In vivo visualization and functional characterization of primary somatic neurons

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ARTICLE INFO

ABSTRACT

In vivo electrophysiological recordings from cell bodies of primary sensory neurons are used to determine sensory function but are commonly performed blindly and without access to voltage- (patch-clamp) electrophysiology or optical imaging. We present a procedure to visualize and patch-clamp the neuronal cell body in the dorsal root ganglion, in vivo, manipulate its chemical environment, determine its receptive field properties, and remove it either to obtain subsequent molecular analyses or to gain access to deeper lying cells. This method allows the association of the peripheral transduction capacities of a sensory neuron with the biophysical and chemical characteristics of its cell body.

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1. Introduction

Peripheral somatic sensory neurons are functionally heterogeneous, encompassing both nociceptive and non-nociceptive types which transduce a variety of stimuli from touch to temperature. An understanding of the mechanistic basis of sensory transduction requires the correlation of ion channel expression and other molecular processes with sensory modality. However, current methodologies have limited ability to achieve this aim. Dissociation of peripheral sensory neurons allows patch-clamp recording for measurement of receptor- and ion-channel activity as well as optical imaging, and the harvesting of single neurons for gene expression analysis. But dissociation eliminates the peripheral field resulting in an inability to test sensory modality or the response of the peripheral field to chemical agents. The neuronal cell body (soma) is unlikely to have exactly the same molecular properties as those of the terminal endings (Zimmermann et al., 2009). In addition, dissociation, by itself, alters the excitability, intracellular signaling and gene expression of neuronal somata (Ma and LaMotte, 2005; Zheng et al., 2007; Zimmermann et al., 2009). Alternatively, sharp electrode recordings from intact ganglia retain peripheral properties (Koerber et al., 1988; Lawson et al., 1997) but offer limited ability for voltage control or manipulation/harvesting of an identified neuron since it is not visualized and its membrane is tightly bound to other cells. The cells are also not accessible for opti-

cal imaging or patch-clamp recording of ion channels and isolated currents.

We provide a procedure to apply the advantages of the in vitro methods in vivo. Using a physiological preparation designed for adult mice and rats, we describe how to (a) visualize the neuronal soma in vivo, (b) control/manipulate its external chemical environment, (c) determine its receptive field properties without damage, (d) image its ionic activities and those of its non-neuronal neighboring cells, (e) make it accessible to patch-clamp electrophysiological recording, and/or (f) remove it for the purpose of subsequent molecular analyses and to gain access to deeper lying cells.

## 2. Materials and methods

2.1. Animals

Adult female Sprague–Dawley rats weighing 180–250 g ($n = 34$) and male CD1 mice weighting 35–40 g ($n = 28$) or C57BL/6 mice weighing 25–30 g ($n = 2$) were purchased from Charles River Laboratories (Wilmington, MA, USA). Groups of three or four animals were housed together in a climate-controlled room under a 12 h light/dark cycle. The use and handling of animals were in accordance with guidelines provided by the National Institutes of Health and the International Association for the Study of Pain and received approval from the Institutional Animal Care and Use Committee of the Yale University School of Medicine.

2.2. In vivo physiological preparation

A surgical procedure for the rat (Ma and LaMotte, 2007) was adapted to the mouse by reducing the size of the ring, vertebral

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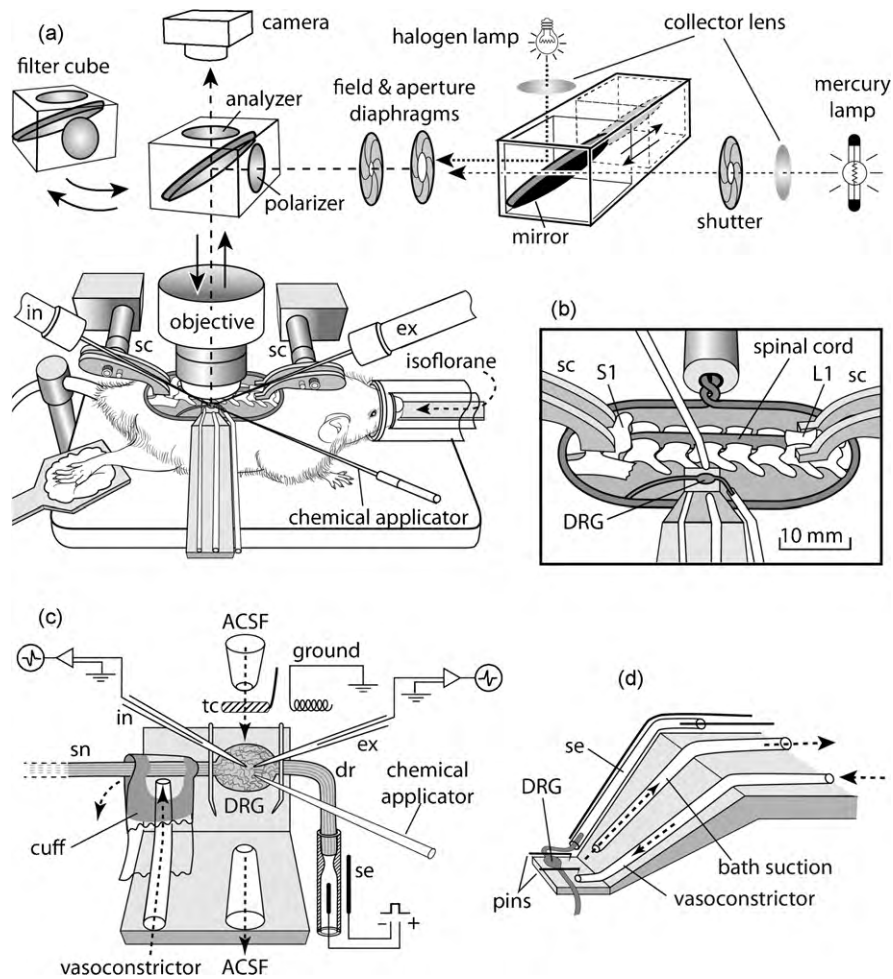
Fig. 1. Physiological preparation shown for the mouse. (a) After laminectomy, the skin was sutured to a ring and the animal rested on a plate attached to which were two spinal vertebrate clamps (sc) on the rostral (L1) and caudal (S1) region. As shown in the enlarged view (b), the L3 (or L4) DRG on the right side was exposed, pinned to a small platform beneath, and superfused with oxygenated ACSF. The surface of the DRG was viewed through a 40× water immersion objective using either reflection microscopy in bright-field via an analyzer-50/50 mirror-polarizer set and halogen lamp (a) or epifluorescence (after switching to a filter cube and mercury lamp). As further enlarged in (c), a cuff around the spinal nerve (sn) delivered a vasoconstrictor via a tube to block the blood flow in the DRG. The DRG was held by two insect pins inserted into a thin layer of silicon rubber attached to the platform. The dorsal root (dr) was transected and its distal end attached to a suction electrode (se). Intracellular (in) and extracellular (ex) electrophysiological recording electrodes and a topical chemical applicator are shown. The vasoconstrictor was applied via a cuff around the nerve to block the blood flow. tc: thermocouple. (d) Oblique view of the platform and perfusion tubing. A similar but larger recording scaffold was used for recordings from rats.

clamps and platform for the DRG (Fig. 1a). Rats and mice were anesthetized, respectively, with pentobarbital sodium (Nembutal, 50 mg/kg i.p. initial dose and 20 mg/kg i.p. supplemental dose) and isoflurane inhalation (1–2% via a nose cone). After a laminectomy at the levels of L2–L6, the L3 or L4 DRG with the corresponding spinal nerve and dorsal root were exposed and isolated from the surrounding tissue. Oxygenated ACSF (Ma et al., 2003) was dripped periodically on to the surface of the ganglia to prevent drying and hypoxia. Two lumbar vertebrae (L1 and S1) were clamped to posts attached to a "base plate" that held the animal (Fig. 1a and b). The skin was sewn to a ring (fixed to a post on the base plate) to hold a pool of warm, oxygenated, artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM): 130 NaCl, 3.5 KCl, 24 NaHCO$_3$, 1.25 NaH$_2$PO4, 1.2 MgCl$_2$, 1.2 CaCl$_2$, and 10 dextrose. The solution was bubbled with 95% O$_2$ and 5% CO$_2$ and had a pH of 7.4 and an osmolarity of 290–310 Osm. A vasoconstricting drug, [Arg^8]-vasopressin (10 μM in oxygenated ACSF, Sigma–Aldrich, St. Louis, MO, USA) was applied via a syringe attached to a soft cuff wrapped around the spinal nerve just distal to the DRG and sealed at each end with Vaseline (Fig. 1c). The vasoconstrictor stopped or substantially reduced the blood flow to the capillary bed in the DRG – thereby preventing the bleeding that otherwise would occur after the sub-

sequent application of collagenase. A successful blockade of blood flow was achieved in 91% (31/34) of rats and 97% (29/30) of mice. The epineurium covering the DRG was removed under a dissection microscope. Then, the base plate with the animal was transferred and mounted to a frame under the objective of an upright, light microscope (BX51WI, Olympus Optical, Tokyo, Japan) on a pneumatic vibration isolation table. The dorsal root was cut and the DRG lifted onto a small platform that was fixed to the base plate. The DRG was continuously superfused at a rate of 3 ml/min by the oxygenated ACSF that was preheated by an in-line heater (TC-344A, Warner Instrument, Hamden, CT, USA) to achieve a desired temperature of 37 °C (via feedback from a thermocouple near the ganglion) (Fig. 1b and d). Suction was used to maintain the level of fluid in the pool. The proximal end of the dorsal root was brought into a suction electrode to electrically stimulate the axon of a recorded neuron and determine its axonal conduction velocity (Fig. 1c). The DRG was viewed through a 40× water immersion objective (magnification of 400×) using reflective microscopy via an analyzer-50/50 mirror-polarizer set (as shown in Fig. 1a) (Ma and LaMotte, 2007). A light source for recording of epifluorescence was delivered from above in a conventional way via appropriate filter sets. A pipette to deliver chemicals (via an 8-channel pressurized chemical delivery system,