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

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## *Title*

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

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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.

## *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.

## *Abbreviations*

ChR2 – channelrhodopsin-2;

## *Keywords*

Optogenetics, light stimulation, patch-clamp, in vivo, optopatcher.

## *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 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, 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 4–8MΩ, 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 Figure 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 x 15 min in TRIS-buffered saline (TBS; 0.05M, pH 7.6) and 2 x 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 0.05M Tris buffer, (2 x 15 min; pH 8.2). Stained sections were mounted on glass slides and coverslipped with Aquapolymount (Fisher Scientific).

The preparations were imaged on a Zeiss AxoImager using the Apotome mode for removing scattered light. For photoreconstruction purposes, z-stacks were collapsed into maximum-intensity-projections.

## *3. Results and discussion*

Our goal was to optogenetically stimulate the neuronal network in the vicinity of the intracellularly recorded cell. This can be achieved by carefully positioning an optical fiber using a second micromanipulator. However, this approach can cause tissue damage, is more costly and less precise since it requires a very

accurate positioning of the fiber. These problems become more severe when recordings are made in deep brain structures such as the thalamus (Brecht and Sakmann, 2002) or the hippocampus (Epsztein et al., 2010). To solve this problem we introduced the optical fiber into the recording pipette, eliminating the need for a second manipulator for the positioning of the illuminating fiber. To this end, we designed an intracellular pipette holder with an optic port through which an optical fiber is inserted into a standard patch-clamp micropipette (Fig. 1A and supplementary Fig. S1, S2).

The optical fiber is inserted via a hollow screw and a rubber gasket through the holder into the glass pipette. This seal allows controlling the pressure inside the pipette as done with standard patch clamp holders. For this procedure one end of the optical fiber was stripped from its protective jackets to a desired length while the other end was left intact and was connected to the laser with standard SMA connector. The actual length of the stripped fiber is determined by the length of the glass pipette in-use plus the length of the fiber's path from the sealing gasket to the pipette's bore within the holder (Supplementary Fig. S1, ~6.3 mm in our holder).

Practically speaking, the fiber tip can be positioned at any depth within the recording pipette but since the pipette is transparent, light would emanate from it according to the half-angle of divergence of the optical fiber (Aravanis et al., 2007), which in turn depends on the fiber's numerical aperture and the medium. Additionally, light transfer through the tapered pipette can cause light diffraction and obstruction. Hence, correct positioning of the fiber optic is crucial for efficient light transmission. To find the optimal positioning of the fiber within the recording electrode, the emitted light power was measured while positioning the fiber at different distances from the electrode's tip. The actual fine positioning was done by moving the electrode within the holder toward the optical fiber. The maximal light intensity and minimal light dispersion were obtained when the fiber was positioned as deep as possible in the pipette (Supplementary Fig. S3). Given the geometry of the tapering pulled pipette and the thickness of the fiber, the tip of the fiber was positioned about 3 mm from the very tip of the pipette (Fig. 1B).

Using the maximal power of the DPSS laser at this distance, the measured light power was 5.4 mW compared to 6.7 mW when measured directly from the fiber in the absence of the glass pipette (20% reduction in light power). Under maximal illumination power a large volume surrounding the pipette will be activated by the electrode, but since the light is focused below the electrode's tip (Fig. 1E, see the high intensity in the center of light signal), the illuminated volume can be controlled by the light intensity. If it is critical to confine the illuminated area, the pipette can be externally coated by non-reflective, non-toxic, opaque lacquer (Cardin et al., 2010, Supplementary Fig. S2). This can be helpful while recording in deep brain structures in which neurons situated in the electrode's path should not be optically activated.

To evaluate the propagation of light in the brain, we performed light power measurements in brain slices (Fig. 1B). Under these conditions the light intensity was attenuated by about 70% at a distance of 1 mm compared to the intensity measured at the tip of the pipette (Fig. 1C). Another important property of this optical system is the light distortion by the pipette and tissue. Hence, we measured the beam profile through the glass pipette in saline and brain slice (Aravanis et al., 2007). The beam profile within saline (Fig. 1D) has a ring-like profile at depth exceeding 150μm (Supplementary Fig. S4A). This is probably caused by the tapering of the pipette which refracts the light away from the center of the pipette. Remarkably, this effect is completely diminished in brain slice (Fig. 1E, S4B) due to light diffraction by the tissue.

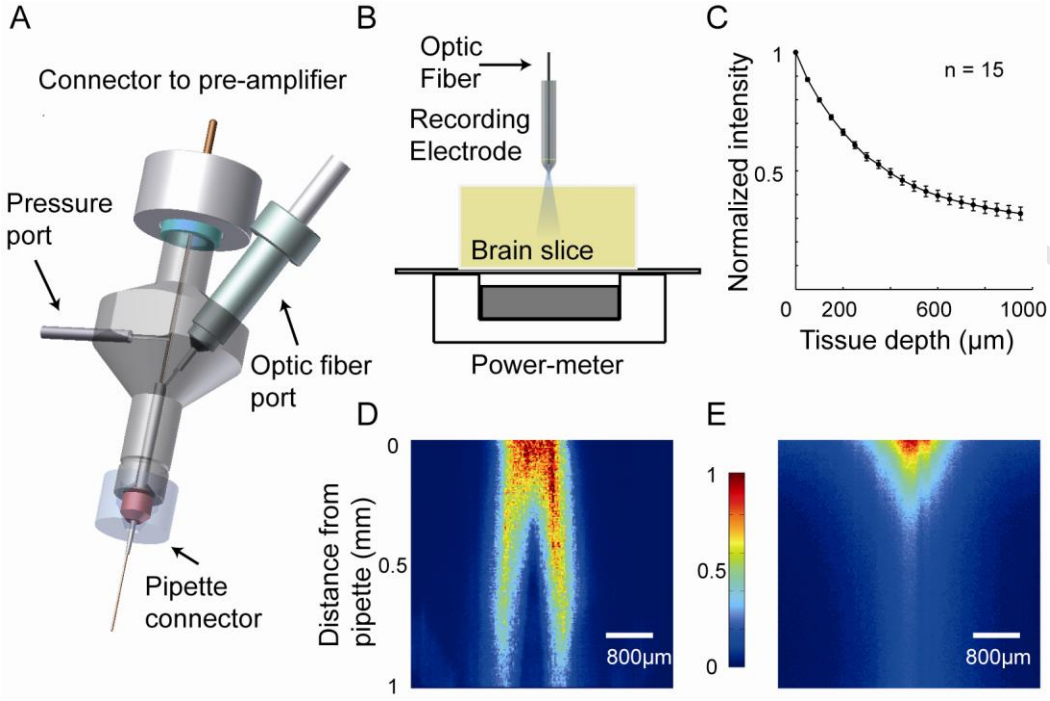


**Fig. 1.** The properties of the optopatcher. (A) Drawing of the electrode holder. The bare optical fiber is inserted through the optic fiber port into the glass pipette which is inserted at the pipette connector. (B) Light transmission through the brain was measured by mounting a brain slice on a cover 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, 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).

The optopatcher was tested *in vivo* using a transgenic mouse line (See Materials and methods) which abundantly expresses ChR2 in pyramidal cells of layer 5 (Arenkiel et al., 2007; LeChasseur et al., 2011), together with membrane-tagged EYFP for visualization of these neurons (Fig. 2A). We examined the activation profile in the intact cortex by using the optopatcher to record the local field potential (LFP) in response to light stimulation (Fig. 2C).The profile of LFP

responses matched the expected laminar expression as stimulation of deeper layers exhibited a stronger response. The response consists of a negative-positive transient waveform likely corresponding to excitation followed by feedforward inhibition (Okun et al., 2010). Maximal activation (LFP negative peak) was evoked at around 800 µm below the dural surface, corresponding to the measured depth of layer 5 cell-bodies. Importantly, in control experiments using animals not expressing ChR2, no LFP or intracellular responses were evoked by illumination of any strength, indicating the specific optogenetic origin of these field potentials. Moreover, since the optical fiber is situated at the tip of the pipette below the silver wire, it allows photoelectric artifact free recording (Cardin et al., 2010).

Next, we used the optopatcher to perform whole cell patch-clamp recordings. The success rate in obtaining a stable recording with the optopatcher, about one out of 3 pipettes, was similar to that achieved with a commercial holder (ST50-200/HS, Siskiyou Corporation, Grants Pass, OR). The response to trains of light stimuli was examined in 14 neurons recorded from different cortical layers. The average membrane potential in these stable recordings was -59.1 ± 3.4mV. Cells were filled with biocytin and an example of a recovered, photo-reconstructed L5 small pyramidal neuron is presented (Fig. 2B). In some cells (2/14) activation was suprathreshold, and showed short-latency responses. These two cells showed firing responses that reliably followed high frequency (50Hz) light pulse trains (Fig. 2D and E) and their depth matched layer 5 (850, 832µm). In many cells (12/14), trains of light stimuli evoked small excitatory responses on top of a prolonged hyperpolarizing response (Fig. 2F), probably induced by inhibitory inputs activated by the light-stimulated cells. The responses were reliable and had sub-millisecond jitter (Fig. 2E, G). Thus, all the recorded cells in our study responded to light stimulation showing precise and reliable responses.

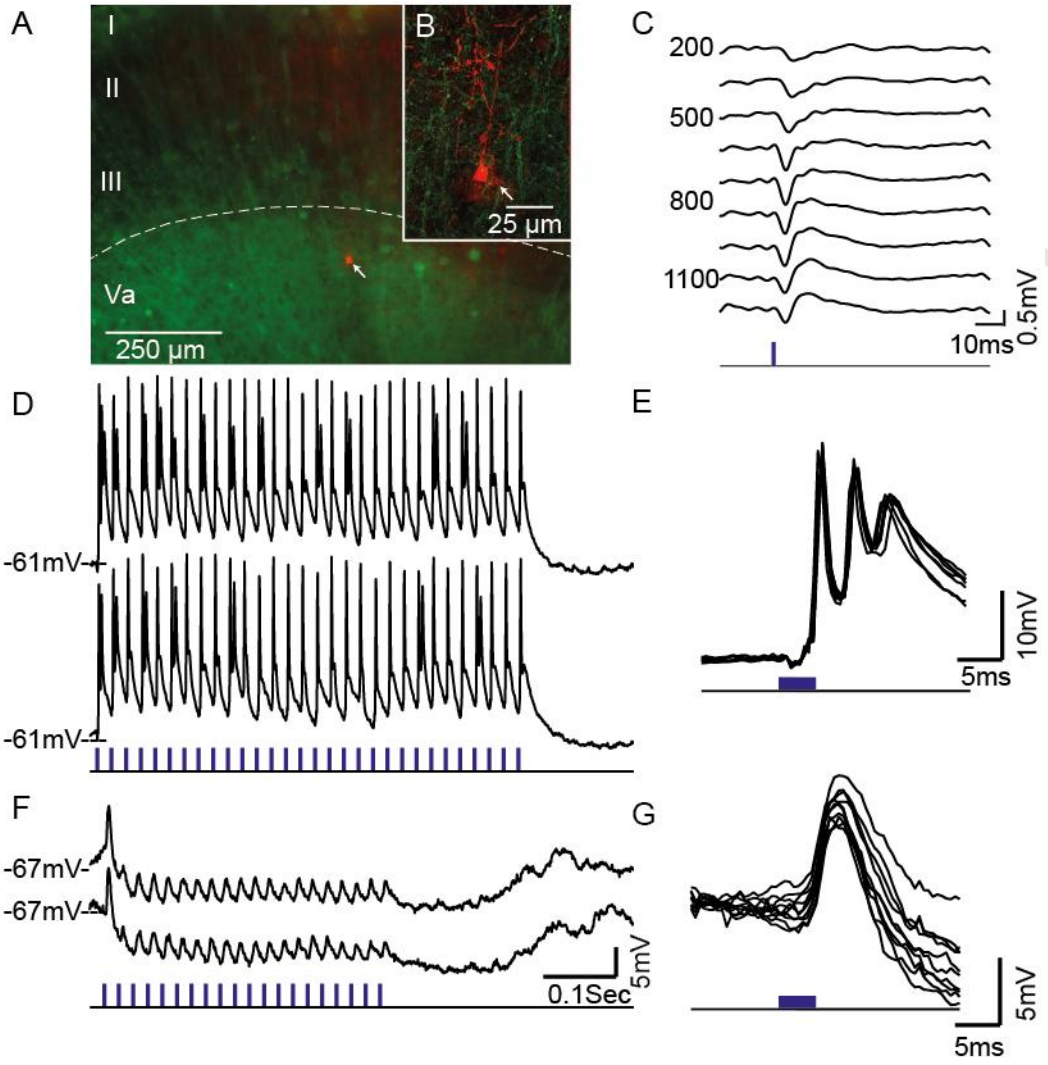


**Fig. 2.** In-vivo optogenetic stimulation and recording of cortical neurons using the optopatcher. (A) Low magnification widefield fluorescence image of the motor cortex showing EYFP fluorescence (in green) localized to pyramidal cells of layer 5a (and b) and their apical dendrites in the supragranular layers 1-3. Arrow points to a patched and biocytin-filled cell (red). (B) Higher magnification of the same neuron (ApoTome, maximum intensity projection) shows the typical features of a small pyramidal cell. (C) A depth profile of LFP recorded by the optopatcher in response to a brief light pulse (1 ms) illuminated by the recording pipette. (D) Two traces showing the response of intracellularly L5 recorded cell to 50Hz light stimulation. The pattern of light stimulation

(blue ticks) is depicted below the voltage traces. (E) Overlay of the 8 first consecutive responses shows a short delay of the highly reproducible responses. (F) Two traces recorded intracellularly from a L2 cell showing the response to 50 Hz light stimulation. Optical stimulation trace as above. (G) Overlay of the 10 first consecutive responses to the light stimulations showing the 2.4 ± 0.55 ms delay for the responses. Scale bars for D, F are similar.

## *4. Conclusions*

In summary, we designed an electrode holder for simultaneous intracellular patch-clamp recording and optical stimulation, and showed examples of recorded cortical neurons in anesthetized mice. The optopatcher prevents the need for a second manipulator and for insertion of the optical fiber into the tissue. It can be also used for any other type of recordings that make use of glass capillaries, such as LFP recording and single unit recording. Without any modifications, the optopatcher can be utilized for in-vitro recordings in brain slices or organotypic cultures and can be also used for discrete or concurrent photolysis of caged compounds. Because it is fully compatible with existing intracellular recording equipment and requires no additional technical training for usage, the optopatcher will facilitate the use of optogenetics in neuroscience research.

## *Conflict of interest*

None.

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