512-electrode MEA System For Spatio-Temporal Distributed Stimulation and Recording of Neural Activity

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Abstract

We present a design and preliminary tests result of a large-scale MEA-based system for spatio-temporal distributed stimulation and recording of neural activity. The system is based on 512-electrode array with 60 µm inter-electrode spacing and dedicated multichannel integrated circuits for independent stimulation and recording on all the electrodes. In the preliminary tests we stimulated individual neurons in rat retina and cultured cortical slices and recorded the evoked spikes as soon as 55 µs after the stimulation pulse on the stimulating electrode, and 5 µs after the pulse on other electrodes and were able to detect and classify even the low-latency spikes from directly activated neurons.

1 Introduction

Modern MEA-based systems can record neural activity with single cell resolution and with hundreds or even thousands of independent channels, but the electrical stimulation is usually limited to a few electrodes or several groups of electrodes repeating the same stimulation protocol. In contrast, two-way electrical communication with large population of neurons with resolution of single cells and individual action potentials requires a system capable of generating independently defined stimulation signals on large number of sites, combined with recording of the network response with minimal stimulus-related artifacts.

Here we present 512-electrode system able to stimulate individual neurons in general spatio-temporal patterns, with simultaneous low-artifact recording of the elicited activity. The system is based on high-density MEA and can elicit complex, precisely defined patterns of action potentials in hundreds of individual neurons. We plan to use the system to investigate the mechanisms of information processing in brain tissue, and to develop stimulation techniques for high-resolution neural prostheses.

2 System design

The presented system is based on custom-designed MEA [1] and Application Specific Integrated Circuits (ASICs). Eight Stimchip ASICs [2] generate the electrical stimulation signals independently on each electrode and the Neuroplat chips [3] simultaneously record the neural activity. The photograph of system Printed Circuit Board is shown in fig. 2A.

The system is controlled by a PC running a Labview application, which generates the stimulation control signals based on user-defined input files and continuously records the neural activity on all the electrodes. The input files are generated with Matlab and allow for independent definition of stimulation amplitude and timing for each channel. The recorded data are analyzed off-line with custom software developed in Matlab and Java.

2.1 Multielectrode array

The MEA consists of 512 microelectrodes arranged in a hexagonal pattern, with 60 µm inter-electrode spacing (fig. 2B). The electrodes have diameter of 5 µm and are electroplated with platinum before the experiment (typically 80 nA for 15 seconds) for impedance reduction.

Arrays of this type have been used previously for large-scale recording of neural activity from retina [4,5] and cultured brain slices [6]. Smaller 61-electrode arrays with the same spatial resolution and electrical properties have been used for electrical stimulation of retinal ganglion cells in rodents and primates, using both old stimulation electronics [7] as well as the Stimchip ASIC [8].

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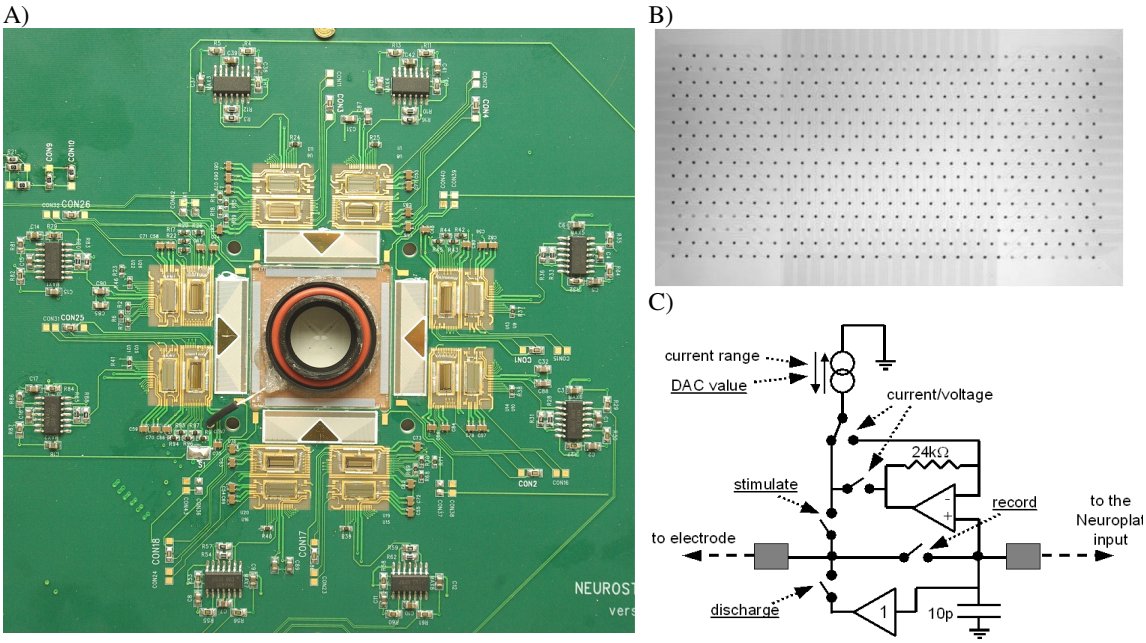

Fig. 1. A) Photograph of the Neuroboard. The array with chamber and eight sets of ASICs are visible in the center. B) Microphotograph of the 512-electrode array. C) Functional diagram of single Stimchip channel with control signals. The real-time control signals are underlined.

2.2 Stimchip

The Stimchip ASIC includes 64 independent stimulation channels, circuitry for generating internal reference voltages, and a logic block that controls the operation of each channel based on digital commands sent from the control PC. Each channel comprises a programmable generator of arbitrary current waveform, a current-to-voltage converter, and artifact suppression circuitry (fig. 2C). The waveform generator is built from an 8-bit digital-to-analog converter and eight selectable output current buffers with maximum current ranging from 60 nA to 1 mA. The current waveform can be sent to the electrode either directly or via the current-to-voltage converter when the voltage stimulation mode is selected [2].

During signal recording the electrode is connected to the input of the external amplifier through the closed *record* switch (fig. 2C). To avoid saturation of the amplifier by the large voltage signal generated by the stimulation pulse (either on the same channel, or one of the neighboring electrodes) the amplifier input can be disconnected from the electrode prior to the pulse and held at the constant potential, sampled before the stimulation pulse. In addition, the electrode-electrolyte interface can be actively discharged via a voltage follower before the electrode is reconnected to the amplifier input.

The shape of the stimulation signal and the states of all the switches in the circuitry are controlled with time resolution of 50 µs, independently for each channel, by a stream of real-time data sent from the control computer (15 Mbit/s for each chip).

2.3 Neuroplat chip

The Neuroplat chip [3] comprises 64 independent recording channels, an analog multiplexer, bias circuitry and control logic. Each channel includes an AC-coupled amplifier, a band-pass filter, and a sample-an-hold circuit. The amplifier gain and the filter frequency range can be set by digital commands. The output signals on all the channels are sampled with a frequency of 20 kHz, multiplexed, and sent to the control computer for digitization and storage.

3 Preliminary tests

To evaluate the system performance, we stimulated and recorded activity from isolated rat retina and organotypic cultures of the rat cortex.

3.1 Stimulation protocol

We used charge-balanced triphasic stimulation pulses generated in current mode, with relative amplitudes of 2:-3:1 and duration of 50 µs or 100 µs per phase (fig. 2A). This pulse shape resulted in very low stimulus artifact that did not require post-pulse discharging. We recorded the elicited neural activity, on the stimulating electrode, as soon as 55 µs after the stimulation pulse, and as early as 5 µs after the pulse on the other, non-stimulating electrodes.

For each electrode, we used stimulation pulses with amplitudes (defined as the current value for the

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negative pulse phase) ranging from 0.2 to 4.0 µA for the 50 µs/phase pulses and 0.1 to 2.0 µA for 100 µs/phase pulses. The amplitude was increased in 5% steps and stimulation pulse of given amplitude was repeated 100 times.

To reduce the experiment time, we grouped 512 electrodes in 64 eight-electrode patterns, with inter-electrode distance of 480 µm in each pattern. The electrodes within one pattern generated stimulation pulses simultaneously with frequency of 2 Hz, and pulses in each next pattern were shifted by 7.5 ms. The whole stimulation procedure took ~1 hour and 50 minutes when both pulse durations were used.

3.2 Results

Stimulation of individual neurons

The four subplots in figure 2A show overlaid responses of a retinal ganglion cell (RGC) to stimulation pulses of different amplitudes recorded on the electrode generating the stimulation signal.

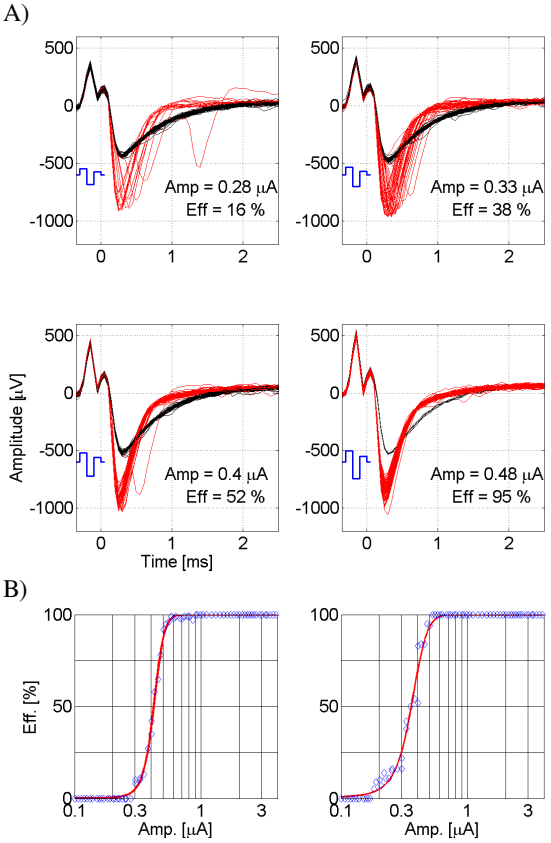

Fig. 2. Stimulation and recording of single RGC activity. A) Each subplot shows overlaid responses of retinal ganglion cell to 100 stimulation pulses recorded on the stimulating electrode. Four subplots correspond to various stimulation amplitudes. Black traces: artifacts only. Red traces: artifact combined with cell response. The blue trace illustrates the shape and timing of the stimulation pulse. B) Stimulation efficiency as a function of current amplitude for 50 µs/phase pulse (left) and 100 µs/phase pulse (right).

Increasing the stimulation amplitude results in both, gradual increase of the stimulation efficiency and stabilization of the elicited spike latency, while the artifact level stays well within linear range of the Neuroplat chip (+/- 3mV for the gain set to 270).

Identification of activated cells

To reconstruct shape of the elicited neuronal signal, we averaged traces within each of the two classes shown in fig. 2A (subplot no. 4, current amplitude 0.48 µA) and subtracted the averaged waveforms. The same procedure was applied to the 6 electrodes adjacent to the stimulating electrode. The elicited waveforms are virtually identical to the signals recorded from the same neuron spiking spontaneously, as shown in fig. 3A.

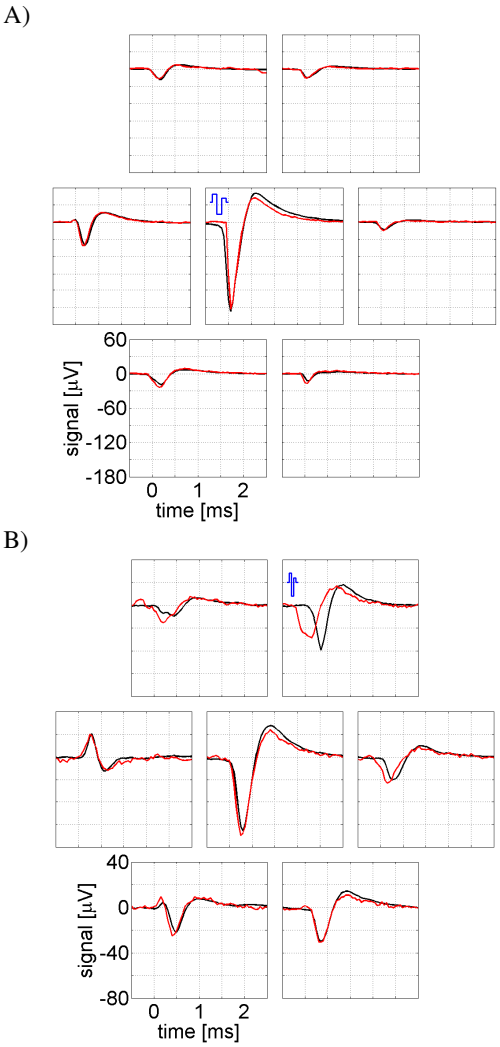

Fig. 3. A) Average shape of recorded elicited spike from retinal ganglion cell shown on seven electrodes (blue traces) compared with shape of signal recorded from the same neuron spiking spontaneously (red traces). Blue diamond marks the stimulating electrode. B) Identical analysis for neuron in cultured rat cortical slice.

Activation of neurons in cultured rat cortical slices required in general higher stimulation currents than retinal ganglion cells. As shown in fig. 3B, the cell response to 4.0 µA pulse recorded on the stimulating electrode is significantly distorted. However, the signals on all the neighboring electrodes are preserved very well and allow for unambiguous identification of the activated cell.

Spatial map of stimulation thresholds

Some of the identified neurons were effectively stimulated by several different electrodes, located near the cell bodies or axons. Fig. 4A shows averaged signals recorded from a retinal ganglion cell on 24 electrodes, including 6 electrodes that stimulated the cell. Four of these electrodes recorded somatic signal from the stimulated neuron, and two remaining electrodes recorded axonal spikes.

The stimulation efficacies as a function of current amplitudes are shown in fig 4B for each of the six electrodes. The cell was stimulated with lowest threshold by the electrode that also recorded the largest signal from this neuron, however, the other electrodes near the soma show similar stimulation threshold to the electrodes close to the axon.

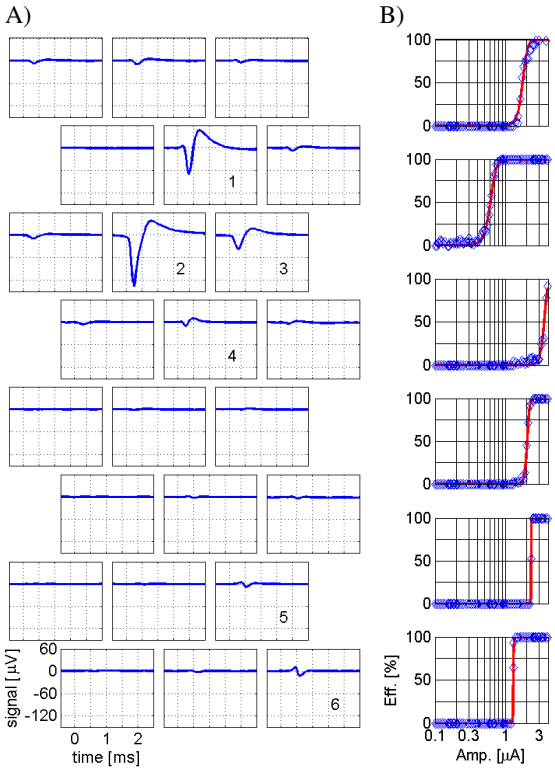

Fig. 4. A) Averaged signal recorded from an RGC. The electrodes that stimulated the cell are labelled (1-6). B) Stimulation efficiency as a function of current amplitude for each of the electrodes 1-6 (top to bottom). Pulse duration: 50 µs/phase.

4 Summary

We have developed a large-scale system that is capable of stimulating hundreds of neurons with arbitrarily defined patterns of stimulation currents, with simultaneous recording of the elicited activity. In the preliminary experiment, we were able to stimulate individual neurons in rat retina and cultured cortical slice, and record the evoked low-latency spikes from directly stimulated neurons with minimal distortions. The software for automated classification of elicited neural responses is currently under development.

Acknowledgement

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