SPINAL NEURONS BURSTING IN PHASE WITH FICTIVE SCRATCHING ARE NOT RELATED TO SPONTANEOUS CORD DORSUM POTENTIALS

C. A. CUELLAR, [a] A. TREJO, [b] P. LINARES, [b]
R. DELGADO-LEZAMA, [a] I. JIMÉNEZ-ESTRADA, [a]
L. M. ABYAZOVA, [c] T. V. BALTINA [c] AND E. MANJARREZ [b*]

[a] *Departamento de Fisiología, Biofísica y Neurociencias CINVESTAV, Mexico*

[b] *Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, 14 Sur 6301, Colonia San Manuel, Apartado Postal 406, CP 72570 Puebla, Pue., Mexico*

[c] *Institute of Basic Medicine and Biology, Kazan (Volga Region) Federal University, Kremlevskaya Street 18, 420008 Kazan, Russia*

Abstract—Spontaneous cord dorsum potentials (spontaneous CDPs) are produced by the activation of dorsal horn neurons distributed along the L4 to S1 spinal cord segments, in Rexed's laminae III–VI, in the same region in which there are interneurons rhythmically bursting during fictive scratching in cats. An interesting observation is that spontaneous CDPs are not rhythmically superimposed on the sinusoidal CDPs generated during fictive scratching episodes, thus suggesting that the interneurons producing both types of CDPs belong to different spinal circuits. In order to provide experimental data to support this hypothesis, we recorded unitary activity of neurons in the L6 spinal cord segment. We found that the neurons firing rhythmically during the sinusoidal CDPs associated with the extensor, flexor or intermediate phases of scratching were not synchronized with the spontaneous CDPs. Moreover, we found that the neurons firing during the spontaneous CDPs were not synchronized with the sinusoidal CDPs. These results suggest that the neurons involved in the occurrence of spontaneous CDPs are not part of the spinal cord central pattern generators (CPGs). This study will be relevant for understanding the relationships between the spinal cord neuronal populations firing spontaneously and the CPGs, in the intact and injured spinal cord. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: CDP, spontaneous, CPG, central pattern generator, dorsal horn neurons, scratching.

*Corresponding author. Tel: +52-22-22-29-5500x7326; fax: +52-22-22-33-4511.

E-mail address: eliasmanjarrez@gmail.com (E. Manjarrez).

INTRODUCTION

Neuronal ensembles in the spinal cord accomplish many physiological roles. The study of the interaction between these neuronal networks allows an integral understanding of the complexity of diverse processes, such as during the generation of stereotyped movements and their modulation by other neighboring neuronal groups. One of these populations of neurons is associated with the spontaneous electrical potentials recorded in the dorsum of the lumbar and sacral spinal cord segments (spontaneous cord dorsum potentials (CDPs); Bremer, 1941; Ten Cate, 1950; Mark and Gasteiger, 1953). Large-amplitude spontaneous CDPs are generated by the synchronous activation of a population of dorsal horn neurons (Rexed's laminae III–VI) that respond monosynaptically to low-threshold cutaneous afferents (Manjarrez et al., 2000). In the anaesthetized cat, Manjarrez et al. (2003) reported that the spontaneous CDPs, lasting 40–60 ms, have characteristically low-frequency components (3–20 Hz) and suggested that such potentials are longitudinally synchronized throughout several lumbosacral spinal segments (see also Manjarrez et al., 2002a,b,c, 2005). Moreover, García et al. (2004) showed that the synchronization between the spontaneous CDPs recorded from neighboring segments was reduced after interposed spinal lesions. Recently, Chávez et al. (2012) reported the occurrence of spontaneous negative cord dorsum potentials (nCDPs) and negative–positive cord dorsum potentials (npCDPs) and analyzed the correlation of both potentials between the L5–L7 spinal segments in the anaesthetized cat. According to this study, under conditions of low synchronization, a set of neurons would generate the spontaneous CDPs and activate the class I interneurons mediating the non-reciprocal Ib inhibition. Otherwise, increased synchronization of the spontaneous activity would produce the spontaneous npCDPs by recruiting an additional set of interneurons which are included in the neuronal pathways producing PAD of cutaneous afferents in the dorsal horn and of muscle afferents, possibly via class II interneurons in the intermediate zone (see Rudomin et al., 1987; Rudomin, 1990). However, although these studies suggest the concomitant participation of cutaneous and Ib neurons during the generation of spontaneous CDPs, it is important to consider recent studies that indicate that

"Ib interneurons" may belong to a population of cells that also have Ia input and, in some cases, group II input (Jankowska and Edgley, 2010); such cells may be multifunctional but modulated in a task-specific manner.

Furthermore, while the neural elements underlying the generation of spontaneous activity are still unknown, Manjarrez et al. (2000, 2002c, 2003) described that these neurons are located within the dorsal horn in Rexed's laminae III and VI, mostly in the spinal cord segments L5 and L6. Moreover, it is still unknown the extent to which the neurons involved in the generation of the spontaneous CDPs and npCDPs are rhythmically active during stereotyped motor patterns (i.e. scratching or locomotion).

Stereotyped motor patterns are produced by spinal networks known as central pattern generators (CPGs) (see Grillner, 1981; Whelan, 1996; Kiehn and Kjaerulff, 1998; Orlovsky et al., 1999; Kiehn, 2006, for reviewing important aspects of the CPGs). Since the seminal studies by Sherrington (1906, 1910), scratching has been a valuable motor pattern to study the organization of the spinal circuitry responsible for generating rhythmic tasks, hence the CPGs. The motor output during scratching in the cat is produced by interneurons located in the hindlimb enlargement of the spinal cord (Berkinblit et al., 1978a,b;Baev et al., 1981; Deliagina et al., 1983; Cuellar et al., 2009; Pérez et al., 2009).

Bayev and Kostyuk (1981) showed that during scratching, the cat spinal cord exhibits sinusoidal cord dorsum potentials (sinusoidal CDPs) after a tonic CDP. Cuellar et al. (2009) and Pérez et al. (2009) described electrophysiological evidence of the phenomenon of traveling electrical waves produced by populations of interneurons within the spinal cord. These authors showed that during fictive scratching, an electrical field potential produced by the sequential activation of spinal interneurons takes the shape of a sinuous wave, "sweeping" the lumbosacral spinal cord rostro-caudally, from L4 to S1. Thus, it was assumed that sinusoidal CDPs during fictive scratching represent the activity of the CPG itself, as well as the activation of its "outputs" or "followers". However, an interesting observation from the Cuellar et al. (2009) and Kato et al. (2013) reports was that spontaneous CDPs are not rhythmically superimposed on the sinusoidal CDPs generated during a fictive scratching episode, thus suggesting that the interneurons producing both types of CDPs, spontaneous and sinusoidal, belong to different spinal circuits (Kato et al., 2013). In order to provide more experimental data to support this hypothesis, the firing activity of single interneurons located mainly in the dorsal horn and intermediate nucleus (Rexed's laminae III–VII) was analyzed within the L6 spinal segment in decerebrate cats. Firing profile was associated with either the spontaneous CDPs or sinusoidal CDPs during the rhythmic scratching phases: flexor, intermediate or extensor. To our knowledge, there are no studies addressing functional significance of the dorsal horn spontaneous spinal cord activity related to rhythmic motor patterns in adult mammals. Therefore, this study is relevant for understanding hindlimb spinal cord circuits, and the interaction between neuronal populations.

EXPERIMENTAL PROCEDURES

Surgical procedures

The methods for surgical procedures were previously described (Cuellar et al., 2009) and the guidelines contained in the Mexican Norm for Care and Use of Animals for Scientific Purposes, NOM-062-ZOO-1999 (SAGARPA), were strictly followed.

Briefly, 10 adult cats (2.0–3.5 kg) were anesthetized with isoflurane (2%) in a mixture with oxygen (98%). Arterial blood pressure was monitored by a cannula inserted in the carotid artery. Bicarbonate (100 mM) and glucose (5%) solutions were administered throughout the experiment. Dextran solution was administered when blood pressure was below 80-mm Hg. Following a laminectomy in the L4–S1 and C1–C2 spinal segments, the dura mater was removed. Several bilateral hindlimb muscles nerves were dissected free and prepared for recording. Specifically, the flexor tibialis anterior (TA), the extensors lateral gastrocnemius plus soleus (LGS) and medial gastrocnemius (MG) electroneurographic (ENG) recordings were selected. The brain was exposed by craniotomy, after which, a precollicular-postmammillary decerebration was performed, removing all rostral brain tissue. After this procedure, the anesthesia was discontinued and the animals were paralyzed with pancuronium bromide (Pavulon; Organon) and artificially ventilated. The temperature of the animals was maintained close to 37 °C by a heating pad and radiant heat lamp.

Electrophysiological recordings

Fictive scratching was induced ipsilaterally by tactile stimulation of the pinna or other scratch receptive fields (behind the ear or around the neck), after the application of a piece of cotton soaked with D-tubocurarine (0.1%, 14 mM) on the dorsal surface of the C1–C2 segments (Feldberg and Fleichhauer, 1960). Spinal cord electrical activity was monopolarly recorded in direct (DC) or alternating current (AC) mode on a Synamps EEG amplifier (NeuroScan). A multielectrode array composed of 30 Ag–AgCl electrodes (200-μm diameter) was positioned on the dorsal surface of the lumbosacral (L4–S1) spinal cord segments (Fig. 1A) against an indifferent electrode placed on the paravertebral muscles (for further details see Manjarrez et al., 2005; Cuellar et al., 2009). After multielectrode recordings, topographic maps (Scan 4.2 software, NeuroScan) were created in order to identify the region in which the maximal spontaneous CDPs and sinusoidal CDPs occurred. Micropipette insertions were then performed in that region. In addition, medial columns of the multielectrode array (9–16 and 17–24) were selected in order to graph the amplitude of the spontaneous CDPs and sinusoidal CDPs versus the electrode position (data not shown).