Journal of Neuroscience Methods 214 (2013) 105–112

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

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

Basic Neuroscience

A time and cost efficient approach to functional and structural assessment of living neuronal tissue

Abduqodir H. Toychiev[a,b], Bakhodir Sagdullaev[a,b], Christopher W. Yee[a,b], Elena Ivanova[a,b], Botir T. Sagdullaev[a,b,*]

[a] Department of Ophthalmology, Weill Medical College of Cornell University, Burke Medical Research Institute, White Plains, NY 10605, United States
[b] Department of Neurology, Weill Medical College of Cornell University, Burke Medical Research Institute, White Plains, NY 10605, United States

HIGHLIGHTS

- The described technique allows for an affordable and fast comprehensive physiological, anatomical, and immunological characterization of living neuronal tissue.
- Intact tissue is placed on a modified Millicell Biopore insert, which reduces physical damage to the tissue, maintains its structural integrity, and allows easy transfer across multiple setups.
- The approach uses affordable alternatives to common techniques to achieve the same results.

ARTICLE INFO

Article history:
Received 20 November 2012
Received in revised form 16 January 2013
Accepted 18 January 2013

Keywords:
Physiology
Live imaging
Immunohistochemistry
Vasculature
Retina

ABSTRACT

In this manuscript, we describe a protocol for capturing both physiological and structural properties of living neuronal tissue. An essential aspect of this method is its flexibility and fast turnaround time. It is a streamlined process that includes recording of electrophysiological neuronal activity, calcium imaging, and structural analysis. This is accomplished by placing intact tissue on a modified Millicell Biopore insert. The Biopore membrane suspends the tissue in the perfusion solution, allowing for complete access to nutrients, oxygen, and pharmacological agents. The ring that holds the membrane ensures its structural stability; forceps can be used to grip the ring without contacting the filter or the tissue, for easy transfer between multiple setups. We show that tissue readily adheres to the surface of the membrane, its entire surface is visible in transmitted light and accessible for recording and imaging, and remains responsive to physiological stimuli for extended periods of time.

1. Introduction

Advanced techniques for assessing physiology of neuronal cells and their networks have been available for many years. In particular, cellular electrophysiology can be performed with single cell patch clamp recordings, or using planar and 3D multielectrode arrays for multicellular responses (Hamill et al., 1981; Meister et al., 1994; Nicolelis et al., 1997; Charvet et al., 2010). Fluorescent imaging with ion and voltage-sensitive indicators is used to assess the properties of cellular compartments and their networks (Tsien, 1981; Wong and Oakley, 1996; Lohmann et al., 2002; Stosiek et al., 2003).

Because many response parameters are cell-specific, the need to concurrently analyze both functional and structural aspects has become widespread in neuroscience research (Yaksi and Friedrich, 2006). To date, this approach demanded costly customized equipment, procedures, and expertise that are not readily available to many laboratories (Wei et al., 2010). Conventionally, dissected tissue is placed on the glass bottom of a physiological chamber and held in place with an anchored nylon mesh. Alternatively, a tissue sample is pressed down with a filter paper or placed in between two halves of the recording chamber (Newman and Bartosch, 1999; Hu et al., 2000). These approaches have several limitations. Physical pressure can result in structural damage and the tissue may strongly adhere to bottom of the recording chamber, causing damage during transfer. While the latter can be avoided by integrating an imaging unit such as a confocal microscope with the physiological setup, this approach requires costly modifications and prevents the imaging unit from being shared as a standalone workstation (Wei et al., 2010). However, tissue transfer cannot be avoided if performing subsequent immunohistochemical analysis, where preservation of structural integrity is critical. Additionally, with these approaches, only limited parts of the tissue are exposed to

* Corresponding author at: 785 Mamaroneck Ave., White Plains, NY 10605, United States. Tel.: +1 914 368 3167; fax: +1 914 597 2225.
E-mail address: bos2005@med.cornell.edu (B.T. Sagdullaev).

0165-0270/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2013.01.016

the bath solution containing oxygen and nutrients, truncating its viability.

To address these limitations, we describe an affordable solution, using an efficient and flexible protocol, that allows for a comprehensive characterization of neural tissue. To accomplish this goal, we searched for a neutral substrate capable of maintaining the structural and physiological stability of the tissue while providing an opportunity to easily transfer the preparation across multiple experimental setups. Modified Biopore polytetrafluoroethylene (PTFE) membrane inserts fit well for this purpose based on several key features. Due to their superb biocompatibility, Biopore inserts are widely used in medical applications such as growing cell cultures (Falsig et al., 2008; Sawamiphak et al., 2010). Importantly, the porous membrane suspends the tissue in the perfusion solution, allowing easy access to nutrients, oxygen, and when necessary, pharmacological agents. When in solution, the membrane is optically clear, allowing easy observation with common microscope techniques. The ability to present light stimuli using the transmitted light pathway of the microscope without obstructing the preparation is of particular importance to retina and when using optogenetic approach in other tissues.

2. Materials and methods

2.1. Animals

In all experimental procedures, the animals were treated according to the regulations in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, in compliance with protocols approved by the Institutional Animal Care and Use Committee Weill Cornell Medical College, and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Mice homozygous for the Thy1-YFP allele (B6.Cg-Tg(thy1-YFP)/J) and wild type mice (C57BL/6J) were obtained from the Jackson Laboratory (Bar Harbor, ME). For the recordings of light responses, 2-month old mice were used.

2.2. Flat mount retinal preparation

After the animal was euthanized, its eyes were enucleated and placed in bicarbonate-buffered Ames medium continuously equilibrated with O_2/CO_2. The retina was dissected as described in detail elsewhere (Sagdullaev et al., 2011) and attached photoreceptor surface down to a modified hydrophilic Millicell Biopore Insert (Cat no. PICM 01250, Millipore, Bedford, MA). During modification the circular holder portion was cut to the final height of 1 mm (Fig. 1A). To reduce the required volume of bathing solution in the chamber, the feet on the underside of the ring were removed or filed down. The neuronal tissue was mounted on the membrane with gentle suction (Fig. 1B). An empty insulin syringe (1 ml) with flat cut needle holding tip was placed tightly against the opposite from the tissue side of the membrane, then the plunger was pulled back to approximately 0.2–0.3 ml. The adhesion does not require any special treatment, because the tissue is physically enmeshed within the membrane pores. With this procedure, we successfully mounted both whole isolated mouse retinas and dissected quadrants. For larger tissue, a larger syringe may be more appropriate. Individual whole mounts attached to the carrier membrane were then transferred to a recording chamber (Fig. 1C) and bath (1 ml/min) with bicarbonate-buffered Ames medium at near physiological level of 32 °C.

2.3. Electrophysiology

Brief characterization of extracellular spiking activity was obtained prior to breaking into the cell for whole-cell current recordings. Synaptic currents were measured using patch pipettes filled with intracellular solution containing (in mM) 120 Cs-2nate, 10 tetraethylammonium chloride (TEA-Cl), 1.0 $CaCl_2$, 1.0 $MgCl_2$, 11 ethylene glycol-bis (beta-aminoethyl ether)-N,N,N′,N′-tetraacetic

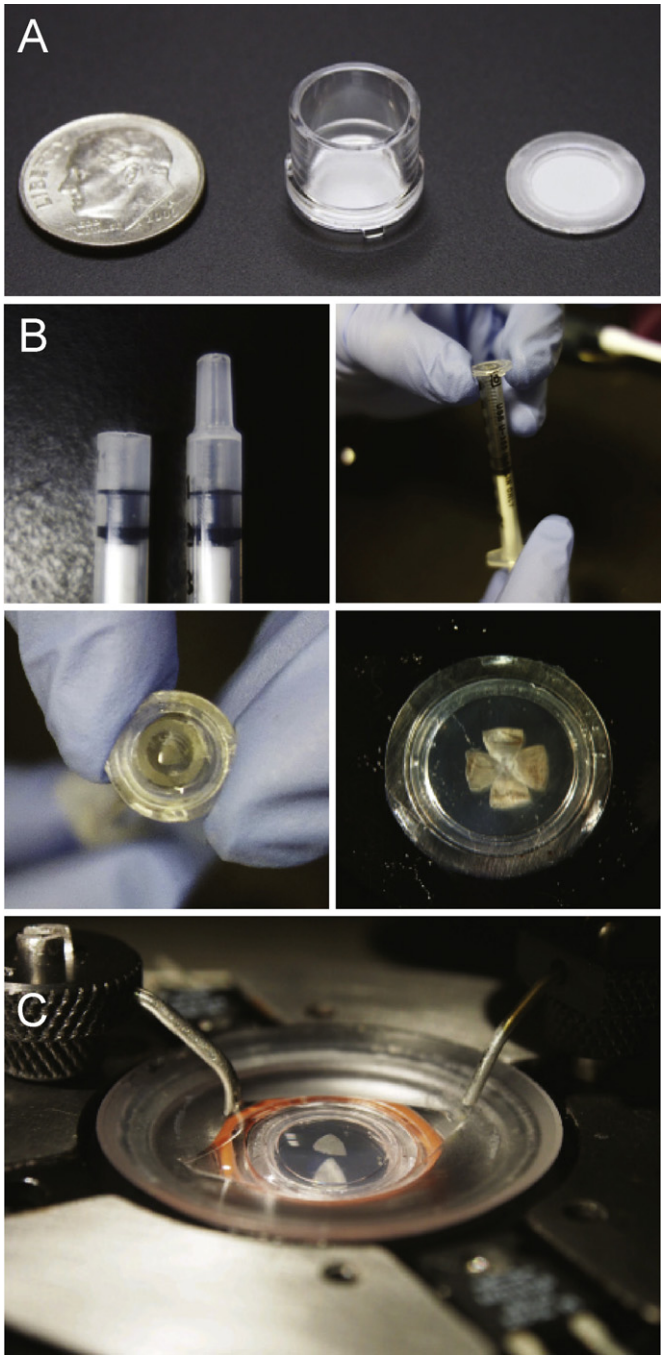

Fig. 1. Application of modified Millicell Biopore culture plate inserts for mounting intact neuronal tissue for physiological and structural assessment. (A) A view of a 12 mm Millicell insert before and after modification. (B) Neuronal tissue/slice is transferred on the top surface of the membrane. Modified insulin syringe is placed on the back of the insert and gentle suction is applied to attach the neuronal tissue to the membrane. In this example, a fraction (*left*) or a whole (*right*) retinal tissue is readily attached to the membrane. The preparation can be manipulated with a pair of forceps by the plastic ring of the insert. Once in physiological media, the hydrophilic Biopore membrane becomes virtually transparent. For larger tissue specimens, a combination of larger Millicell Biopore culture plate inserts with a larger diameter syringe could be used. (C) An insert with the neuronal tissue transferred into the perfused chamber on the microscope stage for patch-clamp recording and calcium imaging.