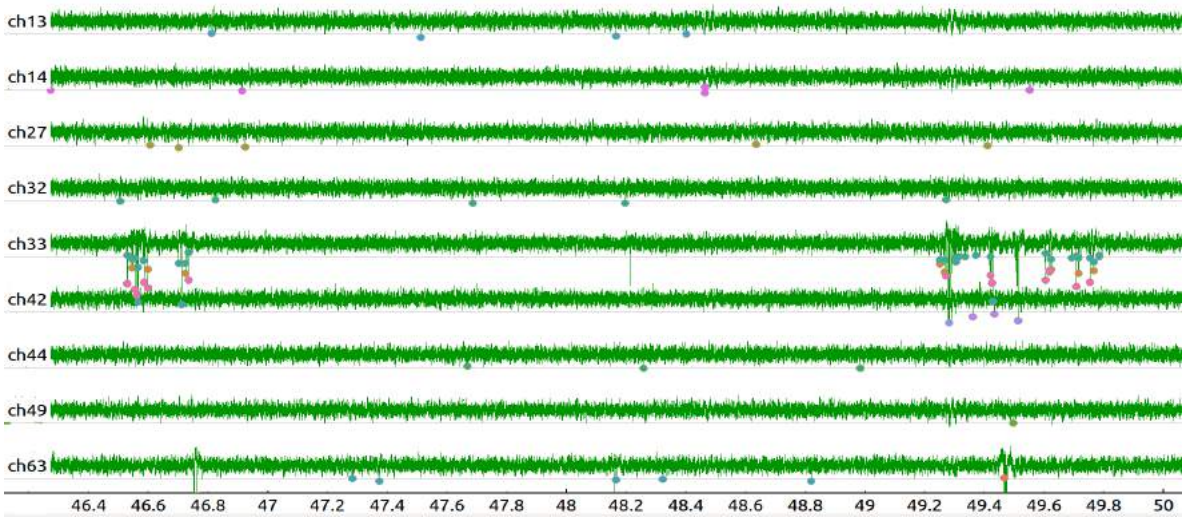
Analysis of electrical activity of biological neural networks is a complex problem including signal filtering, spike detection, classification and spike train analysis [7]. Many algorithms of spikes detection in background noise are based on threshold crossing. Choice of a threshold for spike detection is an important task because too high threshold will lead to losses of spikes, too low – to false positive detection of noise peaks as spikes. In our case, involvement of all channels in network events may indicate satisfactory functioning of the detection algorithm.

Spiking and bursting activation patterns observed in our experiments is a distinct feature of biological neural networks. Single sparse spikes between bursts may look like insignificant in network behavior, but investigations show that such single unit activity may play important role in shaping of bursting events [8]. Bursts of spikes increase reliability of neural information transmission and promote induction of synaptic efficacy changes [9]. Observed in our experiments two types of bursting (Fig. 3a and Fig. 3b, 3c) correspond to local and global network bursting indicating switching of the network behavior into different states.

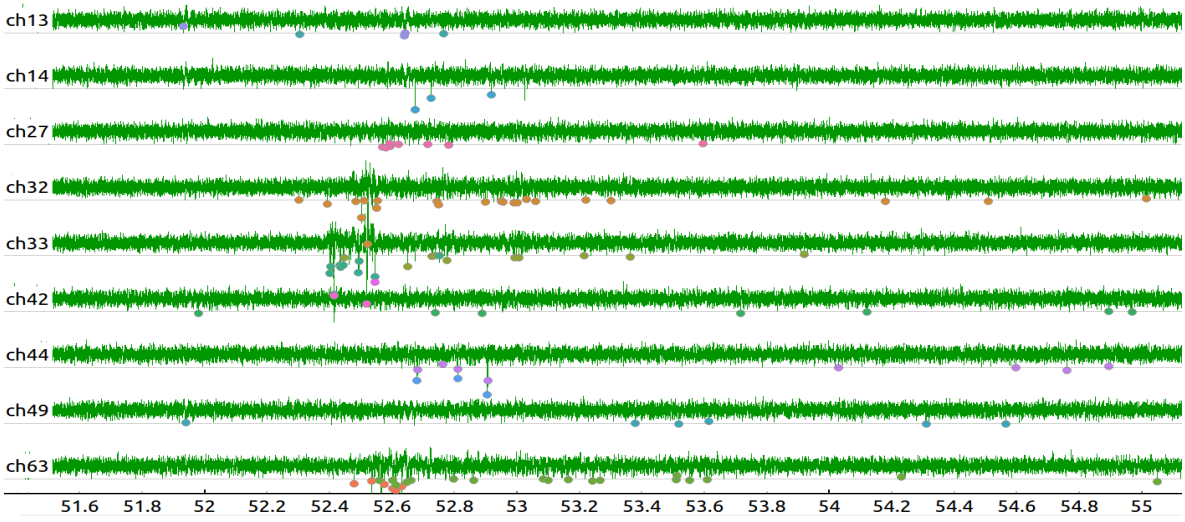
Using topological tools, it is possible to find subnetworks with repetitive and synchronous patterns of activity thus revealing connectivity features of network [10]. Data of [10] show that patterns of spiking propagation induced by external stimulation are topologically similar to spontaneous activation indicating that stimulating electrode can be treated as a biologically realistic input to the network.

A set of external stimulating electrodes can be treated as a vector input to the neural network and it is possible to attempt inducing learning processes [11] on the basis of biological learning rules [12]. In this regard, a question arises about detecting changes induced by learning protocols. Spontaneous activity can interfere with evoked one so several measures can be applied to reduce spontaneous activation such as pharmacological manipulation or low-frequency stimulation [13].

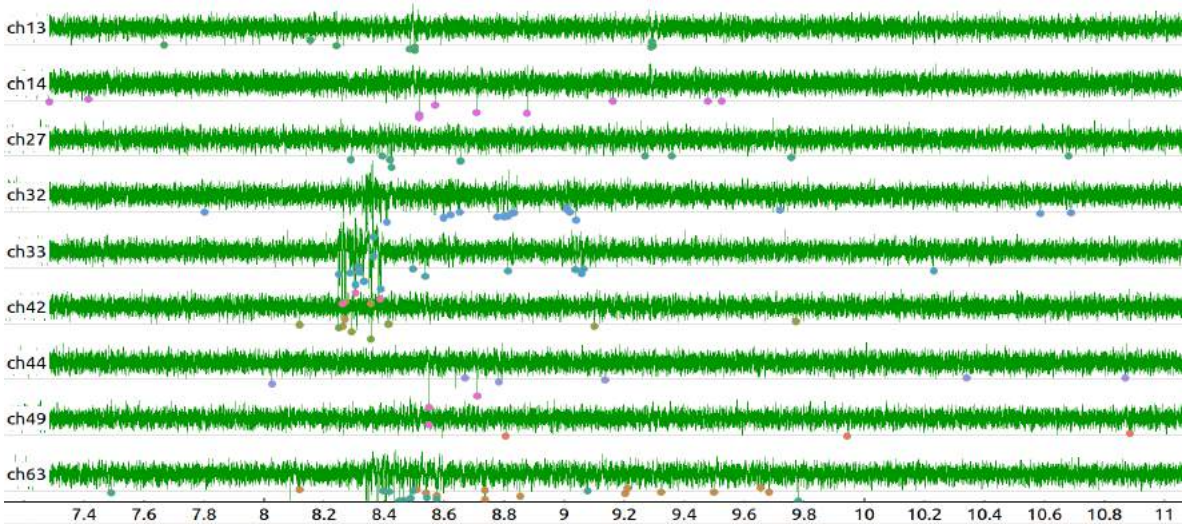
Despite confined simple structure, living network *in vitro* exhibit substantially complex behavior. One of the well-known and distinct features of developing *in vitro* neural networks is an extremely diverse set of electrical activity patterns [14]. Therefore, special techniques are required to distinguish between stochastic and determined network events. Different kinds of activity are distinguished such as spikes, avalanches and fluctuations [15] that can be treated as semantic representations of current network state. Activity patterns presented at Fig. 3b and Fig. 3c have similar overall features but different exact timing of individual spikes. Removal of stochastic part of the response and maximizing information from spike timing is important for analysis approach to completely describe network state.



(a)



(b)



(c)

Figure 3. Examples of electrical activity recorded at different time periods. (a) - Bursting activity at the channel 33. (b),(c) - Activity propagation through the network. Y axis - number of recording channel, X axis - time in seconds from recording frame start.

One of the approaches to reduce variability of electrical activity and to improve response predictability can be based on formation of ordered neural network [16].

The experimental model considered is two-dimensional network with because it is growing on a planar substrate. Modern cell culture techniques offer possibilities to engineer three-dimensional cell structures with topology resembling in vivo neural networks. Such network *in vitro* has more longer and complex response to external stimulation and spontaneous activity is characterized by spatial segregation of bursting with absence of global synchrony [17].

Increasing scale and complexity of modern electrophysiological experiments indicate need for new tools of 'big data' processing [18]. New approaches can be based on powerful tools inspired by intellectual systems designs such as deep learning [19], [20].

V. Conclusion

Diverse natural activity patterns of biological neural networks cultured on planar microelectrode arrays require utilization of sophisticated techniques in order to capture high-order features of the network state on the basis of recorded extracellular potentials. Several tasks are being solved for this purpose such as development of techniques for analysis of complex spatiotemporal patterns of network activation in order to detect response features induced by learning protocols.

Biological neural network *in vitro* is considered as a perspective experimental model for study of the mechanisms of learning. The ultimate goal of experiments with such networks is a reproduction of learning and memory processes specific to brain. However, in spite of extensive scientific work during past two decades this goal currently is not considered as clearly achieved [21]. Our current work is directed towards inducing learning processes by application of appropriate stimulating patterns to the patterned cultured neural network.

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ПАТТЕРНЫ ЭЛЕКТРИЧЕСКОЙ АКТИВНОСТИ БИОЛОГИЧЕСКОЙ НЕЙРОННОЙ СЕТИ in vitro

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Культивируемые диссоциированные нейроны, образующие синаптические связи in vitro, являются уникальной системой, представляющую собой живую биологическую нейронную сеть, развивающуюся в полностью искусственных условиях. Это многообещающая модель для изучения основных механизмов функционирования мозга, которая требует специальных инструментов для исследования и взаимодействия. Нами разработан комплекс устройств и методов для культивирования нейронной сети на поверхности микроэлектродного сенсора и получены специфические закономерности электрической активности живой нейронной сети in vitro.