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Neural signal registration and analysis of axons grown in microchannels

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Neural signal registration and analysis of axons grown in microchannels

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Abstract. Registration of neuronal bioelectrical signals remains one of the main physical tools to study fundamental mechanisms of signal processing in the brain. Neurons generate spiking patterns which propagate through complex map of neural network connectivity. Extracellular recording of isolated axons grown in microchannels provides amplification of the signal for detailed study of spike propagation. In this study we used neuronal hippocampal cultures grown in microfluidic devices combined with microelectrode arrays to investigate a changes of electrical activity during neural network development. We found that after 5 days *in vitro* after culture plating the spiking activity appears first in microchannels and on the next 2-3 days appears on the electrodes of overall neural network. We conclude that such approach provides a convenient method to study neural signal processing and functional structure development on a single cell and network level of the neuronal culture.

1. Introduction

An Action Potential (AP) generated in neuron propagates through an axon with amplitude about 100 mV. Such signal mostly occurs inside cell body while extracellular measurements record AP as 20-100 μV spikes. It was shown that extracellular voltage potentials of axons proportionally depend on the resistance of extracellular medium [1]. The resistance can be significantly increased by reducing extracellular medium volume around axon near recording electrode which can be done by growing axons in microfluidic microchannel with 5-10 μm diameter. AP signals of isolated axons in microchannels combined with extracellular recording on microelectrode arrays (MEA) can be amplified up to 4.5 mV [2]. Such method allows to study axonal signal transmission for various medical applications [3,4], implantable neurointerfaces [1] and information processing in axons. Microfluidic methods allow to control morphology of the neural culture of dissociated neurons to study functional characteristics of realistic unidirected synaptic pathways in the neural tissue. Specific form of the microchannels (figure 1c) can be used to guide axon outgrowth in preferred direction between chambers [5, 6]. Such directed connectivity

provides a directed spike propagations and localization of pre- and postsynaptic neurons in the network for fundamental studies of synaptic plasticity, neural information coding/decoding, memory detalization and learning on the network level of neural tissue.

However axonal signals in asymmetric microchannels with specific geometry [6] has not been yet studied. Axonal signals in microchannels recorded on early stages of the neural culture development during axon growth in specified direction can be used to uncover mechanisms of neuronal connection appearance. We used the method to investigate the relations between electrical activity and morphology on the cellular and network wide level of the neural culture organization.

2. Materials and methods

2.1. Microfluidic device fabrication

General design of the microfluidic device is shown in figure 1 (a). Microelectrode array (MEA) was precisely positioned and connected with poly-dimethylsiloxane (PDMS) microchannels, connecting two chambers of the device. The mcrofluidic chip was fabricated by pouring PDMS (Sylgard 184, Dow Corning) onto the structured molds and curing at 70°C for at least 4 h. Then PDMS chips were removed from molds and chamber structures were punched. Finally, each PDMS chip was positioned and mounted onto the surface of a planar microelectrode array (MEA). For mold fabrication, standard two layer lithography was used: silicon wafers were spin-coated with SU-8 2025 negative photoresist (MicroChem, USA), diluted by the SU-8 Thinner (MicroChem, USA) for the first layer (4.5 μm thick) and SU-8 2075 for the second layer (~200 μm). Exposure was performed through a chromium photomask on MJB4 (SUSS Microtec, Germany) with an UV-filter PL-360-LP (Omega Optics, USA). Patterns were developed by using SU-8 Developer (MicroChem, USA).

2.1. Cell culture and electrophysiology recording

Hippocampal neuronal cells were dissociated from embryonic mice (E18) and plated on microelectrode arrays (MEAs) (Multichannel systems, Germany) at a final density of approximately 15,000–20,000 cells/mm2 [6]. The MEAs consisted of 60 electrodes with the diameter of 30 μm and 200 μm interelectrode space. Electrophysiological signals of spontaneous spiking activity were recorded with USB-MEA120-Inv-2-BC-System (Multichannel systems, Germany) and analyzed with custom made software Meaman developed in Matlab. The experiments were performed from 5 to 12 days in vitro (DIV) after culture plating. For more details of the experimental procedures see [6,7].

3. Results and Conclusions

3.1. Results

In our previous study [6] we developed the microfluidic devices which consisted of two chambers with neuronal subcultures connected by microchannels for axons and dendrites outgrowth (figure 1b, c). The device was placed on microelectrode array to record neuronal bioelectrical activity in microchannels and chambers independently (figure 1a). Because of specific form of microchannels axons grew mostly in one direction: from presynaptic subculture (left chamber) to postsynaptic (right chamber). We investigated electrophysiological signals recorded from the electrodes in microchannels (axonal spikes) and chambers (somata, dendritic and axonal signals).

The signal from the microelectrode composed of extracellular potentials from axon, dendrites and neuron's somata. Amplitude of the potentials proportionally depended on extracellular resistance that mostly depended on extracellular medium volume around axon near the recording electrode. The structure of the microfluidic microchannels limited a volume of extracellular medium around axon increasing its resistance and hence extracellular potential. Example of signal amplitude amplification in the microchannel is shown on figure 1 (d, right), in contrast to regular spiking activity recorded from neurons on the electrode where the signal is lower (figure 1d, left).

Microscopic image (20x) of electrodes in the microchannel and the chamber are shown on figure 1 (e) on the right and left respectively.

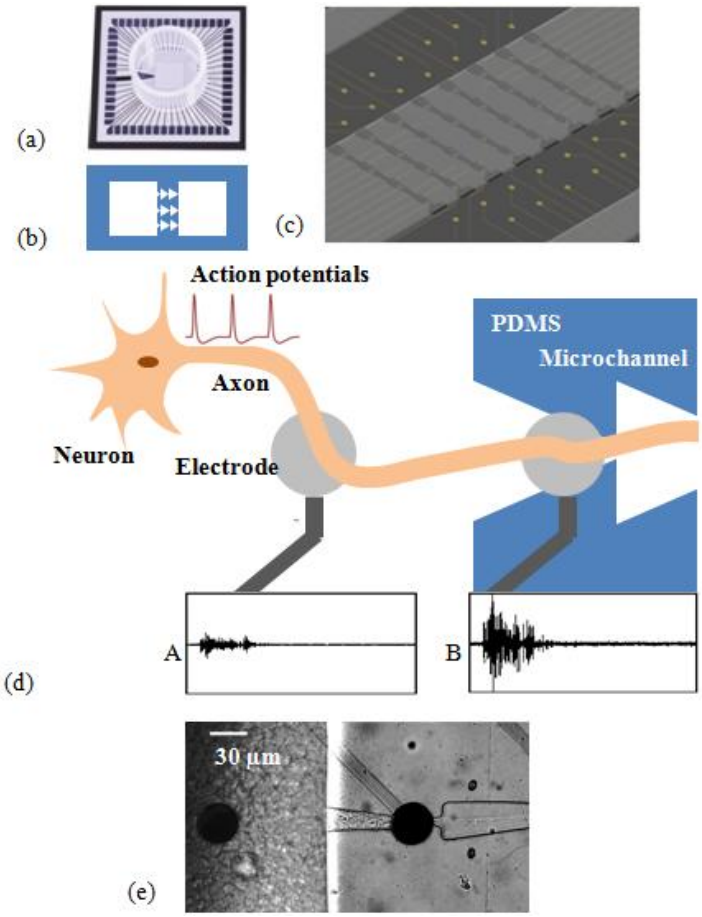

Figure 1. Hippocampal culture grown on MEA in microfluidic chip with two chambers connected by microchannels. (a) Microelectrode array with 60 electrodes combined with microfluidic device (b) with 8 microchannels. (c) Scheme of microfluidic device with two chambers connected by microchannels with length of 600 µm. (d) Schematic representation of signal registration in the microfluidic structure and examples of recorded signals. (e) Hippocampal cells grown on microelectrode array in microchannels at the places illustrated in (d). Microscope photo was made with 10x objective on 5 DIV.

We recorded the spiking activity on the MEA starting from 5th day of development *in vitro* (DIV) every two days until DIV 12. In one culture on 5 DIV the spikes were obtained in all electrodes of the MEA, but a firing rate in the microchannels was greater (40-300 Hz) than in the chambers (20-50 Hz). On 6 DIV spontaneous bursting activity appeared in the microchannels. The bursts were characterized by rapid spiking periods about 200-500 ms that followed by silence phase. The bursts in the chambers appeared on 8 DIV. Example of the recorded signal from electrodes in chamber and microchannels are shown in figure 2 (a). Note that due to the amplification inside the microchannel the noise level of the signal was also higher than in the chamber (figure 2b). Higher noise level leads to increase in absolute value of spike detection threshold estimation. Distributions of spike amplitudes observed for one electrode in the microchannel and one in the chamber were presented in figure 2 (c). The activity from microelectrodes in chambers and microchannels on different days of development is presented on figure 2 (e), (f). Note that even in case where the electrode has a minimal coverage area with axon in the microchannel bursts were still registered (figure 2d).

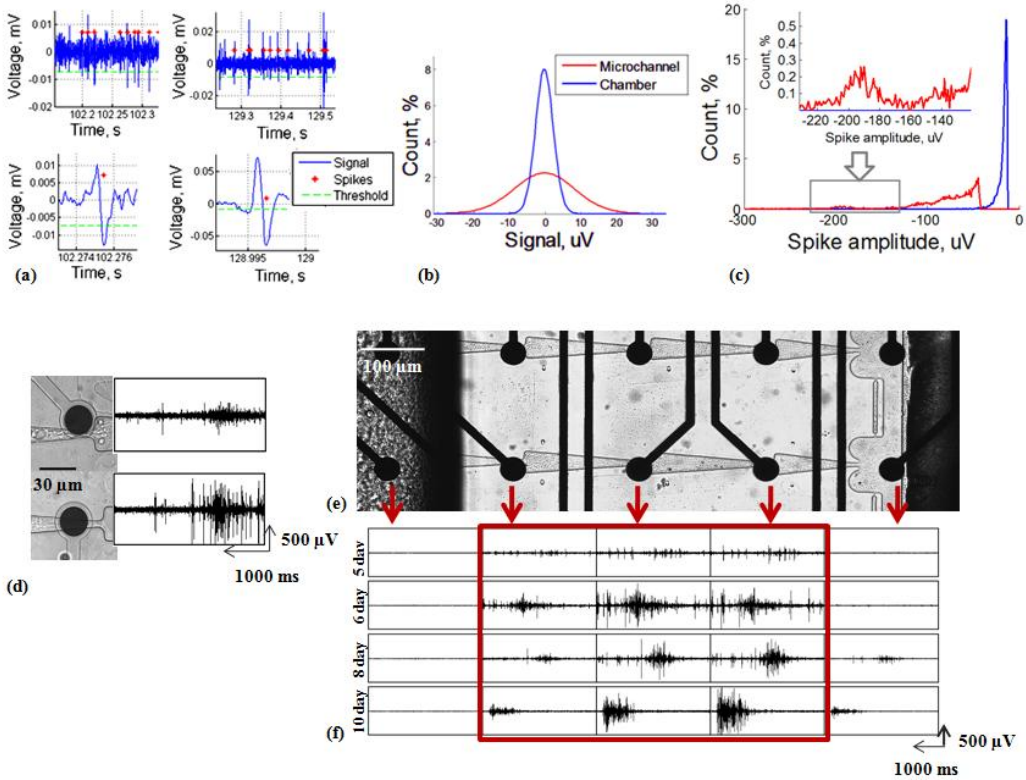

Figure 2. Neuronal signal amplification in microchannels. (a) Example of signal and one spike recorded in chamber (left) and microchannel (right). Spikes marked by asterisks. (b) Distribution of 60 seconds of the signal recorded from microchannel (red) and chamber electrodes (blue). (c) Distribution of spike amplitudes recorded from microchannel (red) and chamber electrodes (blue). (e) Microscope photo of microchannel with axons and two chambers with neurons. (f) Activity recorded from microelectrodes in chambers and microchannels (red box) on different days of development. Bursts in microchannels appeared earlier than in chambers and demonstrated greater amplitude.

Next we investigated electrophysiological characteristics of the 5 neuronal cultures during culture development. Mean firing rate, number of bursts in microchannels and chambers and amplitude amplification during each 2 days are presented on the figure 3 (n=5 cultures). Firing rate increased during culture development and was always greater in microchannels (figure 3a). Number of bursts per minute in microchannels and chambers increased until 8-10 DIV and then decreased. Number of bursts dominated in microchannels. Importantly that the bursting activity appeared in microchannels two days before it was obtained in chambers (figure 3b). We estimated a ratio between the spike amplitude recorded in the microchannels and chambers during each burst. The amplitude ratio in microchannels was about 1.4 in three experiments, 3 and 4.7 in other two cultures (figure 3c). Note that in average the ratio was relatively stable during the culture development.

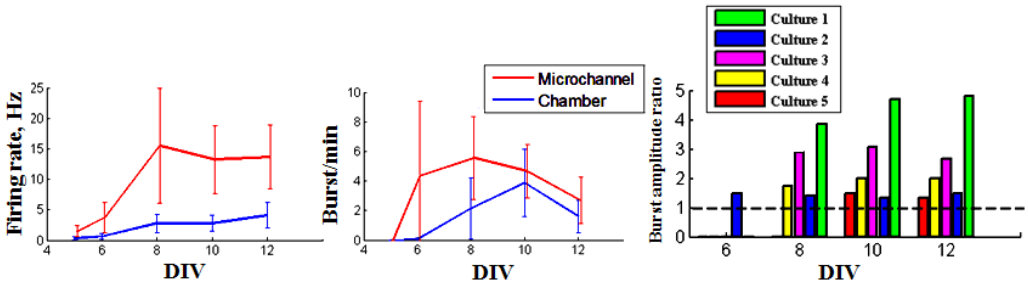

Figure 3. Changes of neuronal activity in microchannels during early culture development. (a) Mean firing rate measured per electrode in micrichannels (red) and chambers (blue). (b) Bursting frequency per minute obtained in microchannels (red) and chambers (blue). (c) Ratio of spike amplitudes registered in microchannels and chambers during bursts.

3.2. Conclusion

In this study we investigated the spiking activity of hippocampal cultures grown on microelectrode arrays combined with microfluidic device with microchannels to guide axon growth in specific direction. Our previously designed microchannels consisted of asymmetric blocks with specific shape in order to maximize probability of axon growth in one direction [6]. We used amplification feature of the extracellular signal in microchannels to obtain single cell activity on early stage of the culture development. Similar to symmetric shaped microchannels [2,3] our structure was also capable to enhance axonal signal due to small amount of the medium around axons (figure 2). Axonal spikes were found to be in the range of 30-300 μV while the spikes from neurons in chambers were 20-50 μV. We obtained a high variability across different microchannels within the single culture. We suggested that the density of survived cells on the 1st and 2nd day after culture plating may vary which contributes to the number of axons growing inside the channel. The signal also depended on the overlap area between the electrode and the microchannel. These questions will be addressed in further study.

The bursting activity in the microchannels was obtained earlier (6-8 days in vitro) than bursting activity of the whole culture network (starting from 8-10 days in vitro). Thus the synchronized bursting activity in the network started to develop in certain fraction of the culture and could not be observed in neuron's somata (chambers). Note that axonal bursts were synchronous in all 8 microchannels which suggest that all neurons in the network contribute to global burst generation. We also obtained the activity in microchannels when it was undetectable in the rest of the culture. The mechanisms of the effect will be explored in future studies and may uncover fundamental features of functional connectivity development in neural networks. The results suggest that presented microfluidic platform provides new physical methods to study spike pattern generation and transmission in neuronal cultures. The amplification effect in microchannels can be also used to develop bioartificial neurointerfaces and neuroimplants. High signal-to-ratio characteristics and spikes in range of millivolts will simplify signal registration methods and reduce applied electronics requirements.

Acknowledgments

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