Journal of Neuroscience Methods 180 (2009) 1–8

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Imaging synaptically mediated responses produced by brainstem inputs onto identified spinal neurons in the neonatal mouse

Karolina Szokol, Marie-Claude Perreault*

University of Oslo, Institute of Basic Medical Sciences (Domus Medica), Department of Physiology, N-0317 Oslo, Norway

ARTICLE INFO

Article history:
Received 6 November 2008
Received in revised form 21 January 2009
Accepted 22 January 2009

Keywords:
Motor control
Descending pathways
Reticulospinal
Spinal cord
Motoneurons
Interneurons
Optical recording
Calcium

ABSTRACT

Descending inputs to spinal cord neurons in mammals have previously been characterized functionally using microelectrode recording of single neurons, a technique with high spatial and temporal resolution but low yield. Consequently our knowledge about the functional connections between the brain and the spinal cord has been accumulating at a very low pace. Here we describe a high throughput optical recording approach in an ex vivo brainstem–spinal cord preparation of the neonatal mouse that permits screening many spinal neurons simultaneously for synaptic inputs from descending axons.

The fluorescent calcium indicator calcium green dextran amine was loaded retrogradely into specific spinal neuron populations, including motoneurons (MNs) of the medial and lateral motor columns and two populations of interneurons with descending axons (dINs) in the ventral funiculus. Focal electrical stimulation of brainstem neuron populations with descending axons generated synaptic responses revealed by transient increases in intracellular calcium concentration in all four populations of spinal neurons. The resultant fluorescence signals could be readily visualized in individual MNs directly through the ventral white matter. In the more deeply located dINs, responses could be readily visualized in individual neurons from the surface of an oblique cut through the spinal cord.

The rapid optical investigation of functional connections between brainstem descending neurons and various populations of spinal neurons in the living mammalian preparation should help uncover some of the key features of supraspinal sensory and motor control and provide a valuable tool for examining the re-innervation of spinal neurons by descending axons after spinal cord regeneration.

1. Introduction

Optical recording techniques permit a more rapid and comprehensive characterization of synaptic connections than electrophysiological recording techniques because activity can be monitored in many neurons simultaneously. Fluorescent calcium probes have been used successfully to detect calcium influxes in spinal neurons during evoked or spontaneous activity in lower vertebrates like the lamprey and the zebrafish (McClellan et al., 1994; Viana di Prisco and Alford, 2004; Fetcho et al., 2008) and in the isolated spinal cord of the chicken embryo and the neonatal mouse (Wilson et al., 2007; O'Donovan et al., 2008). However, the approach has only recently been used to map connections between supraspinal neurons and spinal neurons (Szokol et al., 2008).

Descending pathways play a crucial role in the control of spinal cord functions in mammals (Millan, 2002; Lemon, 2008, for reviews). Yet little is known about the functional connections between supraspinal neurons and neurons in the spinal cord. One reason is that descending inputs onto spinal cord neurons have been investigated by electrical recording from one or a few spinal neurons at a time. Assessing connectivity patterns between descending inputs and specific spinal neuron groups in this way has been laborious. Nearly all available information derives from in vivo experiments in anesthetized intact cats and unanesthetized decerebrated cats and pertains mostly to connections between descending neurons and MNs of the lateral motor column (LMC). Descending connections to MNs of the medial motor column (MMC) (Sasaki, 1999; Peterson et al., 1979; Fukushima et al., 1979) or to spinal INs (Jankowska, 2008; Noga et al., 1995; Davies and Edgley, 1994; Harrison and Jankowska, 1984) have been explored to a much lesser degree.

The goal of the present study was to investigate whether it was feasible to optically record synaptic responses evoked by descending axonal projections to both MMC and LMC MNs and to spinal INs in a living mammalian brainstem–spinal cord preparation. To achieve this goal, we chose as an experimental model the brainstem–spinal cord preparation of the neonatal mouse. A similar preparation from the neonatal rat introduced about 25 years

* Corresponding author at: University of Oslo, Institute of Basic Medical Sciences, Department of Physiology, Sognsvannsveien 9, PB 1103 Blindern, N-0317 Oslo, Norway. Tel.: +47 22 85 12 90; fax: +47 22 85 12 49.
E-mail address: m.c.perreault@basalmed.uio.no (M.-C. Perreault).

0165-0270/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2009.01.018

ago (Suzue, 1984) has become an excellent model for investigating the mechanisms involved in the control of motor rhythms such as respiration (Feldman and Del Negro, 2006) and locomotion (Jordan et al., 2008). The development of a brainstem–spinal cord preparation from the mouse not only extends these capabilities but also gives access to newly available tools from the fields of genetics and optogenetics.

We describe two brainstem–spinal cord preparations for the recording of optical signals generated by descending inputs onto ventral horn MNs and INs, respectively. To exemplify the utility of these preparations, we examined the functional connections in reticulospinal pathways originating from the medullary reticular formation. However, the same preparations could be used to investigate functional connections in other descending pathways onto other types of spinal neurons such as dorsal horn sensory interneurons and sympathetic preganglionic neurons.

Our optical recordings were performed using an electron multiplying video camera and standard wide-field epi-fluorescence microscopy. Recently, two-photon laser scanning microscopy (2p-LSM) and calcium imaging has been combined successfully to image the activity of identified neuronal populations in the isolated mammalian spinal cord (O'Donovan et al., 2005; Wilson et al., 2007). However, compared to single-photon microscopy, the instrumentation required for 2p-LSM may still be too costly for many laboratories and may not provide the wide-field excitation and rapid image acquisition necessary to investigate spatially extended neuronal networks (see however Lillis et al., 2008). For explorative studies of brain–spinal cord connections, for instance, it is an advantage to be able in a single experiment to scan rapidly as many spinal cord segments as possible, i.e., an area that may easily extend over tens of millimeters. Fast calcium imaging of neuronal populations using "scanless" two-photon microscopy (Nikolenko et al., 2008) may be a promising avenue but in the immediate future this option is likely to remain cost-prohibitive for the great majority of laboratories. We provide here the details of a procedure to study long-range connections between the brain and the neurons located throughout the spinal cord that is simple, straight forward and inexpensive and which could be implemented easily in many laboratories without delays.

2. Materials and methods

2.1. Animals

Experiments were performed on 20 wild-type ICR neonatal mice (postnatal day (P) 0–P4) obtained from Harlan, France. All efforts were made to minimize the number of animals used and their suffering in accordance with the European Communities Council directive 86/609/EEC and the National Institutes of Health guidelines for the care and use of animals. All procedures were approved by the National Animal Research Authority in Norway.

2.2. Brainstem–spinal cord preparations and retrograde loading of calcium indicator

After deep anaesthesia with isoflurane, animals were decerebrated by transecting the brain just rostral to the superior colliculus and eviscerated. To expose the brainstem and the spinal cord, both a craniotomy and a laminectomy were performed. The brainstem and spinal cord (including down to at least the S2 segment) with the dorsal and ventral roots attached were then gently dissected out in ice-cold (1–5 °C), oxygenated (95% O_2 and 5% CO_2) glycerol-based dissecting solution containing in mM: glycerol 250, KCl 2, D-glucose 11, $CaCl_2$ 0.15, $MgSO_4$ 2, NaH_2PO_4 1.2, HEPES 5, and $NaHCO_3$ 25. We then proceeded with the retrograde loading of spinal MNs and INs with the fluorescent calcium indicator, calcium green-1 (CG-1) conjugated dextran amine (CGDA; 3000 MW, Molecular Probes). All spinal neuron groups investigated in this study (L2 and L5 MMC and LMC MNs, L2 ipsilateral and contralateral descending INs (dIINs and dCINS)) were labelled in the same way, by applying CGDA crystals to their transected axons using tungsten or stainless steel pins (Glover, 1995). For the MNs, the axons were cut close to the ventral root exit from the spinal cord (Fig. 1A) to minimize the labelling time. For the L2 INs, the axons were severed by cutting the ventral funiculus unilaterally between the L3 and L4 ventral roots (Fig. 2A). Retrograde labelling continued in the dark at room temperature (23–25 °C) for 3 h (MNs) or 5 h (INs) after replacing the dissecting solution by oxygenated artificial cerebrospinal fluid (ACSF) containing in mM: NaCl 128, KCl 3, D-glucose 11 $CaCl_2$ 2.5, $MgSO_4$ 1, NaH_2PO_4 1.2, HEPES 5,

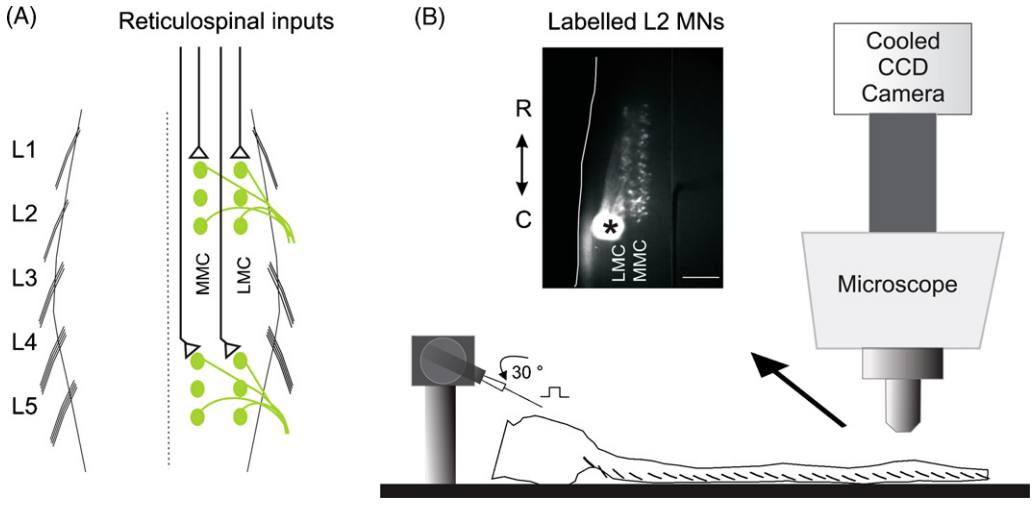

Fig. 1. Brainstem–spinal cord preparation to study descending inputs to spinal MNs. (A) Diagram of putative functional connections between reticulospinal neurons with axons descending ipsilaterally and MNs in the MMC and LMC of the L2 and L5 segments. The four groups of MNs were labelled by applying CGDA to the cut L2 and L5 ventral roots. The connections may represent both mono- and polysynaptic connections, include both excitatory and inhibitory components and arise from the same or different reticulospinal axons. (B) Schematic of the experimental arrangement for optical recording of Ca^{2+} transients in spinal MNs during focal electrical stimulation of the brainstem. The recording chamber (not shown) where the preparation is positioned ventral side up is set on a custom-built plate (130 mm × 320 mm, shown in black) screwed on the XY specimen stage of the microscope. This plate accommodates both the recording chamber and the XYZ micromanipulator holding the stimulating electrode. The electrode is aligned parallel to the ventral midline and inserted in the brainstem with an angle of 30° from the horizontal with the tip pointing caudally. Inset: microphotograph of CGDA labelled MNs in the MMC and the LMC of the L2 segment showing that the MNs are readily visualized through the ventral white matter. Asterisk indicates the location of the VR stump that remained after cutting and labelling the MN axons. Scale bar is 100 μm.