Journal of Neuroscience Methods 262 (2016) 21–31

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Basic neuroscience

An optimized surgical approach for obtaining stable extracellular single-unit recordings from the cerebellum of head-fixed behaving mice

Joshua J. White, Tao Lin, Amanda M. Brown, Marife Arancillo, Elizabeth P. Lackey, Trace L. Stay, Roy V. Sillitoe*

Department of Pathology and Immunology, Department of Neuroscience, Baylor College of Medicine, Jan and Dan Duncan Neurological Research Institute of Texas Children's Hospital, 1250 Moursund Street, Suite 1325, Houston, TX 77030, USA

HIGHLIGHTS

- We describe a surgery that allows for routine isolation of single units in behaving mice.
- The high quality of the single units permits the analysis of action potential waveforms.
- The excellent wound healing of the craniotomy allows us to record over several weeks.
- The setup is stable enough for recording individual neurons in severely tremulous mice.

ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form
16 December 2015
Accepted 6 January 2016
Available online 14 January 2016

Keywords:
Electrophysiology
Action potential
Surgery
Behavior
Cerebellum
Tremor

ABSTRACT

Background: Electrophysiological recording approaches are essential for understanding brain function. Among these approaches are various methods of performing single-unit recordings. However, a major hurdle to overcome when recording single units *in vivo* is stability. Poor stability results in a low signal-to-noise ratio, which makes it challenging to isolate neuronal signals. Proper isolation is needed for differentiating a signal from neighboring cells or the noise inherent to electrophysiology. Insufficient isolation makes it impossible to analyze full action potential waveforms. A common source of instability is an inadequate surgery. Problems during surgery cause blood loss, tissue damage and poor healing of the surrounding tissue, limited access to the target brain region, and, importantly, unreliable fixation points for holding the mouse's head.
New method: We describe an optimized surgical procedure that ensures limited tissue damage and delineate a method for implanting head plates to hold the animal firmly in place.
Results: Using the cerebellum as a model, we implement an extracellular recording technique to acquire single units from Purkinje cells and cerebellar nuclear neurons in behaving mice. We validate the stability of our method by holding single units after injecting the powerful tremorgenic drug harmaline. We performed multiple structural analyses after recording.
Comparison with existing methods: Our approach is ideal for studying neuronal function in active mice and valuable for recording single-neuron activity when considerable motion is unavoidable.
Conclusions: The surgical principles we present for accessing the cerebellum can be easily adapted to examine the function of neurons in other brain regions.

1. Introduction

There are now many methods available for studying brain function. Among these are various approaches for recording neurons *in vivo* (Chorev et al., 2009; Gilja et al., 2010; Long and Lee, 2012). The signals may be collected from populations of neurons using electrode arrays (Buzsaki, 2004; Khodagholy et al., 2015), tetrodes (Gao et al., 2012; Chaumont et al., 2013; Sauerbrei et al., 2015), or single electrodes to collect either local field potentials (LFPs; Miller and Wilson, 2008; Servais and Cheron, 2005) or action potentials from single cells (Bryant et al., 2009; White et al., 2014). For recording from individual neurons, signals can be acquired by using whole-cell techniques in which the electrode penetrates the cell to measure the internal milieu and cell membrane properties (Long

* Corresponding author. Tel.: +1 832 824 8913; fax: +1 832 825 1251.
E-mail address: sillitoe@bcm.edu (R.V. Sillitoe).

http://dx.doi.org/10.1016/j.jneumeth.2016.01.010
0165-0270/

and Lee, 2012), or the electrode can be placed in very close proximity to a cell of interest to record extracellular signals (Arancillo et al., 2015). In either case, the preparation has to be very stable in order to hold the cell for a sufficient length of time so that an adequate number of spikes can be collected for statistical analyses. Since limiting the animal's movements and eliminating vibrations are major hurdles to overcome, stability is a particular challenge in acute recordings in behaving animals.

One of the most common sources of stability problems is an inadequate surgery. Low-quality surgeries can cause excessive blood loss, hypothermia (especially in smaller animals that lose body heat rapidly), major tissue damage at the surgical site resulting in limited healing of the surrounding tissues, and bone fragments that lacerate the brain due to poor bone drilling technique. These bone fragments, along with other tissue debris and excessive bleeding, also obscure visibility when trying to make a craniotomy of precise size and location. Ultimately, these issues make it very difficult to firmly implant head plates, which are essential for stabilizing the animal during the recording. If the animal is not stable, the signal-to-noise ratio becomes too low, single units become difficult to isolate (especially in brain regions that have a high density of neurons), and, even if spike sorting is possible, the single-unit activity (Wise et al., 2010; Viskontas et al., 2007; Cazakoff et al., 2014; Nakamura et al., 2014; Zhou et al., 2014) cannot be analyzed further as it is often hard to see the full action potential waveform (White et al., 2014). The exact shape of a waveform is important for accurately differentiating individual units and in some regions also for separating excitatory and inhibitory neurons. In this paper, we present a surgical procedure that overcomes many of these problems. We introduce a method for making clean craniotomies and describe how to firmly implant head plates in order to acquire stable extracellular single-unit recordings *in vivo* in behaving mice.

We use the cerebellum as a model to demonstrate the efficacy of our surgery and implantation techniques for recording neurons *in vivo*. The cerebellum is essential for diverse functions, including motor coordination and learning, posture, and balance. Therefore, damage to its circuits causes a number of motor disorders, such as ataxia, dystonia, and tremor (Orr, 2012; LeDoux and Lorden, 2002; Wilson and Hess, 2013; Louis et al., 2011). Cerebellar function in both normal and abnormal states has been intensely studied. As a result, its anatomy, circuitry, and neuronal firing properties are among the best understood in the brain (Ruigrok et al., 2011). We delineate how to obtain cerebellar recordings in moving mice (Fig. 1) and demonstrate the stability of our approach by injecting the tremorgenic drug harmaline (Handforth, 2012). Harmaline induces tremor within minutes after injection. Despite the continuous shaking of the animal during tremor, we were able to isolate clean single units from Purkinje cells with recognizable, quantifiable complex spike and simple spike action potential waveforms.

2. Materials and methods

2.1. Animals

C57BL6/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained in our animal colony. We bred the mice using timed pregnancies, and we designated noon on the day a vaginal plug was detected as embryonic day (E) 0.5. Some mice were mixed-background controls from previous breeding (White et al., 2014). Mice of both sexes were studied at postnatal day (P) 60 or beyond. All animal studies were carried out under an approved IACUC animal protocol according to the institutional guidelines at Baylor College of Medicine.

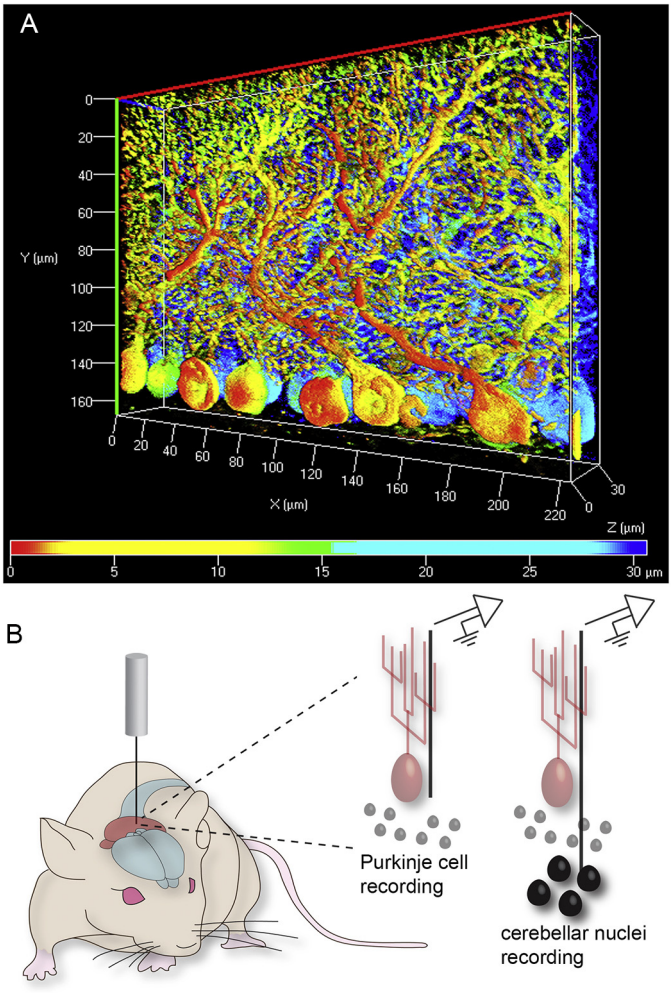

Fig. 1. (A) Purkinje cells stained with an anti-CAR8 antibody plus an anti-IP3R1 antibody demonstrating the cellular density and structural complexity of the cerebellum. In this 3D reconstruction, the red pseudocolor indicates the structures that are closest to the surface whereas the blue reveals deeper structures. Extracellular recoding electrodes have to traverse these structures in order for a single-unit to be isolated, typically with the electrode tip near the soma. (B) Schematic of an electrode targeting the cerebellum for *in vivo* recordings in an alert adult mouse. The schematics on the right illustrate the basic architecture of the cerebellum with the electrode targeting either the Purkinje cells (left, pink) or the cerebellar nuclear neurons (right, black). The granule cells are gray. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Perfusion and tissue preparation

Mice were deeply anesthetized with 2,2,2-tribromoethanol (commonly known as Avertin) and then perfused with 0.1 M phosphate-buffered saline (PBS; pH 7.4), followed by 4% paraformaldehyde (4% PFA) diluted in PBS. The brains were post-fixed for 24 to 48 h in 4% PFA and then cryoprotected stepwise in buffered sucrose solutions (15% and 30% diluted in PBS). Serial 40-μm thick coronal or sagittal sections were cut on a cryostat and collected as free-floating sections in PBS. Some brains were embedded in paraffin and cut on a microtome at 10 μm. The staining was conducted directly on electrostatically coated slides (Sillitoe et al., 2008).

2.3. Tissue staining and histology

Immunohistochemistry was conducted on frozen sections as previously described (Sillitoe et al., 2003, 2010; White and Sillitoe,