Microelectronic system for high-resolution mapping of extracellular electric fields applied to brain slices

U. Frey a,∗, U. Egert b, c, ∗∗, F. Heer a, S. Hafizovic a, A. Hierlemann a

a ETH Zurich, Bio Engineering Laboratory, Department of Biosystems Science and Engineering, Mattenstrasse 26, CH-4058 Basel, Switzerland
b Bernstein Center for Computational Neuroscience Freiburg, Neurobiology & Biophysics, Faculty of Biology, Albert-Ludwigs-University, Hansastr. 9a, 79104 Freiburg, Germany
c Bioimicrotechnology, Department of Microsystems Engineering, Faculty of Applied Sciences, Albert-Ludwigs-University, Georges-Köhler-Allée 102, 79110 Freiburg, Germany

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A B S T R A C T

There is an enduring quest for technologies that provide – temporally and spatially – highly resolved information on electric neuronal or cardiac activity in functional tissues or cell cultures. Here, we present a planar high-density, low-noise microelectrode system realized in microelectronics technology that features 11,011 microelectrodes (3,150 electrodes per mm²), 126 of which can be arbitrarily selected and can, via a reconfigurable routing scheme, be connected to on-chip recording and stimulation circuits. This device enables long-term extracellular electrical-activity recordings at subcellular spatial resolution and microsecond temporal resolution to capture the entire dynamics of the cellular electrical signals. To illustrate the device performance, extracellular potentials of Purkinje cells (PCs) in acute slices of the cerebellum have been analyzed. A detailed and comprehensive picture of the distribution and dynamics of action potentials (APs) in the somatic and dendritic regions of a single cell was obtained from the recordings by applying spike sorting and spike-triggered averaging methods to the collected data. An analysis of the measured local current densities revealed a reproducible sink/source pattern within a single cell during an AP. The experimental data substantiated compartmental models and can be used to extend those models to better understand extracellular single-cell potential patterns and their contributions to the population activity. The presented devices can be conveniently applied to a broad variety of biological preparations, i.e., neural or cardiac tissues, slices, or cell cultures can be grown or placed directly atop of the chips for fundamental mechanistic or pharmacological studies.

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1. Introduction

As a complement to the well-established patch clamp technique and optical techniques extracellular electrophysiological recordings contain a wealth of information on the properties of, e.g., individual cells or neurons and on the dynamics in neuronal networks. This is due to the fact that they offer the potential to simultaneously record from a large number of spots at high temporal resolution. The simultaneous recording from a large number of spots is comparably difficult to realize by means of patch-clamping, whereas optical methods, such as 2-photon microscopy and calcium imaging, provide high spatial resolution (even in 3-dimensions) (Gobel et al., 2007), however, still suffer from poor temporal resolution or low signal-to-noise ratio. Information acquired through extracellular recordings has enabled the reconstruction of activation sequences in cultured networks, slice preparations and intact brains (Buzsaki, 2004), and it potentially enables the characterization of ion channel properties and ion channel distributions in single cells. As yet, the detailed structure of the extracellular-potential landscape of a neuron and its dynamics are still mostly unknown, since the signal-to-noise ratio and the spatial resolution in the recordings of extracellular potentials are, in most cases, low. The low resolution prevents a clear separation of individual sources and an unambiguous reconstruction of the neuronal networks (Plenz and Aertsen, 1993). Commercially available planar microelectrode arrays (MEAs) for in vitro experiments usually comprise ∼60 electrodes, with diameters up to 30 μm and feature up to 100 electrodes per mm² (Gross et al., 1995; Pine, 1980). Recently, high-density microelectrode arrays (HD-MEAs), realized in standard microelectronics or CMOS (complementary metal oxide semiconductor) technology have emerged (Berdonzini et al., 2005; Eversmann et al., 2003), that bear the potential to perform recordings at single-cell resolution. This is mostly due to the possibility to place thousands of tightly-spaced electrodes and the respective addressing and read-out circuitry on a single chip.
To achieve the desired results, however, these devices have to meet two stringent requirements: (i) a high signal-to-noise ratio in the recordings of partially very small signals and, at the same time, (ii) a high spatial resolution. The dilemma is that the two requirements are diametrically opposed in a technical realization. High spatial resolution entails the use of small electrodes featuring higher thermal noise, and it entails that only very few and small circuitry elements for addressing and signal amplification can be realized and repeated with each electrode. The smaller the available space for electrodes and circuitry, however, the larger is the noise, since the noise of a transistor scales with its size. This means that the signal quality is compromised by the circuitry that, in the first place, enables the readout of such high-density arrays. The result for devices to date is a comparably high noise level on the order of 70–250 $\mu$V RMS (Eversmann et al., 2003; Lambacher et al., 2004), which prevents the revelation of electrophysiological details.

Here we present an elegant workaround for this dilemma and show, how extracellular potential landscapes can then be recorded at subcellular resolution and at high signal quality for low signal levels. Instead of simultaneously reading from all electrodes, which requires the front-end amplifiers to be located directly at each recording electrode, the workaround includes the utilization of a matrix of switches and memory cells underneath the electrodes to route a subset of these electrodes to circuitry units (channels) placed outside the array, where no area constraints apply (Fig. 1A), (Frey et al., 2007). This way we combine high-density electrodes with high-performance, low noise circuitry. The implementation enables an almost arbitrary selection of electrodes (cohesive blocks, lines, distributed single electrodes) to be connected to the 126 available readout channels (Fig. 1B). The electrode selection can be changed within 2 ms.

Signal shapes and amplitudes recorded by extracellular electrodes depend on the spatial arrangement of the electrodes and the nature of the cells under investigation. Larger cells tend to yield larger signals, as the membrane current that is needed to depolarize the cell is larger in comparison to small cells. A second factor is the distance between the electrode and the neuron: the closer the cell is to the electrode, the larger is the signal. A third factor includes the resistivity of the extracellular space: the larger the resistivity is, the larger are the signals that can be observed. A fourth factor is the electrode size. Ideally, the electrodes should be as small as possible, since large electrodes will measure a potential averaged over a larger area, which reduces the peak signal amplitudes. On the other hand the use of smaller electrodes may entail higher noise levels in the signals, which may counterbalance or even outbalance the advantage. In dissociated cultures the cells are usually very close to the electrodes, yielding large signals. Moreover, when large neurons, such as snail neurons (Lambacher et al., 2004), are used, the signals can reach up to tens of mV, in the case of cultures of smaller mammalian neurons they still can reach several mV (Voelker and Fromherz, 2005). High-density cultures provide larger signals in comparison to low-density cultures, as the resistance in the extracellular space is larger due to many tightly spaced cell membranes. Slices generally produce considerably smaller signals, as a layer of cell debris is in between the intact cells and the electrodes.

APs and, more frequently, field potentials from brain slices have previously been recorded by means of MEAs. Local field potentials (LFP) are electrophysiological signals that are generated by the collective activity of many cells. LFPs have typically larger amplitudes, since many cells contribute to the signal so that they can be recorded over larger distances in comparison to single-cell APs. The signal frequencies of LFPs are lower than those of single APs, and the LFPs exhibit a characteristic duration of tens of milliseconds. Field potentials can be triggered by forcing the cells to fire synchronously, e.g., by collective stimulation.

The recording of single APs from acute slices with MEAs is difficult, as the amplitudes can be rather small (tens to hundred $\mu$V). When recording individual neuronal activity using microwires, it is typically distinguished between ‘single unit’ recordings and ‘multi-unit recordings’. Spike sorting is applied to ‘multi-unit recordings’ to separate the individual neurons according to their spike shape. The use of devices with multiple electrodes is advantageous, as each electrode picks up a different version of the same spike as a consequence of the different spatial arrangement or relative position of the electrode with regard to the cell.

2. Materials and methods

2.1. CMOS microelectrode system and setup

The microsystem chip has been realized in 0.6 $\mu$m CMOS technology (3M2P), is $7.5 \times 6.1 \text{ mm}^2$ in size, and features $11,011$ metal electrodes as well as $126$ bidirectional circuitry channels, each of which is equipped with recording and stimulation electronics (Frey et al., 2007) (Fig. 1A and B). The electrodes feature a diameter of 7 $\mu$m and are placed at a pitch of 18 $\mu$m (honeycomb pattern) in an area of $2.0 \times 1.75 \text{ mm}^2$ yielding a density of 3150 electrodes per $\text{mm}^2$. The electrodes have been realized during a 3-mask post-CMOS processing step. A 0.5 $\mu$m thick passivation layer ($\text{SiO}_2$) was deposited to improve the CMOS passivation and the pad openings are reopened using reactive ion etching (RIE), TiW (20 nm) and platinum (Pt, 200 nm) as the electrode material were sputtered onto the wafer and patterned using a lift-off process. Afterwards, a 1.6 $\mu$m thick passivation layer stack (4 alternating layers of $\text{SiO}_2$ and $\text{Si}_3\text{N}_4$) was deposited for corrosion protection (Heer et al., 2006). The Pt electrode openings have been shifted away from the location of the original CMOS aluminum contacts to ensure chip long-term functionality.
stability. Pt-black was afterwards grown on the electrodes to reduce the electrode impedance (Heer et al., 2006).

Flexibility in the electrode selection is attained through an analog switch matrix integrated underneath the electrode array, which consists of 13 k SRAM cells and analog switches to define the routing from the electrodes to the amplifiers of the channel units. The memory cells store the information whether the respective switch is closed or not, i.e., whether an electrode is connected via several switches to one of the readout/stimulation channels. Dedicated algorithms are used to optimize the routing for a defined selection. To obtain the settings of the switches for a selected set of electrodes, the switch matrix is represented as a graph, and a max-flow, min-cost optimization problem is solved using an integer-linear program. Arcs from spots of interest to a set of electrodes, on which the signal can be measured, are assigned a cost, such as the Euclidean distance or a value that is inversely proportional to the signal quality. Simulations for the task of routing 126 randomly distributed spots to the 126 readout channels using the Euclidean distance as a cost measure were carried out to assess the ability to select spots of interest. The average distance from any spot of interest to its closest electrode was 6.75 μm for the set used (n = 2000). The implemented routing scheme provides an average distance to the connected electrode of 7.1 μm, where 114.6 of the 126 spots can be read out via the closest electrode. 102 electrodes in a 6 × 17 rectangular configuration constitute the largest obtainable coherent electrode block.

A block diagram of the overall device is shown in Fig. 1B. The 126 readout channels provide signal conditioning with programmable amplification in 18 steps to account for the large variation in the signal amplitudes of the different cell types (0–80 dB). A first-order high-pass filter with adjustable cutoff frequency (0.3–100 Hz) removes the DC offset and fluctuations resulting from the electrode-saline interface. A tunable second-order low-pass filter defines the upper frequency limit (3.5–14 kHz). The signals are sampled at 20,000 samples/s per channel by means of 8-bit analog-to-digital converters (ADCs). The data are transferred off chip along with the chip-status information and a CRC (cyclic redundancy check) for transmission error detection. Electrical stimuli can be delivered through two 10-bit digital-to-analog converters (DACs). The data are transferred off chip along with the chip-status information and a CRC (cyclic redundancy check) for transmission error detection. Electrical stimuli can be delivered through two 10-bit digital-to-analog converters (DACs). Two additional channels are used to record the on-chip temperature and the electrode DC potential. The low-power design delivers through two 10-bit digital-to-analog converters (DACs). The data are transferred off chip along with the chip-status information and a CRC (cyclic redundancy check) for transmission error detection. Electrical stimuli can be delivered through two 10-bit digital-to-analog converters (DACs).

The noise level in the recordings was 7–9 μVRMS (100 Hz–3 kHz), which includes 3 μVRMS from the preamplifier (Frey et al., 2007). Additional noise is generated by the ADC (quantization noise), the measurement setup and neuronal background activity. Worst-case electrode-to-electrode crosstalk within the array is 67 dB measured in saline solution using Pt-black electrodes. Measurements shown here were obtained with a gain of 2000 or 3000 and an ADC range of 3 V (LSB of 5.9 or 3.9 μV).

2.2. Acute slice preparations

Parasagittal cerebellar slices (0.3 mm thick) were prepared from Long-Evans rats at postnatal days 16–18 as described previously (Heck, 1993). Animals were anesthetized with isoflurane and decapitated. The brain was quickly removed and rinsed in ice-cold (≤4 °C) artificial cerebrospinal fluid (ACSF; contents in mM: NaCl 132.0, KCl 2.0, KH₂PO₄ 1.2, MgSO₄ 1.1, NaHCO₃ 19.0, CaCl₂ 2.5, D-glucose 10.0, continuously gassed with 95% O₂, 5% CO₂). Rats were housed and handled in accordance with the guidelines of the University Freiburg and German law. Slices were cut with a vibratome, stored at 37 °C for 1 h and kept at room temperature thereafter. Recordings were performed at 28 °C with eight buffer exchanges/min.

To improve tissue adhesion, the HD-MEAs were coated with cellulose nitrate (Schleicher & Schuell, Dassel, Germany), dissolved in methanol (0.14 mg/ml) (Egert et al., 2002). Slices were cut in half along the main stem of the white matter, and one of the pieces of the caudal section was positioned above the HD-MEA in a drop of ACSF. ACSF was then quickly and completely removed so that the slice settled onto the HD-MEA surface and adhered.

2.3. Spike sorting

The application of independent-component analysis (ICA) (Brown et al., 2001; Lewicki, 1998; Takahashi et al., 2003) to the recorded multi-electrode data can drastically improve the spike sorting performance. One limitation of ICA is that the number of sources, i.e., the number of neurons, must be equal to or less than the number of sensors, i.e., electrodes. The large number of electrodes in our system relaxes this constraint and allows us to directly apply ICA for spike sorting. ICA has been applied as a preprocessing stage to a standard spike sorting procedure (Snellings et al., 2006). The overall procedure is briefly outlined here: (1) band-pass filtering (500–3000 Hz) of the data, (2) ICA using fast ICA (Hyvarinen, 1999) yielding separated independent components (ICs), (3) threshold detection on the ICs using ±4.2 standard deviations, (4) alignment of the events for maximal correlation with a template spike. (5) Events have then been clustered according to the shape of the ICs (Harris et al., 2000), and (6) spike-triggered averages have been calculated for each cluster. (7) Alternatively, the procedure can be run iteratively by subtracting the spike-triggered averages from the data and repetition of steps (2) through (6). (8) Finally, spike footprints have been calculated by re-extracting and averaging the events from the raw data, which have been band-pass filtered at 3–4000 Hz.

2.4. Extracellular action potential model

To calculate the extracellular potential induced by an AP in the model we modified the approach described by Gold et al. (Gold et al., 2006). It consisted of two stages. First, the dynamic transmembrane potential and the currents were calculated using the NEURON 5.9 simulation environment (Hines and Carnevale, 1997) with a detailed compartment model (Vetter et al., 2001) using the ‘extracellular’ mechanism included in NEURON. The obtained membrane currents, I_m(n), were used to calculate the DC electric field in a conductive medium. For point-current sources in an infinite isotropic, homogenous volume conductor, the electrical potential, \( \Phi_e \), can be calculated as (Klcc and Rall, 1977; Lorente de No, 1947; Rall, 1962):

\[ \Phi_e = \frac{1}{4\pi \sigma} \sum_n \frac{I_m(n)}{d(n)}, \]

where \( \sigma \) is the conductivity of the medium and \( d \) the distance from the current source, \( I_m(n) \). The extracellular conductivity was shown to be inhomogeneous and anisotropic in the cerebellum (Kwan and Murphy, 1974; Okada et al., 1994; Yedlin et al., 1974), accurate values are, however, difficult to obtain. We hence assumed a homogeneous and isotropic conductivity regime with a conductivity of \( \sigma = 0.22 \text{ S/m} \).

Based on this model the ideal extracellular potential was calculated on a grid in three dimensions around the neuron (MATLAB R2007b, MathWorks, Natick, MA). Next, the simulated neuron was semi-automatically positioned to match with the measured neuron. Finally, we solved a finite-element model (FEM) using the calculated membrane currents and taking into account the chip surface
as an insulator using FEMLAB’s conductive media DC application mode (COMSOL 3.3). The assumptions described above allow for a quasi-static description of the electric field in the extracellular space (Mitzdorf, 1985). The model was solved in a cylindrical domain with an insulator, as the boundary condition for the bottom plate (chip surface), whereas the upper plate and the cylinder wall were grounded. The conductive platinum electrodes (10% of the array surface) have been neglected. Equipotential surfaces were calculated and visualized using POV-Ray 3.6 (Persistence of Vision Raytracer Pty. Ltd., Victoria, Australia).

The morphology of a PC was taken from Fig. 2 in (Roth and Hausser, 2001), cell 3 in ref. (Roth and Hausser, 2001). The Na reversal potential has been set to 71 mV. Na, 1.1 channels (Akemann and Knoppel, 2006; Khalil et al., 2003) feature a maximum density on the soma of 0.22 S/cm² and decay rapidly to zero in the dendritic trunk (∼0.1 S/cm² at 50μm and below 0.025 S/cm² at 100μm and further away from the soma) (Bekkers and Hausser, 2007; Migliore and Shepherd, 2002; Stuart and Hausser, 1994; Trimmer and Rhodes, 2004). The Na,1.6 channel modified from (Akemann and Knoppel, 2006) with a slightly lower activation threshold has been placed at high density on the initial segment (0.6 S/cm²) and on the nodes of Ranvier (1.5 S/cm²) (Boiko et al., 2003; Kole et al., 2008). The K⁺ channels were implemented using K, D, A, BK types of voltage- and calcium-activated channels as well as I₉ (Gold et al., 2006). K⁺ channels were placed at high density on the nodes and on the initial segment (Kole et al., 2007). Intracellular resistivity, Rᵢ, was set to 70 Ωcm in the soma and the dendrites and to 50 Ω in the axon (Gold et al., 2006). A passive leakage current with a reversal potential of −65 mV and a membrane resistance of Rₘ = 15 kΩ cm² (myelin: 40 kΩ cm², nodes: 2 kΩ cm²) was included. The membrane capacitance, Cₘ, was set to 1 μF/cm². Spines were accounted for by scaling membrane capacitance and conductivity. The axon was modeled similarly to (Mainen et al., 1995). We simulated synaptic activation by lowering the membrane resistance at the distal dendrites and increased the reversal potential to −40 mV (Destexhe and Pare, 1999). Channel types that do not significantly contribute to the signal shape were omitted.

### 3. Results and discussion

Recordings from a variety of biological preparations, including dissociated cardiomyocytes and neurons, have been performed (Sanchez-Bustamante et al., 2008), however, the focus is here on acute sagittal cerebellar slices with the aim to demonstrate the performance of the developed microelectrode system. The chip is immersed in the culture solution of the biological preparation, and the cells are placed or grown directly on the chip surface so that a good passivation and corrosion protection is necessary.
3.1. Single-cell localization

As 126 out of 11,011 electrodes can be selected for recording, a two-phase strategy was adopted: (1) an activity map was generated from partially overlapping blocks of regularly spaced electrodes, covering patches of $0.3 \times 0.3 \text{mm}^2$ up to $0.5 \times 0.5 \text{mm}^2$; This produced up to 50 recordings of roughly 1 min duration; (2) recordings at a higher resolution were then performed in selected regions of interest. In total, we recorded from five cerebellar slices. The raw signal traces as simultaneously recorded for 126 electrodes are shown for 6 exemplary electrodes in Fig. 2A and B. The relative spatial arrangement of the six electrodes labeled 1–6 in Fig. 2A is shown in Fig. 2D. The electrodes appear in black due to an electrodeposited platinum-black layer. The spikes in the voltage traces of these six electrodes varied considerably in shape (Fig. 2A and B), and from the close-up in Fig. 2B it is evident that the signals on neighboring electrodes showed some degree of synchronicity. These findings indicate (i) that the same neuron provides signals on various electrodes, and (ii) that more than one or different neurons contribute to the overall signal on each electrode (Fig. 2A and 2B).

Spike amplitudes ranged from the detection limit ($\sim 35 \mu V$, $\sim 4 \times$ noise level for chip immersed in biological preparation) up to a few $100 \mu V$, with firing rates of 7–35 Hz. Detectable spike activity was predominantly found in the cerebellar cortex, as shown in the activity map in Fig. 3A, and the rate of supra-threshold events and their amplitudes were maximal in the region of the Purkinje cell layer (PCL), (Fig. 3C). The PCs are spontaneously active and they are effectively disconnected from each other as the parallel fibers have been cut in a sagittal slice. In addition, the excitatory input from the deep cerebellar nuclei via climbing fibers is missing (Fig. 3C). The PC electrical fields can thus be considered independent from each other, which facilitates efficient spike sorting and eases waveform interpretations.

The largely redundant information in the recordings allows for an effective application ICA (Hyvarinen, 1999) as a preprocessing stage to a standard spike sorting procedure. The result of the spike sorting allows assigning “footprints” to individual cells, which constitute a band-pass filtered spike-triggered average of the raw event data (Fig. 2E and F). The spike-triggered average signals of two different cells recorded from on the six selected electrodes marked in Fig. 2D are shown in Fig. 2C. The footprints of the two cells producing the blue and red patterns in Fig. 2B and C are shown in Fig. 2E. The six electrodes in Fig. 2B and 2C are labeled 1–6 in Fig. 2E. In general, the cell footprints spread over an area that extends well beyond the soma and partially covers the extension of the dendritic tree (Fig. 2F, 3B–C). Spikes were typically bipolar with large negative peaks in the somatic region and smaller positive peaks in the dendritic region (Fig. 2F, 3D). Spike shapes at positions in between soma and dendrites represent combinations of these basic types. The cellular footprints obtained here provide an unprecedentedly comprehensive and detailed picture of the electrical activity of a single cell during an AP and they substantiate estimates from in

![Fig. 3. Recordings from an acute slice preparation of the caudal half of the cerebellar vermis. (A) Spatial distribution of detectable spike activity in the recording area. All electrodes used for recording are marked with a small dot (approximately 30% of the available electrodes). Events exceeding a threshold of $\pm 36 \mu V$ were used to calculate the color-coded event rate; scale bar: 0.3 mm. Spike activity is found in a broad band following the cerebellar cortex. (B) Close-up of a region with high activity delimited in (A). All units identified by spike sorting are marked in analogy to Fig. 2F, i.e., the somatic region is blue, the dendritic region is red; scale bar: 0.1 mm. (C) Schematic of the basic cellular structures in the plane of the slice (Gray, 1818); ML: molecular layer, PCL: Purkinje cell layer, GL: granular layer, CF: climbing fiber, MF: mossy fiber, PF: parallel fiber, PC: Purkinje cell, GC: Golgi cell, SC: stellate cell, BC: basket cell; scale bar: 0.1 mm. (D) Footprint of a PC selected from the region shown in (B); scale bar is 200 $\mu V$, 1.9 ms. (E) CSD analysis for the cell shown in (D) at several points in time (green: sink; yellow: source). The sink moves from the soma at 0.4 ms to the proximal dendrites at 0.6 ms and covers the dendritic area, while the soma repolarizes. Frequency band: 180 Hz–3.5 kHz.]
vivo and in vitro recordings performed, however, with much less electrodes (Egert et al., 2002). The spatio-temporal characteristics of the potential of a certain cell or PC in the parasagittal slice were found to be those of a dipole with the negative pole in the PCL and the positive pole in the molecular layer (ML) during an AP onset. During further spike evolution, the soma became more positive, while proximal parts of the dendrites became more negative, and the potential in the distal dendritic tree subsided. The potential distribution at the time of the negative peak potential as shown in Fig. 2F was used to estimate the orientation of each neuron relative to the device surface. At a threshold value of \(-35 \mu\)V, the negative-potential area of a cell extended 50–150 \(\mu\)m along the surface of the chip. Its location coincided with that of the PCL and the positive-potential region extended across most of the adjacent ML. Consequently, the somata of the identified cells in the slices (e.g., 27 cells in Fig. 3B) have been assigned to the PCL, the respective dendritic trees to the ML as can be seen in Fig. 3B and C. Evidence suggests that we predominantly recorded from PCs, since we did not identify any units exclusively in the granular cell layer, the ML, or in the white matter. In some regions a slight displacement of presumptive PC somata near the chip surface with regard to the PCL visible at the upper surface of the slice was observed, since the slice was slightly slanted with respect to the sagittal plane.

3.2. Analysis of the cell dynamics

In a next step we looked at the highly-resolved temporal signal evolution of single identified PC. To this end we performed a current source density CSD (Nicholson and Freeman, 1975) analysis on the data, since extracellular potentials reflect the dynamics of transmembrane currents during an AP. CSD distributions are obtained through the second spatial derivative of the potential field. CSD analysis was used by other authors to determine the location of synaptic currents, in particular in layered brain structures, such as the neocortex or the lateral geniculate nucleus. As yet, CSD analysis was used by other authors to determine the location of the electrical activities of different PCs can be considered independent due to the lack of intercellular connections between them in a parasagittal slice (parallel fibers were cut), individual time-resolved CSD distributions were determined. The CSD structure revealed distinct dipole fields oriented in parallel to the chip surface. At spike onset there was a pronounced sink around the presumptive location of the PC soma and an equivalent, but spatially less focused source in the dendritic region in the ML (Fig. 3E). The sink then shifted towards the ML into the region of the proximal dendrites, where it slowly faded away. This backpropagation of the AP into the proximal dendrites was clearly visible and reproducible. The whole process of dipole field generation, sink and source shift and passive inversion during an AP takes less than 1 ms as can be seen in Fig. 3E.

3.3. Combination with a Purkinje cell model

Finally, we combined our findings with a compartmental model of a PC (Fig. 4) (Roth and Hausser, 2001; Vetter et al., 2001) for a more detailed interpretation of the spatio-temporal features of the extracellular recordings (Gold et al., 2007; Gold et al., 2006; Moffitt and McIntyre, 2005). The footprints, obtained in our recordings, featured very similar shapes, and the PC dendritic tree was oriented approximately plane-parallel to the chip surface (Fig. 4B). In the specific example shown in Fig. 4, the soma center was slightly closer to the surface (center 40 \(\mu\)m above the surface) than the center of the dendritic tree. Even though many cellular parameters are not available in literature, and although the morphology of the specific PC here is not precisely known, the spatio-temporal structure of the simulated cell signals was in good agreement with the experimental results (Fig. 4A; simulation green, recorded results black). Moreover, the simulations confirmed that the density of 3150 electrodes/mm\(^2\) of the HD-MEA is sufficient to capture details of the extracellular signal distribution (Fig. 4C) of neurons located at a depth corresponding to the thickness of the cell layer damaged by the slicing procedure (20–50 \(\mu\)m).

The overall signal amplitude is sensitive to the intracellular resistivity, \(R_i\) (Gold et al., 2007), the extracellular conductivity, \(\sigma\), and gives access to the current dynamics of individual neurons. As the electrical activities of different PCs can be considered independent due to the lack of intercellular connections between them in a parasagittal slice (parallel fibers were cut), individual time-resolved CSD distributions were determined. The CSD structure revealed distinct dipole fields oriented in parallel to the chip surface. At spike onset there was a pronounced sink around the presumptive location of the PC soma and an equivalent, but spatially less focused source in the dendritic region in the ML (Fig. 3E). The sink then shifted towards the ML into the region of the proximal dendrites, where it slowly faded away. This backpropagation of the AP into the proximal dendrites was clearly visible and reproducible. The whole process of dipole field generation, sink and source shift and passive inversion during an AP takes less than 1 ms as can be seen in Fig. 3E.
Fig. 5. Comparison of measured and simulated evolution of the CSD during an AP. (A) Average spikes (averaged over several APs) recorded from the three electrodes labeled (1–3) in Fig. 5B; black dots on the curves in Fig. 5A mark the exact point in potential evolution time, for which the CSDs from measurements in (B) or the CSDs resulting from the compartment model in (C) are displayed; scale bar is 50µV, 1 ms. (B) CSDs resulting from the measured spikes and (C) CSDs from simulations at the points in time specified in (A). The spatio-temporal structures of the measured and simulated CSD distribution are very similar, although there are differences in some details. This is most probably due to the fact that a rather general PC model has been used in the simulation. (D) Equipotential surfaces at −35 V in blue and +35 V in red of the simulated PC. Note, that the insulator nature of the chip distorts the equipotential balloons, which increases the signal at the electrodes. A time lapse video of the dynamics of the equipotential surfaces is available as the Supplementary Video S1.

and the distance between cell and chip surface. These parameters predominantly influence the overall amplitude and, to a lesser extent, the shape of the spike, which is much more sensitive to the distribution and dynamics of the active ion channels (Gold et al., 2006). The distribution of especially the Na⁺ channels in the compartment model was found to be pivotal in reproducing the observed footprints, the shape of which was highly depending on the concentration of Na⁺ channels in the axon initial segment and the exponential decay of the Na⁺-channel density into the dendritic trunk.

With regard to AP dynamics, the CSD distributions calculated from simulations were in good agreement with the measured CSD distributions. The measured and simulated evolution of the CSD during an AP is shown in Fig. 5A–D. Average spikes recorded from the three electrodes labeled (1–3) in Fig. 5B are shown in Fig. 5A. Fig. 5B shows the CSDs from measurements and Fig. 5C the CSDs resulting from the compartment model. The spatio-temporal structures of the measured and simulated CSD distribution are very similar. With the help of comprehensive, temporally and spatially highly resolved measurements as shown here it will be possible to refine the model in the near future. The sinks in the model represent a large influx of positive ions into the cell somatic region through opened Na⁺ channels. Simultaneously to the sinks in the somatic area, a spatially extended source region develops in the dendritic region, which can be assigned to passively drawn currents. Also the backpropagation of the AP into the dendrites can be confirmed in the model. As PC dendrites lack active sodium channels in the distal dendrites, this backpropagation is mostly passive and limited to the proximal stem of the dendrites, in agreement with findings from single-cell patch-clamp studies (Stuart et al., 1997). The extent of the exponential decay of the Na⁺-channel density on the dendritic trunk was found to be a crucial model parameter to accurately reproduce the spike shape (Stuart and Hausser, 1994). Fig. 5D shows simulated equipotential surfaces at −35 V in blue and +35 V in red of the respective PC, again at the same points in time marked in Fig. 5A. The equipotential balloons in Fig. 5D are distorted and drawn towards the chip, the surface of which was modeled as an insulator. This effectuated a 1.6-fold increase of the signal amplitude in comparison to models without an insulating chip surface. Additional simulations showed that the signal amplitude is reduced by only a few percent upon the usage of electrodes with diameters up to 30 µm, depending on the surface-cell distance and the dynamics of signal propagation within the cell. Similar findings have been reported for in vivo applications of shaft-type neuronal probes (Moffitt and McIntyre, 2005). A time-lapse video of the temporal evolution of the extracellular equipotential surfaces of a single cell as shown in Fig. 5D is available as the Supplementary Video S1.

4. Conclusion

The presented CMOS-based microelectrode systems offer great flexibility in recording from biological preparations. Due to the large number of available and selectable recording sites there is no need for a precise alignment of the biological sample with respect to the chip. The device allows performing overview scans of a larger cell ensemble but also offers the potential to gain detailed insights into the mechanisms underlying the AP dynamics of single cells. The
subset selection method is likewise applicable to in vivo shaft-style probes (Csicsvari et al., 2003; Najafi and Wise, 1986) or neuronal interfaces. The presented system can be conveniently applied to a broad variety of biological samples: acute or organotypic slices of the brain or the spinal cord, retinal preparations, cultures of dissociated neuronal cells, layers and microtissues of cardiac myocytes, or other samples involving electrogenic cells.

The devices can be used to address fundamental functional questions in the context of systems biology or to test the influence of drugs or chemicals on the cell activity, they can be cleaned and re-used, and are stable in culture over months. In combination with detailed morphological and immunohistochemical analyses the obtained high-spatiotemporal-resolution electrophysiological data can be used to fine-tune compartment models that describe the characteristics of single cells and their contributions to the population activity. The resulting models will provide important information for spike sorting and will facilitate the identification of neuronal cell types in extracellular recordings.

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Appendix A. Supplementary data


References