LARGE-SCALE RECORDING FROM AXONAL ARBORS OF SINGLE NEURONS WITH CMOS BASED HIGH-DENSITY MICROELECTRODE ARRAYS

Jan Müller[1], Marco Ballini[1], Paolo Livi[1], Yihui Chen[1], Douglas J. Bakkum[1], Milos Radivojevic[1], Urs Frey[2], Alexander Stettler[1] and Andreas Hierlemann[1]

[1]ETH Zurich, Basel, SWITZERLAND and

[2]RIKEN, Kobe, JAPAN

ABSTRACT

We report on the identification and tracking of the electrical activity of processes of single neurons. With a newly designed complementary metal-oxide-semiconductor (CMOS) based integrated high-density microelectrode array (HD-MEA) [1,2], we were able to simultaneously use more than 1000 electrodes and low-noise readout channels to monitor electrophysiological signals of many individual branches of a single axonal arbor of a specific neuron.

KEYWORDS: HD-MEA, Single neurons, Axons, CMOS

INTRODUCTION

Originating from the cell body of a neuron, axons are tiny processes with diameters below 800 nanometers. As amplitudes of extracellular recorded axonal signals (2–10µV) are an order of magnitude smaller than amplitudes of somatic signals (hundreds of µV), their detection and unequivocal assignment is difficult. The availability of new tools to study such axons will reveal further insights into how neuronal networks function [3].

EXPERIMENTAL AND RESULTS

The HD-MEA system (Figure 1) features 26'400 platinum microelectrodes (5 x 9 μm^2), arranged in a grid-like configuration (3.8 x 2.1 mm^2 overall electrode area; electrode center-to-center pitch 17.5 µm). Through a configurable network of wires and switches, an almost arbitrary subset of the bidirectional microelectrodes can be routed to 1024 low-noise readout channels (2.4 μV_{rms} in the spike band, 300 Hz–10 kHz) and 32 stimulation units. Electrode routing can be reconfigured within milliseconds to adapt the electrode selection to different experimental scenarios and to scan neural activity on all electrodes. Signals are sampled at 20 kHz with 10 bit on-chip analog-to-digital converters.

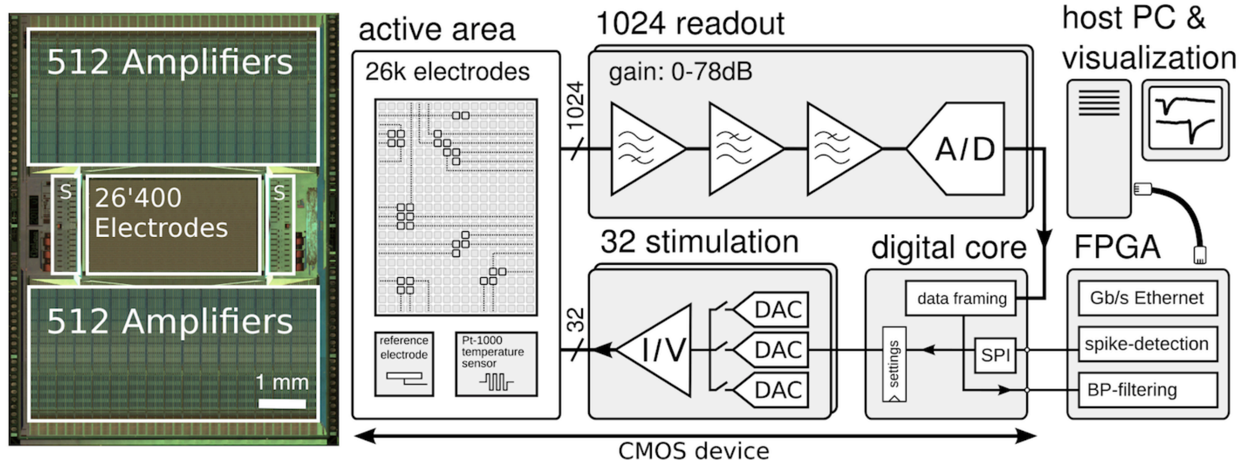

Figure 1: Micrograph (left) and block diagram of the CMOS-HD-MEA setup (right). The 1024 readout channels and the 32 stimulation units (S) can be connected through a set of switches to an arbitrarily selectable subset of the 26'400 Pt electrodes.

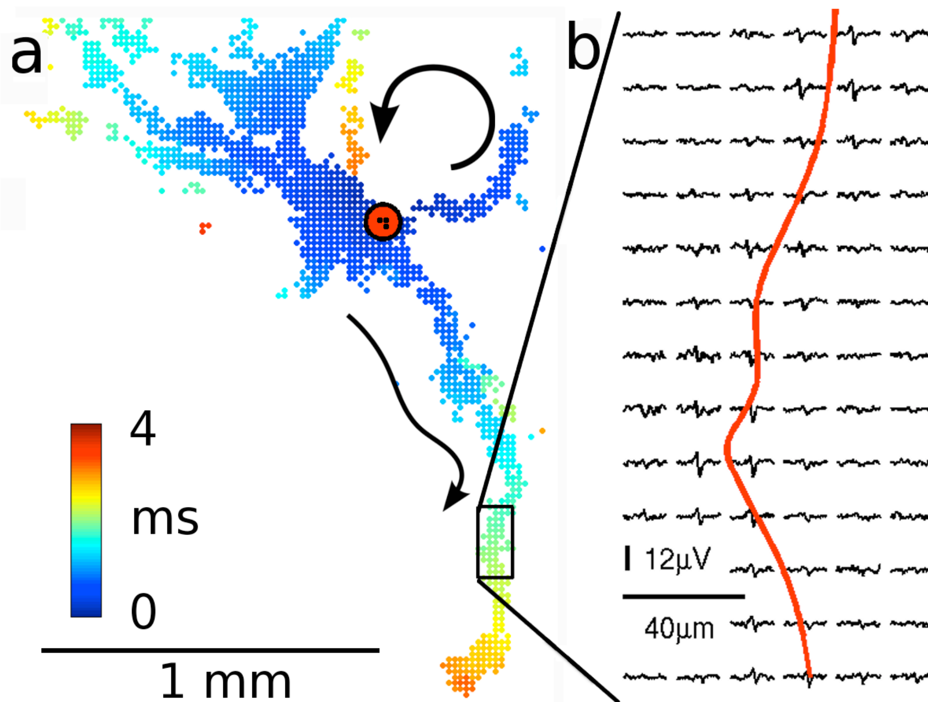

Figure 2: Axonal arbor of a single cell. a) All colored dots represent electrodes, which detected activity attributed to the cell located at the red circle. The arrival time of the action potential at each electrode is color-coded. For some signals, it takes as much as 4 ms to travel from the soma down the axon. Arrows indicate the propagation direction of action potentials in two branches of the axonal arbors. b) Close up of one branch of the axon. A red line indicates the putative position of the axon, along which the low-amplitude axonal action potential signals proceed.

Networks of cortical neurons and glial cells were cultured on top of the MEA for 4 weeks. In order to find the axonal arbor of a single cell, three electrodes below the putative soma of that cell were identified (black dots in the red circle of Figure 2a). These electrodes were kept fixed, while a series of different electrode-readout configurations was used to scan through all other electrodes. Figure 3 shows traces recorded with these 3 electrodes that reveal two distinct sets of waveforms (red, blue) from two putative single cells. Thanks to the high resolution of recording electrodes, the waveforms can easily be assigned to individual neurons. For such an identified neuron, electrode traces can be averaged to remove noise and to reveal the location and waveform of smaller axonal action potentials (Figure 2a,b). As can be seen, at least 6 axonal branches are visible. The negative peak value of the averaged action potential traces was used to assign the putative position of the axon, which is drawn as a red line in Figure 2b.

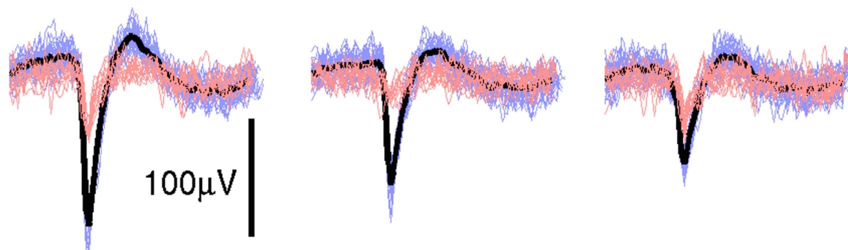

Figure 3: Overlaid raw signal traces from 3 electrodes marked with black dots inside the red circle of Figure 2a. The electrodes exhibit waveforms from two distinct single cells. The axonal arbor of the blue cluster is shown in Figure 2.

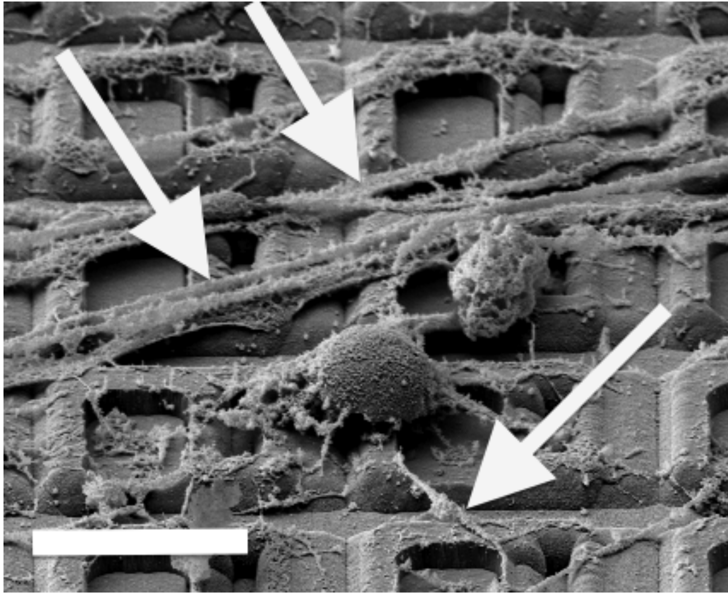

Figure 4: SEM of axons and dendrites (marked with arrow heads) and somas of neurons growing over Pt microelectrodes. Scale bar is 10 µm.

DISCUSSION AND CONCLUSION

The high spatial resolution and large number of addressable Pt microelectrodes, together with the low-noise recording characteristics of the CMOS system, allow for identifying the position and morphology of single neurons and their axonal arbors. Multiple axonal branches can be simultaneously recorded from by using a single electrode configuration. Spatial oversampling enables more reliable recordings from single axonal action potentials without the need for averaging so that we now have unprecedented capabilities to study how single action potentials spread through individual branches of axonal arbors.

ACKNOWLEDGEMENTS

We thank Jörg Rothe, David Jäckel and Marta Lewandowska for their help with the experimental set-up and cell culture preparations. This work was supported by the ERC Advanced Grant “NeuroCMOS” under contract number AdG 267351. Milos Radivojevic and Douglas J. Bakkum received funding support from the Swiss National Foundation through an Ambizione Grant (PZ00P3_132245).

REFERENCES

[1] Müller, J. et al. "Conferring flexibility and reconfigurability to a 26,400 microelectrode CMOS array for high throughput neural recordings," *Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS & EUROSENSORS XXVII), 2013 Transducers & Eurosensors XXVII: The 17th International Conference on* , vol., no., pp.744,747, 16-20 June 2013

[2] Ballini, M. et al. "A 1024-channel CMOS microelectrode-array system with 26'400 electrodes for recording and stimulation of electro-active cells in-vitro," *VLSI Circuits (VLSIC), 2013 Symposium on*, vol., no., pp.C54,C55, 12-14 June 2013

[3] Debanne et al. "Axon physiology"; 2011; Physiol Rev.; 91(2): 555-602.

CONTACT

* J. Müller; phone: +41-61-387-31-78; jan.mueller@bsse.ethz.ch