Journal of Neuroscience Methods 212 (2013) 17–27

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

Biolistic delivery of voltage-sensitive dyes for fast recording of membrane potential changes in individual neurons in rat brain slices

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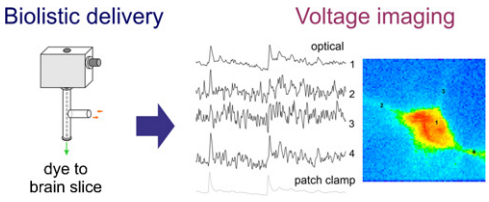
HIGHLIGHTS

- Biolistic delivery allowed rapid staining with hydrophobic voltage-sensitive dyes.
- The dyes di-12-ANEPPQ and di-8-ANEPPS stained the neuronal soma, dendrites and axon.
- Biolistic delivery provided the fluorescence suitable for fast voltage imaging.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 July 2012
Received in revised form 3 September 2012
Accepted 5 September 2012

Keywords:
Biolistic delivery
Voltage-sensitive dyes
Optical recording
Neuron
Membrane
Brain slices

ABSTRACT

Optical recording of membrane potential changes with fast voltage-sensitive dyes (VSDs) in neurons is one of the very few available methods for studying the generation and propagation of electrical signals to the distant compartments of excitable cells. The more lipophilic is the VSD, the better signal-to-noise ratio of the optical signal can be achieved. At present there are no effective ways to deliver water-insoluble dyes into the membranes of live cells. Here, we report a possibility to stain individual live neurons with highly lipophilic VSDs in acute brain slices using biolistic delivery. We tested four ANEP-based VSDs with different lipophilic properties and showed their ability to stain single neurons in a slice area of up to 150 μm in diameter after being delivered by a biolistic apparatus. In the slices of neocortex and hippocampus, the two most lipophilic dyes, di-8-ANEPPS and di-12-ANEPPQ, showed cell-specific loading and Golgi-like staining patterns with minimal background fluorescence. Simultaneous patch-clamp and optical recording of biolistically stained neurons demonstrated a good match of optical and electrical signals both for spontaneous APs (action potentials) and stimulus-evoked events. Our results demonstrate the high efficiency of a fast and targeted method of biolistic delivery of lipophilic VSDs for optical signals recording from mammalian neurons in vitro.

1. Introduction

Voltage imaging in live neurons plays a central role in resolving scientific problems in modern neuroscience at many levels, providing a unique tool to probe the membrane potential from the level of single cellular compartments up to the scale of large brain areas with many neuronal networks. Although these methods were extensively used and developed during decades of research (Cohen and Salzberg, 1978), they still present a significant technical challenge to acquire an appropriate optical recording of the neurons in a specific preparation (Peterka et al., 2011). One of the main problems is to choose a right sensor which provides the best sensitivity of recording for the chosen object, and at the same time causes minimal side effects such as photodamage, phototoxicity, dye internalization, dye leak, non-uniform staining or undesired pharmacological effects. For most preparations the correct choice could be made only empirically (Ross and Reichardt, 1979; Tsau

Abbreviations: ANEP-based dyes, aminonaphthylethenylpyridinium-based dyes; AP, action potential; ASCF, artificial cerebrospinal fluid; CCD camera, charge-coupled device camera; DIC, differential interference contrast; N.A., numerical aperture; ROI, region of interest; RT, room temperature; S/N ratio, signal-to-noise ratio; VSD, voltage-sensitive dye; LSM, laser scanning microscope; LED, light emitting diode; fps, frames per second.

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http://dx.doi.org/10.1016/j.jneumeth.2012.09.008

et al., 1996; Vignali et al., 2010). To minimize possible side effects, the VSDs are frequently used at the lowest possible concentrations to provide the acceptable sensitivity and meet the minimal requirements of spatiotemporal resolution. Many attempts were dedicated to improve the methods of voltage imaging via synthesis of potentially better dyes (Acker et al., 2011; Theer et al., 2011), development of new imaging equipment (Bouevitch et al., 1993; Nishimura et al., 2006), or suggesting completely new approaches (Kralj et al., 2012; Miller et al., 2012). In the present study we introduce a new technique of loading neurons in rat brain slices with highly sensitive lipophilic VSDs that are undeliverable by conventional means.

Acute brain preparations and slices are commonly used for in vitro study of mammalian brain functions (Ballanyi, 1999) and optical imaging (Cohen, 1988; Canepari and Zecevic, 2010). Water-soluble fluorescent ANEP-based VSDs, like di-1-ANEPPS (JPW3028), di-2-ANEPEQ (JPW1114) or di-4-ANEPPS, are often chosen for intracellular loading and bath-application in cortical slices (Grinvald et al., 1987; Antic et al., 1997; Foust et al., 2011; Palmer and Stuart, 2006; Sinha and Saggau, 1999; Tominaga et al., 2000). However, a series of lipophilic VSDs with significantly higher sensitivity to membrane potential changes has been synthesized (Tsau et al., 1996). The highly hydrophobic characteristics of the new dyes reduce the number of possible applications to mostly retrograde loading through the distal neurites within peripheral ganglia (Grinvald et al., 1987; Vignali et al., 2010; Wenner et al., 1996; Wu et al., 1998). Reported successful examples of loading of hydrophobic dyes by injection or bath application have used moderate concentrations of surfactants (Pluronic F-127 in DMSO) to improve dye solubility (Obaid et al., 1999; Stein et al., 2011) but with a likely adverse effect on normal cell physiology. Bath application of dyes was successfully used in cortical slices for recording the population activity in large networks, but single-cell resolution was unattainable (Peterka et al., 2011) mostly because of small cell size and dye diffusion and internalization that greatly increased the background intensity. As a result intracellular injection remains the only suitable way of dye delivery for optical recording of single cells and cellular compartments in acute brain slices. The loading technique through the patch pipette was successfully used with water soluble VSDs revealing voltage changes even in small neuronal compartments like presynaptic buttons and dendritic spines (Foust et al., 2011; Palmer and Stuart, 2009). To load a cortical neuron with a VSD by conventional protocol (Antic et al., 1999) one needs to first deliver it into the cell through the patch pipette and then to repatch the cell with a dye-free pipette to control the electrical properties of the membrane during optical recording. Adding to the complexity of the method, a successful loading and incubation with di-2-ANEPEQ or its analogs should be performed at room temperature (RT) (Antic et al., 1999). After incubation, the cells are repatched and recorded at RT or lower (Palmer and Stuart, 2006; Volgushev et al., 2011) or the temperature in the slice chamber must be increased to physiological levels (34–36 °C) immediately before repatching and imaging (Foust et al., 2010; Popovic et al., 2011). During the staining procedure, some amount of dye may leak out of the pipette and stain elements near the recorded cell, thus increasing the background fluorescence and masking the optical signals from thin neurites (Zhou et al., 2007). Thus, the technique of dye loading through the pipette has many complications restricting its routine use. Two possible ways were considered to improve the dye delivery to distant neuronal compartments: the use of more soluble ANEP dyes and cyclodextrin polymer rings as carriers of molecules of lipophilic VSDs instead of conventional surfactants (Antic et al., 1999). Dyes with increased solubility have been successfully employed recently (Zhou et al., 2007), but to the best of our knowledge, no experimental evidence has been presented that confirms the new polymer surfactants are more efficient for VSD delivery.

In the present study, we loaded the neurons of rat brain slices with highly sensitive lipophilic VSDs by applying a technique of biolistic delivery. The biolistic delivery was first introduced for transformation of cells with genetic vectors several decades ago (Sanford, 1988), and was recently shown to be applicable for morphological staining of live and fixed tissues with lipophilic carbocyanine and dextran-based dyes (Gan et al., 2000, 2009; Lichtman et al., 2008; Morgan and Kerschensteiner, 2011; Seabold et al., 2010). This technique was also used to deliver calcium sensitive probes in brain slices and mouse brains in vivo (Kettunen et al., 2002). Several recent key modifications of the apparatus of biolistic delivery decreased the harm of pneumatic impact and permitted its use in acute brain slices (O'Brien et al., 2001; Rinberg et al., 2005; Shefi et al., 2006). Using a custom-made device (Aseyev et al., 2012) designed and fabricated by adapting published principles and schemes we tested a range of 4 different ANEP-based VSDs varying the lipophilic properties of the probe to find the best candidate for neuronal staining with biolistic delivery. Here we report the two most lipophilic dyes, di-8-ANEPPS and di-12-ANEPPQ, provide cell-specific loading and Golgi-like staining patterns with minimal background intensity. Simultaneous patch-clamp and optical recordings of stained cells showed excellent optical signal for imaging of both spontaneous APs and stimulus-evoked events in single neurons.

2. Materials and methods

2.1. Preparation of brain slices

All experimental protocols were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Department of Humanitarian Expertise and Bioethics of RAS. Wistar rats (15–21 days old) were deeply anaesthetized with diethyl ether and decapitated. Brains were rapidly removed and placed in ice-cold ACSF. Brain slices (300 μm) were cut using a vibratome VT1200S (Leica, Germany) from the primary visual cortex of right hemisphere or hippocampus. ACSF contained (in mM): 125 NaCl, 25 $NaHCO_3$, 27.5 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$ and 1.5 $MgCl_2$ (all Sigma Ultra graded), pH 7.4 and aerated with 95% O_2, 5% CO_2 (details in Volgushev et al., 2000). The slices were incubated at RT for at least 1 h 30 min before the experiments.

2.2. Patch-clamp recording

Patch pipettes were pulled on a horizontal puller P-97 (Sutter Instruments, Novato, CA, USA) from borosilicate glass (GB 150F-8P, Science Products GmbH, Germany) with a tip resistance of 6–7 MΩ. Patch pipettes were filled with a solution containing (in mM) 132 K-Gluconate, 20 KCl, 4 Mg-ATP, 0.3 Na_2GTP, 10 Na-Phosphocreatine, 10 HEPES, pH 7.25 (all from Sigma, St. Louis, MO, USA). Alexa Fluor 488 hydrazide (100 μM, Invitrogen, Carlsbad, CA, USA) was added to the pipette solution for double labeling of the recorded cells in control experiments. Pre-selection of stained pyramidal neurons for recording was performed using Nomarski DIC optics and infrared videomicroscopy. Membrane potential was recorded in whole-cell current clamp mode with an amplifier ELC-03XS (NPI Electronic GmbH, Germany), low-pass filtered at 3 kHz and sampled at 5 kHz with DigiData 1440A running under the Axoscope 10 software (both from Molecular Devices, Sunnyvale, CA, USA). For precise positioning of the patch pipette, the rig was equipped with a motorized micromanipulator Junior (Luigs and Neumann, Germany) mounted on an air table (Scientifica, UK). The whole rig