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Fluorescent pipettes for optically targeted patch-clamp recordings

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ABSTRACT

Targeted patch-clamp recordings are a promising technique that can directly address the physiological properties of a specific neuron embedded in a neuronal network. Typically, neurons are visualized through fluorescent dyes or fluorescent proteins with fluorescence microscopy. After switching to transmitted light microscopy, neurons of interest are re-identified and visually approached *in situ* with patch-clamp pipettes. Here we introduce a simpler method for neuron targeting. With fluorophore-coated pipettes, fluorescently labeled neurons and the pipette tips are simultaneously imaged at the same fluorescence wavelength in the same microscope field, so that the neurons and even their neurites are targeted without suffering from chromatic aberration or mechanical complication in optics. We did not find that the coated fluorophores affected the electric properties of pipettes or neurons. The novel technique will be wildly available for pipette micromanipulation under online visual control.

1. Introduction

As the brain functions through collective dynamics of various types of neurons, electrophysiological recordings from identified neurons are required to reveal neuron type specific behaviors in neuronal networks. One of the methods is optically targeted patch-clamp recordings, in which specific neurons can be selectively recorded through visualization. In brain slice preparations, conventionally, neurons are identified and targeted with infrared differential interference contrast (DIC) microscopy (Dodt & Zieglgansberger, 1990) or Dodt gradient contrast microscopy (Dodt, Frick, Kampe, & Zieglgansberger, 1998), which facilitate high-quality whole-cell patch-clamp recordings. These techniques are also applicable to patch-clamp dendrites by visually tracing them arising from the somata (Stuart, Dodt, & Sakmann, 1993).

Recent advances in genetic engineering have realized specific expression of fluorescent proteins in neurons, allowing specific labeling of neuron populations, depending on cell type (Galarreta & Hestrin, 2002; Metzger et al., 2002; Meyer, Katona, Blatow, Rozov, & Monyer, 2002; Oliva, Jiang, Lam, Smith, & Swann, 2000; Tamamaki et al., 2003), location (Dittgen et al., 2004; Miyoshi, Blomer, Takahashi, Gage, & Verma, 1998), development (Saito & Nakatsuji, 2001; van Praag et al., 2002), and activity history (Barth, Gerkin, & Dean, 2004; Wang et al., 2006). The genetic marking with fluorescent protein is experimentally powerful, because those 'fluorescent' neurons can be directly searched with online live imaging (Dittgen et al., 2004; Mainen et al., 1999; Margrie et al., 2003; Shi et al., 1999). In this case, however, the fluorescently identified neurons have to be re-observed with a transmitted light microscope (such as a bright-field or DIC microscope) by switching from a fluorescent microscope, which cannot visualize transparent glass pipettes. This switching procedure often produces optical aberration and makes it difficult to couple images obtained with different microscope modes or different wavelengths.

In the present work, we overcome this problem by coating pipettes with commercially available fluorophore-conjugated albumin. Albumin is a water-soluble form of biological protein, especially found in blood serum and egg white. It is stable in water and non-toxic, but physically adhesive, and thus it has been used for glass coating. By taking advantage of this nature, we coated the surfaces of patch-clamp pipettes with Alexa Fluor-conjugated bovine-serum albumin (BSA). The coating was conducted immediately before each experiment by briefly immersing the pipette tip into the albumin solution. We believe that the method provides a new tool for physiological analyses of neuronal microcircuitry.

2. Materials & methods

2.1. Cultured brain slices

Hippocampal slice cultures were prepared from postnatal day 7 Wistar/ST rats (SLC, Shizuoka, Japan) as described previously

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(Koyama et al., 2007). Experiments were performed with the approval of the animal experiment ethics committee (approval number, 19–43), according to The University of Tokyo guidelines for the care and use of laboratory animals. Briefly, rat pups were chilled, and the brains were removed and horizontally cut into 300 μm-thick slices using a DTK-1500 vibratome (Dosaka, Kyoto, Japan) in aerated, ice-cold Gey's balanced salt solution (Invitrogen, Carlsbad, CA, USA) supplemented with 25 mM glucose. Entorhino-hippocampal stumps were cultivated for 10–14 d on Omnipore membrane filters (JHWP02500, φ 25 mm; Millipore, Bedford, MA, USA). Cultures were fed 1 ml of 50% minimal essential medium, 25% Hanks' balanced salt solution (Invitrogen), 25% horse serum (Cell Culture Laboratory, Cleveland, OH), and antibiotics in a humidified incubator at 37 °C in 5% CO_2. The medium was changed every 3.5 d.

2.2. Acute brain slices

Using a vibratome (Vibratome 3000; Vibratome company, St. Louis, MO, USA), horizontal slices of 400 μm in thickness were prepared from postnatal 10–12-day-old Thy1-GFP mice (De Paola, Arber, & Caroni, 2003), which express membrane-targeted green florescent protein (GFP) in a small number of CA3 neurons. The mice were deeply anesthetized with ether and immediately decapitated. The brain was isolated quickly (within less than 30 s) and placed in ice-cold sucrose-based artificial cerebrospinal fluid (aCSF) consisting of (in mM) 27 $NaHCO_3$, 1.4 NaH_2PO_4, 2.5 KCl, 0.5 Ascorbic acid, 7.0 $MgSO_4$, 1.0 $CaCl_2$, and 222 sucrose. aCSF was continuously bubbled with 95% O_2 and 5% CO_2. The leptomeninx was carefully removed with a pair of microforceps. After dissection, hippocampal slices were maintained for 90 min at room temperature in aerated aCSF that consisted of (in mM) 127 NaCl, 26 $NaHCO_3$, 3.5 KCl, 1.24 KH_2PO_4, 1.4 $MgSO_4$, 1.2 $CaCl_2$, and 10 glucose. The same surface of each slice was kept upside throughout the following experiments.

2.3. Electrophysiological recordings

A slice preparation was placed in a recording chamber and perfused with aCSF at 2–3 ml/min. CA3 pyramidal neurons were whole-cell recorded with a MultiClamp 700B amplifier and a Digidata 1320A or 1440 digitizer controlled by pCLAMP 9 or 10 software (Molecular Devices, Union City, CA, USA). Patch pipettes were pulled from borosilicate glass tubes (CG-1.5, Ken Enterprise, Kanagawa, Japan) with a P-97 horizontal puller (Sutter Instruments, Novato, CA, USA), and the tips were heat-polished with a MF-830 microforge (Narishige, Tokyo, Japan). For whole-cell recordings, pipettes (5–8 MΩ for soma, 12–18 MΩ for dendrites) were filled with internal solution consisting of (in mM) 135 K-gluconate, 4 KCl, 10 HEPES, 10 phosphocreatine-Na_2, 4 Mg-ATP, and 0.3 Na_2-GTP (pH 7.2). With these external and internal solutions, the reversal potential for $GABA_A$ receptor-mediated current was calculated to be −90 mV. For loose cell-attach recordings from axons, borosilicate glass pipettes (12–18 MΩ) were filled with aCSF. Signals were low-pass filtered at 2 kHz and digitized at 20 kHz. In some experiments, the pipette tips were evaluated with scanning electron microscopy (5000×; S-4800, Hitachi, Tokyo, Japan).

2.4. Optical imaging

Fluorescence images of CA3 pyramidal neurons were acquired at 10 frames/s with a Nipkow-disk confocal unit (CSU-X1, Yokogawa Electric, Tokyo, Japan), a cooled CCD camera (iXon DV897, DCS-BV, Andor, Belfast, Northern Ireland, UK), a 16× or 40× water-immersion objective lens (numerical aperture 0.80, Nikon, Tokyo, Japan), and Solis image acquisition software (Andor).

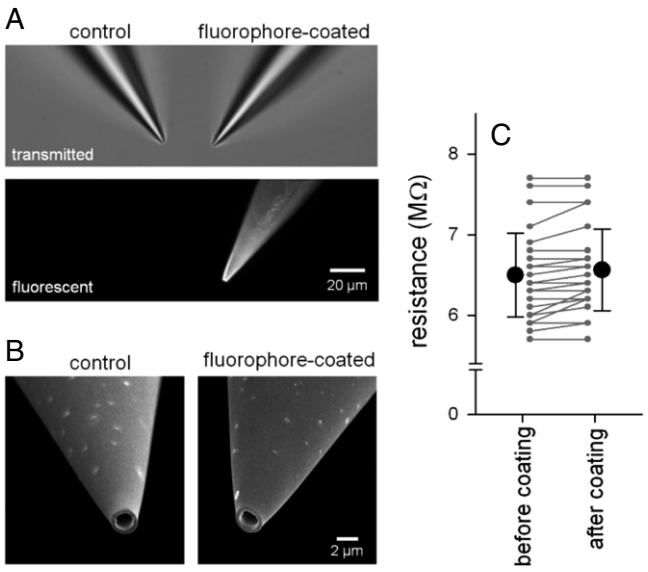

Fig. 1. *Fluorophore-coated glass pipettes.* (A) Bright-field (top: transmitted) and fluorescence images (bottom: fluorescent) of the tips of control and fluorophore-coated pipettes. (B) Scanning electron microscopic images of the tips of control (left) and fluorophore-coated pipettes (right). (C) Comparison of pipette resistance before and after fluorophore coating. Gray lines represent individual pipettes. Black symbols are the means ± standard deviation of all 30 pipettes tested.

Fluorophores were excited at 488 nm with an argon laser (5–10 mW, HPU50101, Furukawa electric, Tokyo, Japan) and visualized with a 507-nm long-pass emission filter. For tracing neurites, CA3 pyramidal cells were whole-cell recorded with normal patch-clamp pipettes containing 40 μM Alexa Fluor 488 hydrazide (A-10436, Invitrogen).

3. Coating pipettes with fluorophores

Glass pipettes were coated with BSA Alexa Fluor 488 conjugate (BSA-Alexa; A-13100, Invitrogen). In order to prevent non-specific adsorption of BSA-Alexa, all plastic tips and tubes used for preparation and stock were rinsed for 60 s with 0.1% BSA (A-4161, Sigma-Aldrich, St. Louis, MO, USA) in advance. BSA-Alexa and NaN_3 (Nacalai Tesque, Kyoto, Japan) were dissolved in 0.1 M phosphate-buffered saline at the final concentration of 0.02% and 3 mM, respectively.

Immediately before use, a pipette was backfilled with internal solution, and the tip was immersed in the BSA-Alexa solution in 1.5 ml tube for 5–10 s under a positive intra-pipette pressure of 50–60 hPa. This procedure reliably made the pipettes fluorescently visible with confocal microscopy (Fig. 1A). Electron microscopic inspection reveals that the coating did not affect the appearance of the pipette tip in terms of dirt or blemish on the glass surface (Fig. 1B). Indeed, no marked change in pipette resistance occurred by coating (Fig. 1C).

4. Patch-clamp recordings with fluorescent pipettes

To examine whether the coated pipettes affect the quality of patch-clamp recordings, CA3 pyramidal cells in cultured slices were whole-cell recorded with control pipettes and fluorophore-coated pipettes. We measured the membrane capacitance (C_m), membrane resistance (R_m), and series resistance (R_s) and did not find any statistical differences in these parameters between control and fluorophore-coated pipettes (Table 1).

Three example cases for application of fluorescent pipettes are presented in Fig. 2. First we prepared acute hippocampal