Biomimetc Spike Timing Based Ionic Micro-Stimulation for Neuron Culture

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Abstract

Neurodegenerative diseases are incurable and debilitating conditions that influence cognitive and/or motor functions in millions of people worldwide. Neuroprosthesis are used today to support the quality of life but have yet to improve in their power consumption and biocompatibility issues. For the future realization of neuroprosthesis, understanding the neurophysiological behaviors and the investigations on the interaction of neuronal cell assemblies is therefore essential. Here, we propose a novel microfluidic system to investigate the response of the neurons directly stimulated by the potassium ions in vitro in biomimetic timing.

Keywords: Ionic micro-stimulation, Neurons, Spiking Neural Network, Microfluidics

1. Introduction

Most of neurodegenerative diseases are incurable and debilitating conditions that influence cognitive and/or motor functions in millions of people worldwide. Neuroprosthesis are used today to support the quality of life but have yet to improve in their power consumption and biocompatibility issues. To realize a neuroprosthesis that are close to biological behavior, understanding the neurophysiological behaviors and the investigations on the interaction of neuronal cell assemblies is therefore essential. Often being silicon based, this kind of neuromorphic engineering is called biomimetic artificial neural systems [1], [2], [3]. The main goal of such systems is to design tools for biomedical applications including neuroprosthesis and to understand the human nervous system. This project reveals a new bio-hybrid system which includes a real-time Spiking Neural Network (SNN) and biomimetic ionic micro-stimulation coupled to living 'in vitro' neuron culture. To simplify the interactions with biological neurons and to reduce the bio-compatibility issues, we designed a biomimetic ionic micro-stimulation using microfluidic techniques and ionic exchange. Furthermore, to obtain a stimulation close to biological one, a neuromorphic system called Spiking Neural Network (SNN) is used. Firstly, the SNN was integrated in a digital platform FPGA. Then, the spike-timing coming from SNN system is used for triggering the microfluidic ionic micro-stimulation to make the stimulation the closest to biological behavior.

2. Biomimetic spike-timing based microfluidic system

There are three parts that compose the neuro-hybrid system (Fig. 1): (1) Spiking Neural Network (SNN), (2) neuron culture and (3) ionic micro-stimulation in microfluidic system. This system characterizes the neural network and its evolution by using biomimetic spike-timing based ionic stimulation. Each part will be described in detail throughout this article.

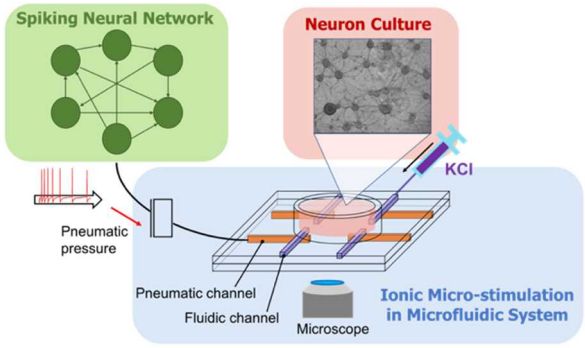

Fig. 1. Neuro-hybrid system with real-time digital SNN (green), biomimetic ionic micro-stimulation in microfluidic system (blue) and biological neuron culture (pink). SNN electrical output is converted into air pulse.

3. Spiking Neural Network (SNN)

The biomimetic SNN network is a neuromorphic system that is the closest detailed level of analogy to the nervous system [4]. A network of silicon neurons is connected via silicon synapses under plasticity rules. In neuro- hybrid experiments, spike timing and shape of the action potential (AP) must reproduce the same dynamics of a biological nerve impulse. This tunable biomimetic system works in real time and is based on Hodgkin-Huxley (HH) formalism [5] which is the most bio-plausible neuron model. We integrated the SNN in a digital platform FPGA. This spike timing-based system, SNN, was connected to the air pressure controller to trigger ionic micro-stimulation on the microfluidic device with stimulation closer to biological behavior.

4. Ionic micro- stimulation in microfluidic device

This device is structured with fluidic channels and pneumatic channels that allow the control of a stimulation sent to the neurons. The structure is described in Fig. 2 [7]. This device has four fluidic channels to input potassium chloride (KCl) of 10mM with each having a pneumatic valve to control the stimulation timing. The channels are equipped with ion selective permeable membrane (Sigma-Aldrich, Nafion® perfluorinated resin solution 5wt.%) to mimic the chemical exchange of biological neurons. It also has an integrated cell dish for the placement of living neurons with a thinness of less than 5mm allowing the observation through microscope during calcium imaging.

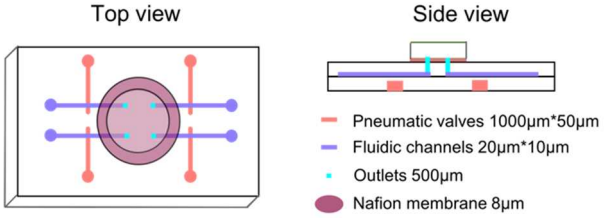

Fig.2. Structure of ionic micro-stimulation device. Set of four fluidic channels with under layer of pneumatic channel. The crossing of these two different types of channels will allow to control the input of solution in fluidic channel.

5. Neuron Culture

This bio-hybrid system contains ‘in vitro’ primary cultures of mice hippocampal neurons. Neurons were carefully dissected from embryos and were prepared as described [6]. Neurons isolated from embryonic day 17 ICR mouse were plated in Neurobasal medium. They were used in this experiment after two weeks of maturation inside the cell dish of the microfluidic device.

6. Results

6.1. *Integration of Spiking Neural Network into FPGA*

Regular and simplified equations of biomimetic SNN are implemented on a FPGA [8]. Electrical activities of different classes of cortical neurons can be simulated in biological time scale with strong correlation between hardware simulations (Fig.3. red) and software simulations (Fig.3. blue). The Central pattern generators (CPGs) provide bursts of spikes with frequency, variability and spike width in the ranges that are commonly observed in nature [9]

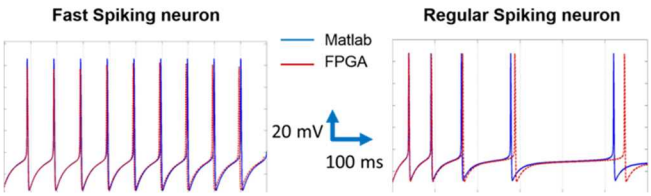

Fig. 3. Comparison between model (blue) and FPGA (red). FPGA implementation and Matlab software simulations are nearly similar.

6.2. *Micro stimulation in the microfluidic system*

After two weeks of cell maturation, neuronal activities were visualized by calcium imaging with Fluo-4 AM (Molecular Probes, United States). To observe the spatial and temporal changes in calcium resulting from spontaneous activity, the raw sequences were processed to observe changes in fluorescence intensity of when the biomimetic ionic micro-stimulation is activated (Fig. 4).

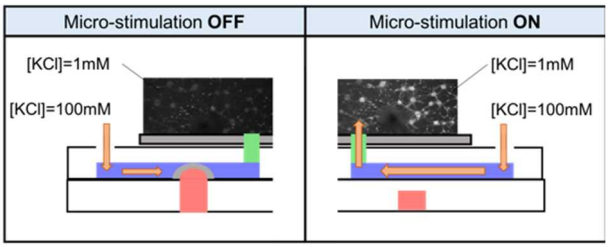

Fig. 4. Bio-hybrid experiments using biomimetic ionic micro-stimulation. When micro-stimulation is ON, the neuron culture is active. Calcium imaging technique is used for detecting neuronal activity.

When the 250ms length pulse of ON/OFF were sent to the pneumatic valve, the ionic stimulation was received by the neurons which in turn were stimulated by potassium ion. This was confirmed by staining the neurons with calcium imaging. The neural activities (Fig.5.) successfully responded accordingly to the programmed ionic stimulation of 250ms. When the pneumatic microvalve is open, the fluorescent intensity increases. On the other hand, when it closes, the fluorescent decreases. During when the pneumatic valve is closed, the neurons activity returns back to its original intensity range.

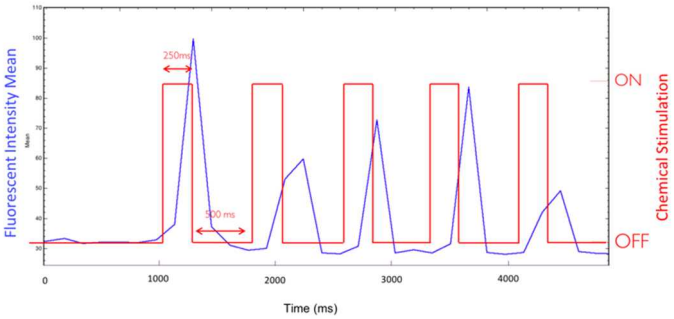

Fig. 5. Stimulations were sent to the device with neuron culture. After calcium imaging, the activity was seen accordingly to the programmed air pulse sent to the pneumatic valve.

After this successful stimulation, the device was integrated with the Spiking Neural Network (SNN) to acquire a more bio-inspired stimulation. This was done by connecting the SNN implemented FPGA unit to the valve of the air controller.

6.3. *Neural response to spike-timing based micro-stimulation*

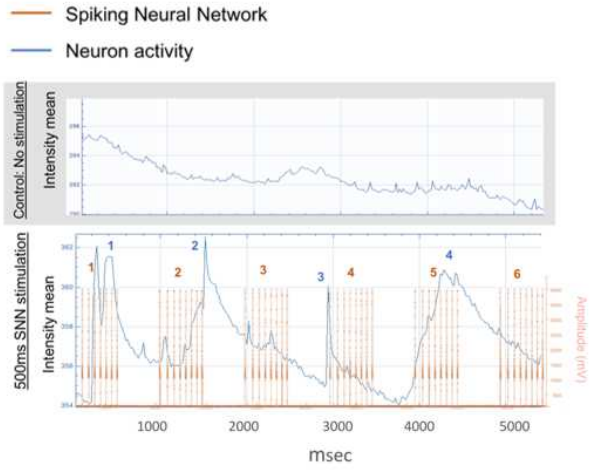

Fig.6. After calcium imaging, the neural activity without any stimulation (top) and during the stimulation using SNN system (bottom) were recorded. The top graph shows the control activity of neurons at resting state. The bottom graph shows the neural activity when CPGs-based burst-like stimulations were sent for 500ms. Images were taken at 200ms frame rate and the fluorescent intensity mean was plotted. The neural activity is visualized when the florescent intensity increased spontaneously. Activity was seen accordingly to the programmed spikes with added delays of approx. 250ms each spike.

The matured neurons were stained with calcium specific fluorescence to visualize the neural activity. The SNN stimulation sent has 8 bust-like spikes for 500ms with 500ms of no signals between stimulation. Fluorescence response observed shows a timely neural activity. However, there were delays in the response time for neuron activity (Fig. 6.). This may have been caused by the insufficient performance of the camera that was used.

7. Conclusion

Our device can generate a biomimetic stimulation accordingly to the sequenced air pressure programmed with Spiking Neural Network (SNN). The activity of neurons observed through calcium imaging showed timely response to SNN signals sent with certain delays.

The neural response was as expected when triggering the stimulation manually and by programmed air pressure of 250ms with air pressure controller. During the 500ms of OFF stimulation, the neural activities decreased as its fluorescent intensity diminished. On the other hand, as we integrated the Spiking Neural Network in the system, delays in response time for the activity of neuron after each stimulation were observed. However, during this recording, the computer had error for insufficient working space (RAM) and the recording showed some lags. This might have caused the error of response time in neural activities.

In the future, we will try to record the neural activity with a camera capable of capturing higher frame rate per second without having a lag during the recording of the neurons. On the other hand, the next step of our work is to create a closed-loop bio-hybrid system where the recorded neuronal activity is used as a feedback for SNN.

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