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Single-Cell Electrical Stimulation with CMOS-based High-Density Microelectrode Arrays

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Motivation

The main goal of this work was to explore electrical stimulation parameters that reproducibly and precisely elicit action potentials in single neurons (Wagenaar *et al.* 2004). We compared voltage and current modalities' and their efficacy in activating single neurons; we also studied the related stimulation artifacts. For our studies, we used a CMOS-based MEA featuring 26400 electrodes at 17.5 µm pitch (Ballini *et al.* 2014).

Material and Methods

Cell preparation and plating: E-18 Wistar rat cortices were dissociated in trypsin with 0.25% EDTA followed by trituration and counting. To coat the surface and to induce cell adhesion, Poly(ethyleneimine), 0.05% in borate buffer (w/v) at 8.5 pH, followed by 0.02 mg ml^{-1} laminin in Neurobasal medium were used. We seeded 20'000 cells over an active area of approx. 8 mm^2 on each chip.

Microscopy and staining: NeuroFluor NeuO live staining was used to localize isolated neurons on the array. Subsequent fixation of the neurons after stimulation experiments was performed by using 4% paraformaldehyde. Antibodies against βIII-tubulin, Ankyrin G, and the fluorescent dye Hoechst were used to stain neurons, axonal initial segments (AIS), and the nuclei.

Stimulation and data analysis: The CMOS-based MEA featured 26400 bidirectional electrodes within a sensing area of 3.85 x 2.10 mm^2 at a pitch of 17.5 µm, 32 stimulation buffers and 1024 reconfigurable readout channels (Fig. 1A). Electrical stimulation was controlled via a custom-made python script, while the collected data was analyzed by MATLAB. A randomized voltage stimulation protocol, made up of 4 different waveforms (biphasic cathodic-anodic, biphasic anodic-cathodic, monophasic anodic, monophasic cathodic) (Wagenaar *et al.* 2004), 4 durations (50, 100, 150, 200 µs) and 5 amplitudes (20, 40, 60, 80, 100 mV peak-to-peak), was used. For current stimulation, a randomized protocol of 2 waveforms (biphasic cathodic-anodic, triphasic cathodic-anodic-cathodic, charge balanced) (Grosberg *et al.* 2016), 5 durations (2, 6, 10, 14, 20 µs) and 8 amplitudes (210, 420, 630, 840, 1050, 1260, 1470, 1680 nA) was applied.

Results

Stimulation electrodes were selected after identification of the spatial distribution of single-neuron extracellular action potentials. The electrode recording the highest action potential signal amplitude is considered the most sensitive site for stimulating the given neuron (Radivojevic *et al.* 2016) and was used as a stimulation electrode (Fig. 1C-D). Neurons were stimulated at DIV 10, 15, 20. In voltage mode, the artifact associated with monophasic waveforms was significantly larger in comparison to the artifact of biphasic waveforms. Furthermore, voltage biphasic anodic-cathodic waveforms were less efficient than biphasic cathodic-anodic waveforms in eliciting action potentials (Fig. 1E). We found that the artifact associated with current stimulation had a shorter duration and less spatial extent in comparison to voltage stimulation artifacts (Fig. 1B). Therefore, in case of current stimulation, it was possible to detect action potentials directly at the cell soma, which was not possible with voltage stimulation. After having conducted the electrical stimulation protocols, the neurons were stained for correlating neuron morphologies with their spatial extracellular-action-potential distribution.

Discussion / Conclusion

We showed that it is possible to selectively and reliably stimulate individual neurons by using a high-density MEA chip with 26400 electrodes. In voltage mode, the biphasic anodic-cathodic waveform featured lower efficiency at eliciting action potentials than the biphasic cathodic-anodic waveform. The duration and the spatial extent of the stimulation artifact in the current stimulation mode were smaller, which rendered the readout of the evoked action potentials easier and more reliable.

References

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Figure 1

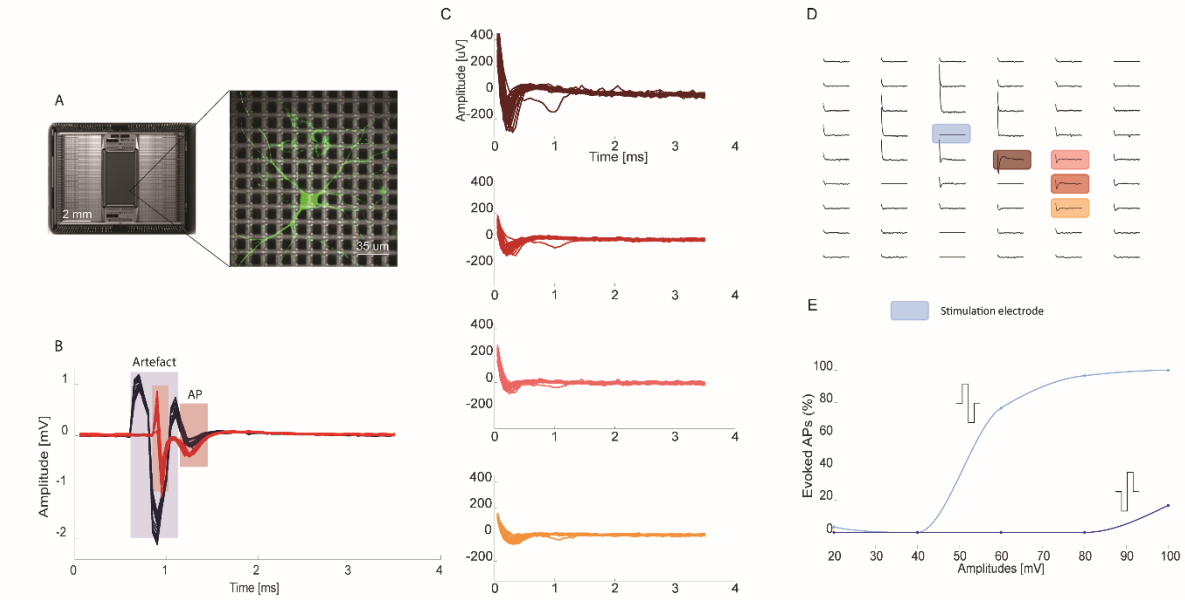

Figure Legends

(A) Chip micrograph (left) and close-up of the array, with a neuron labeled with NeuO (green).

(B) Comparison between current (red) and voltage (black) stimulation artifacts and evoked APs. The current stimulation waveform is biphasic positive-negative with an amplitude of 1.26 µA and a total duration of 40 µs. The voltage stimulation waveform is biphasic positive-negative with an amplitude of 80 mV and a duration of 400 µs.

(C) 30 stimulation repetitions that evoked APs with an efficiency of 100%, plotted from the 4 readout channels of figure (D).

(D) Map of some of the routed electrodes; blue indicates the stimulation electrode; the other highlighted electrodes are readout electrodes shown in (C).

(E) Activation curves in voltage-stimulation mode show the efficacy of biphasic positive-negative and biphasic negative-positive stimuli. The result was computed from 30 stimulation repetitions.

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