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Recording from defined populations of retinal ganglion cells using a high-density CMOS-integrated microelectrode array with real-time switchable electrode selection

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HIGHLIGHTS

- Selection of defined retinal neurons after large-area extracellular screening.
- Distinct light patterns produce specific neuronal activity without signal artifacts.
- High-density electrodes enable precise cell allocation and type assignment.

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ABSTRACT

In order to understand how retinal circuits encode visual scenes, the neural activity of defined populations of retinal ganglion cells (RGCs) has to be investigated. Here we report on a method for stimulating, detecting, and subsequently targeting defined populations of RGCs. The possibility to select a distinct population of RGCs for extracellular recording enables the design of experiments that can increase our understanding of how these neurons extract precise spatio-temporal features from the visual scene, and how the brain interprets retinal signals. We used light stimulation to elicit a response from physiologically distinct types of RGCs and then utilized the dynamic-configurability capabilities of a microelectronics-based high-density microelectrode array (MEA) to record their synchronous action potentials. The layout characteristics of the MEA made it possible to stimulate and record from multiple, highly overlapping RGCs simultaneously without light-induced artifacts. The high-density of electrodes and the high signal-to-noise ratio of the MEA circuitry allowed for recording of the activity of each RGC on 14 ± 7 electrodes. The spatial features of the electrical activity of each RGC greatly facilitated spike sorting. We were thus able to localize, identify and record from defined RGCs within a region of mouse retina. In addition, we stimulated and recorded from genetically modified RGCs to demonstrate the applicability of optogenetic methods, which introduces an additional feature to target a defined cell type. The developed methodologies can likewise be applied to other neuronal preparations including brain slices or cultured neurons.

1. Introduction

The retina is a multilayered, light-sensitive sheet of neural tissue that encodes visual stimuli as complex spatio-temporal patterns of action potentials. The final output of the retina is encoded in the ganglion cell layer, in which densely packed neurons, called retinal ganglion cells (RGCs), generate action potentials that proceed along the optic nerve to higher brain regions (Masland, 2001; Wassle, 2004).

Microelectrode arrays (MEAs) are electrophysiological devices for simultaneously recording the extracellular activity of electrogenic cells at multiple spatial positions (Gross et al., 1995; Jimbo et al., 1998; Rutten, 2002; Stett et al., 2003). MEA technology has been widely applied to record electrical activity in the retina (Meister et al., 1994; Segev et al., 2004; Zeck et al., 2011), to investigate retinal development (Anishchenko et al., 2010; Elstrott et al., 2008), retinal connectivity (Field et al., 2010), visual encoding (Gollisch and Meister, 2008; Pillow et al., 2008; Puchalla et al., 2005;

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Schwartz et al., 2007), to evaluate the efficacy of visual restoration techniques (Bi et al., 2006; Lagali et al., 2008), and for the design of artificial retinal implants (Sekirnjak et al., 2008).

Commercially available MEAs usually comprise up to 256 electrodes and feature up to 300 electrodes per mm^2 (Gross et al., 1995; Pine, 1980) (www.multichannelsystems.com, www.ayanda-biosys.com, www.plexon.com). This density of electrodes is significantly lower than the density of RGCs in many mammals, including rabbits (Oyster et al., 1987), mice (Jeon et al., 1998) and monkeys (Perry and Cowey, 1985). Furthermore, when using conventional MEA technology, it can be challenging to actively target specific cell types for recording, because the electrodes are in a fixed-configuration block, and only the activity of cells that are in the vicinity of these electrodes can be detected.

Recently, high-density MEAs, fabricated in standard microelectronics or CMOS (Complementary Metal Oxide Semiconductor) technology have emerged (Berdondini et al., 2009; Eversmann et al., 2003; Lambacher et al., 2004) and bear the potential to perform recordings from dense populations of neurons at single-cell resolution.

In order to understand how specific features of the visual scene are encoded by the retina, a first step is to examine the synchronously elicited action potentials of defined populations of RGCs (Ackert et al., 2006; Pillow et al., 2008; Schwartz et al., 2007; Trong and Rieke, 2008). To record the activity of a defined population of RGCs, we capitalized upon the electrode configurability capabilities of a CMOS based high-density MEA (Frey et al., 2009). In contrast to an earlier study on blind retinae (Jones et al., 2011), we here used light stimulation to evoke electrical activity from wild type retinae and selected cells according to their light response, which adds to the complexity of the setup and entails the risk of producing artifacts in the recorded signals (see below).

The accurate characterization of a population of neurons is dependent upon the extracellular recording of action potentials with a high signal-to-noise ratio that can be easily differentiated and sorted (Lewicki, 1998). However, light-induced artifacts can introduce erroneous signals and noise into electrophysiological recordings and disrupt the signal analysis process. Such artifacts may arise from the interaction of photons with the electronic components of the CMOS-based circuitry of the MEA chip. Despite the presence of light-sensitive elements in the CMOS-based circuitry, we demonstrate that it is possible to project a light stimulus directly onto the CMOS-based MEA without generating such artifacts in the recorded signals.

Consequently, the absence of light artifacts and the high signal-to-noise ratio allowed us to characterize densely packed RGCs according to their response to light stimulation.

Furthermore, the real-time switchable electrode selection of the MEA allowed the assignment of electrodes to defined physiological types of RGCs. This made it possible to stimulate and record the action potentials from a defined type of RGCs. Finally, it was possible to perform light stimulation of genetically modified RGCs that can be used as optogenetic tools directly on the CMOS-based MEA.

2. Methods

2.1. Data acquisition system

The CMOS-based MEA features 11,011 platinum electrodes with diameters of 7 μm and electrode center-to-center distances of 18 μm over an area of 2 mm × 1.75 mm (Frey et al., 2009). The centrally located electrode array is surrounded by the signal amplification (0–80 dB), filtering (high pass: 0.3–100 Hz, low pass: 3.5–14 kHz) and analog-to-digital conversion (8 bit) units (Fig. 1a).

Extracellular action potentials can be recorded at high temporal resolution (20 kHz) and with low noise levels (~7–9 μV_{rms}, band: 100 Hz–3 kHz, perfusion system operational but without retinal tissue). In the maximum-density recording scenario (3161 electrodes/mm^2), each mouse RGC lies in close vicinity to multiple electrodes, which allows for recording single-cell action potentials at different spatial locations (Fig. 1b).

A switch matrix circuitry is located under the electrode array and connects the electrodes to 126 readout channels (Frey et al., 2010). An arbitrary subset of 126 electrodes at any location and desired inter-electrode spacing can be routed to the 126 readout channels that surround the electrode array (Fig. 1c).

To reduce the electrode impedance and to improve the signal-to-noise ratio, a layer of Pt-black has been electrochemically deposited onto the electrodes at a current density of 0.5 nA/μm^2 in a solution containing 7 mM hexachloroplatinic acid, 0.3 mM lead acetate, and hydrochloric acid with an adjustment of the solution pH to 1.

For the purpose of interfacing with the semiconductor chips, custom-designed printed circuit boards have been used. The recorded data are multiplexed and sent via a single twisted-pair cable to a field-programmable gate array (FPGA) board at a rate of 16 MB/s. The FPGA provides data processing features, such as error detection, digital filtering, event detection, and data reduction/compression. The preprocessed data are then sent to a personal computer for further data processing, visualization and storage.

2.2. Projection and alignment of images with the MEA

The light stimuli were designed using Psychtoolbox (http://psychtoolbox.org) within the software application MATLAB™ and were projected onto the electrode array by an LED projector with a refreshing rate of 60 Hz (Acer K10). The light stimulus was focused only on the electrode array area of the MEA chip by two camera lenses (Nikkor 60 mm 1:2.8 G ED, Nikon), a mirror (U-MBF3, Olympus) and a 5× objective (LMPLFLN5X Olympus) (Fig. 1d). The light projection setup was assembled on an upright microscope (BX5IWI, Olympus). The MEA chip was positioned with a joystick-controlled system (20 nm resolution along *X* and *Y* axis, Scientifica). A video camera provided a real-time view of the electrode array. This procedure enabled the precise projection of a light stimulus exclusively on the electrode array area.

2.3. Preparation of mouse retina and light induced activity recordings

Wild-type C57BL/6J mice (P30) were obtained from Charles River Laboratories (L'Arbresle Cedex, France). All animal experiments and procedures were approved by the Swiss Veterinary Office. The retina was isolated under dim red light in Ringer's medium (in [mM]: 110 NaCl, 2.5 KCl, 1 $CaCl_2$, 1.6 $MgCl_2$, 10 D-glucose, 22 $NaHCO_3$), continuously bubbled with 5% CO_2/95% O_2. The remaining vitreous was removed to improve the contact of the retinal ganglion cells to the electrodes and, finally, a retina patch was placed ganglion-cell-side-down on the electrode array (Fig. 1e). In order to stably secure the retina directly above the MEA, a permeable membrane (polyester, 10 μm thickness, 0.4 μm pore size) was lightly pressed against the tissue; continuous perfusion with oxygenated Ringer's medium at a flow rate of 2.8 ml/minute and at a temperature of 35 °C was provided to maintain tissue viability. Each isolated section of the retina was light-adapted to a "gray" background (blue LED, 460 ± 15 nm, intensity ~ 1.0×10^{13} photons cm^{-2} s^{-1}; green LED, 525 ± 23 nm, intensity ~ 1.67×10^{13} photons cm^{-2} s^{-1}) for a duration of 30 minutes prior to light stimulation and recording. We recorded from mouse