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Short communication

Optopatcher—An electrode holder for simultaneous intracellular patch-clamp recording and optical manipulation

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HIGHLIGHTS

- The optopatcher: a new holder for simultaneous patch-clamp recording and light stimulation.
- We used the optopatcher for in vivo cortical patch-clamp recording and optogenetic activation.
- The holder can be used in multiple platforms whenever a glass pipette is used.

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ABSTRACT

Optogenetics has rapidly become a standard method in neuroscience research. Although significant progress has been made in the development of molecular tools, refined techniques for combined light delivery and recording in vivo are still lacking. For example, simultaneous intracellular recording and light stimulation have only been possible by using two separate positioning systems. To overcome this limitation, we have developed a glass pipette holder which contains an additional port for the insertion of an optical fiber into the pipette. This device, which we called "*optopatcher*" allows whole cell patch-clamp recording simultaneously with direct projection of light from the recording pipette. The holder spares the use of an additional manipulator and, importantly, enables accurate, stable and reproducible illumination. In addition, replacement of standard pipettes is done as easily as with the available commercial holders. Here we used the optopatcher in vivo to record the membrane potential of neurons from different cortical layers in the motor cortex of transgenic mice expressing channelrhodopsin-2 under the Thy1 promoter. We demonstrate the utility of the optopatcher by recording LFP and intracellular responses to light stimulation.

1. Introduction

Enormous advances have been made in optogenetics since it was introduced only several years ago (Zemelman et al., 2002; Boyden et al., 2005). New hardware technologies have been introduced in order to facilitate the efficiency and accuracy of viral vector delivery into the brain and in order to improve the precision of light-based manipulation of neural activity (Gradinaru et al., 2007). Simultaneous extra-cellular recording and optical stimulation can be achieved using commercial silicone optrodes (Zhang et al., 2009). Additional methods for combined optical manipulation and extracellular recordings have been developed by several laboratories (Stühmer and Almers, 1982; Diester et al., 2011; LeChasseur et al., 2011), but few studies have been performed with combined patch-clamp recordings and light stimulation in vivo (Cardin et al., 2009; Mateo et al., 2011). Light delivery in these studies was performed either by stimulation of superficial cortical layers through the microscope or using a separate optical fiber for stimulation. Using an optical fiber requires a second positioning system and poses a challenge in delivering reproducible amounts of light to the recorded cells, a parameter which could greatly affect the reliability of activation and the latencies to spike, both important quantitative parameters in many experiments. Importantly, simultaneous recording and illumination using a single device allows high repeatability and accuracy and can significantly reduce tissue damage caused by the positioning of an optical fiber within the tissue. However, a similar solution for light stimulation and intracellular patch-clamp recording is currently not available. Here we introduce a new device for combined whole cell patch-clamp

Abbreviation: ChR2, channelrhodopsin-2.

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recording and light stimulation and demonstrate recordings made using this device in transgenic mice expressing channelrhodopsin-2 (ChR2) in cortical neurons.

2. Materials and methods

2.1. Animal preparation for recording

All surgical and experimental procedures were approved by the Weizmann Institute Animal Care and Use Committee. Two Thy1-COP4 mice (6 weeks old, B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J, The Jackson Laboratories, Bar Harbor, ME) were initially anaesthetized with ketamine (i.p., 100 mg kg^{-1}; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine mixture (Eurovet, Bladel, The Netherlands). The animals were placed in a standard stereotaxic device using modified zygomatic ear-bars. Lidocaine (2%) was applied to the pressure points and around the area of surgery. Body temperature was kept at 37.0 ± 0.1 °C using a heating blanket and a rectal thermometer (TC-1000; CWE, Ardmore, PA). The skin over the skull was incised and the periosteum was removed. Bregma and lambda were leveled, and a craniotomy (~1.0 mm in diameter) for unit recordings was made over the motor cortex (1 mm rostral, 1 mm lateral to bregma).

2.2. Anesthesia

Following the initial anesthesia, animals were tracheotomized (22G FEP polymer catheter) and machine ventilated with oxygen enriched air under deep halothane anesthesia (0.8–1.4%).

2.3. Recording technique and light stimulation

Except for the use of a modified pipette holder, whole cell recordings were performed as previously described (Katz et al., 2006). Borosilicate micropipettes were pulled to produce electrodes with a resistance of 4–8 MΩ, and filled with an intracellular solution containing (in mM): 136 K-gluconate, 10 KCl, 5 NaCl, 10 HEPES, 1 MgATP, 0.3 NaGTP, and 10 phosphocreatine (310 mOsm/L). For histological identification of the recorded cells, 0.4% biocytin was added to the solution. Whole-cell patch-clamp recordings were obtained in current-clamp mode, and electrodes were inserted perpendicularly to the cortex. Intracellular signals were acquired using an Axoclamp-2B amplifier (Molecular Devices) and low pass filtered at 3 kHz before being digitized at 10 kHz.

For light stimulation, an analog modulated blue DPSS laser (λ = 473 nm, Shanghai Dream Lasers Technology Co. Ltd., Shanghai, China) coupled to a multi-mode fiber (NA = 0.22, 62 μm core) was used. The distal end portion of the fiber (~6 cm) was stripped and inserted into the glass capillary through the holder as described below (see also Fig. 1 and Supplementary data).

2.4. Histology

At the end of the experiment, mice were over-anaesthetized with halothane and perfused transcardially with 4% paraformaldehyde, after rinsing with physiological saline. The brain was removed and postfixed in the perfusion solution for up to 24 h. The brain was extensively washed with 0.1 M phosphate buffer (pH 7.4) and cut at 100 μm thickness in the coronal plane on a vibratome (Leica VT 1200S). Thereafter, the sections were rinsed 2× 15 min in TRIS-buffered saline (TBS; 0.05 M, pH 7.6) and 2× 15 min with TBS containing 0.5% Triton X-100 (TBST). Then, with an intermediate blocking step, streptavidin Alexa-594 (1:300 in TBST; Invitrogen) was incubated for 3 h at room temperature, under gentle agitation. The histochemical reaction was stopped by rinsing with TBST (15 min; pH 7.6), TBS (15 min; pH 7.6) and 0.05 M Tris buffer (2× 15 min; pH 8.2). Stained sections were mounted on glass slides and coverslipped with Aquapolymount (Fisher Scientific).

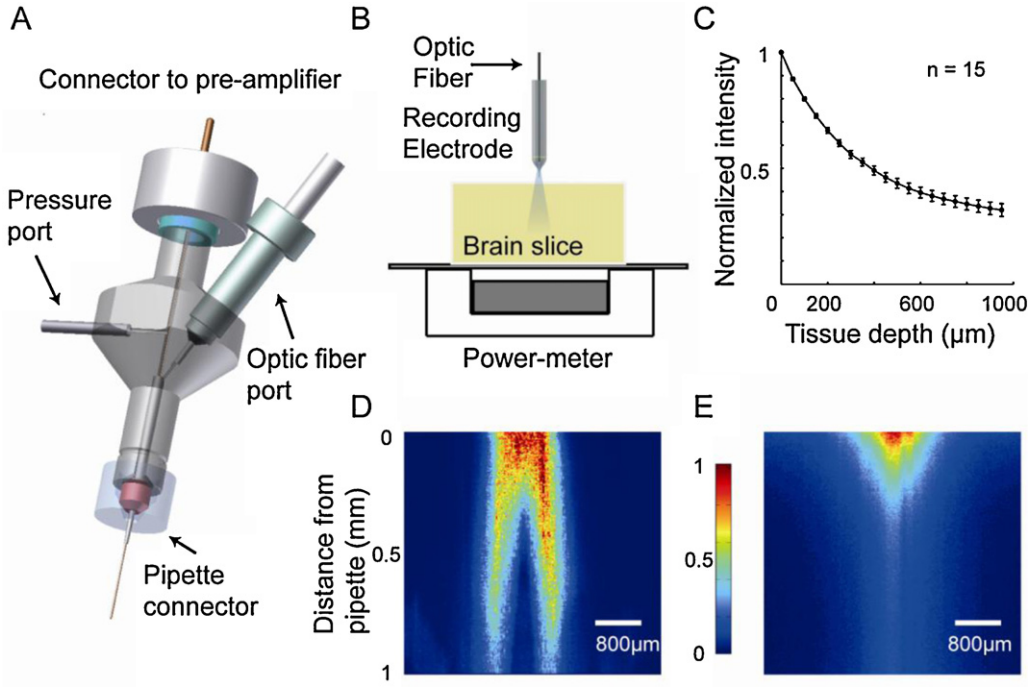

Fig. 1. The properties of the optopatcher. (A) Drawing of the electrode holder. The bare optical fiber is inserted through the optic fiber port into the glass pipette which is inserted at the pipette connector. (B) Light transmission through the brain was measured by mounting a brain slice on a cover glass and advancing the electrode into the tissue. (C) Light intensity was reduced on average by about 70% at 1000 μm depth (n = 15). (D and E) Beam profile in saline (D) and cortex (E). Images are sections of a 3D map along the illumination axis trough the pipette's tip and are normalized to the maximal intensity. Notice the decreased light transmission in the center of the beam profile in saline which results due the glass pipette (see Supplementary Fig. S4).