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Research Report

Activation of spinal locomotor circuits in the decerebrated cat by spinal epidural and/or intraspinal electrical stimulation

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ABSTRACT

The present study was designed to further compare the stepping-like movements generated via epidural (ES) and/or intraspinal (IS) stimulation. We examined the ability to generate stepping-like movements in response to ES and/or IS of spinal lumbar segments L1–L7 in decerebrate cats. ES (5–10 Hz) of the dorsal surface of the spinal cord at L3–L7 induced hindlimb stepping-like movements on a moving treadmill belt, but with no rhythmic activity in the forelimbs. IS (60 Hz) of the dorsolateral funiculus at L1–L3 (depth of 0.5–1.0 mm from the dorsal surface of the spinal cord) induced quadrupedal stepping-like movements. Forelimb movements appeared first, followed by stepping-like movements in the hindlimbs. ES and IS simultaneously enhanced the rhythmic performance of the hindlimbs more robustly than ES or IS alone. The differences in the stimulation parameters, site of stimulation, and motor outputs observed during ES vs. IS suggest that different neural mechanisms were activated to induce stepping-like movements. The effects of ES may be mediated more via dorsal structures in the lumbosacral region of the spinal cord, whereas the effects of IS may be mediated via more ventral propriospinal networks and/or brainstem locomotor areas. Furthermore, the more effective facilitation of the motor output during simultaneous ES and IS may reflect some convergence of pathways on the same interneuronal populations involved in the regulation of locomotion.

Abbreviations: ASIA, American Spinal Injury Association; DLF, dorsolateral funiculus; ES, epidural stimulation; IS, intraspinal stimulation; MLR, mesencephalic locomotor region; PLR, pontomedullary locomotor region

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1. Introduction

There are multiple sites within the brain and spinal cord that can be stimulated to induce, control, and facilitate locomotion in a variety of invertebrate and vertebrate species (Grillner and Zangger, 1979; Shik et al., 1966, 1967; Shik and Yagodnitsyn, 1977; Barthélemy et al., 2006, 2007; Mushahwar et al., 2002; Iwahara et al., 1992; Orlovsky et al., 1999; Lemay and Grill, 2004; Ichiyama et al., 2005; Lavrov et al., 2006, 2008a, b; Rossignol et al., 2008). The circuitries responsible for these effects, however, remain unclear, particularly in mammalian species. The experiments of Shik et al. (1966) demonstrated that specific areas within the mesencephalic nucleus identified as the mesencephalic locomotor region (MLR) could be stimulated tonically to initiate quadrupedal stepping in decerebrated cats. Subsequently, Noga et al. (1991) performed a series of experiments in decerebrated cats that further identified some of the neuronal pathways involved in this phenomenon. These and other results suggest that descending spinal pathways originating from the MLR project to the medial reticular formation and the ventral half of the spinal cord and appear to be important in initiating stepping (Steeves and Jordan, 1984; Garcia-Rill, 1986; Noga et al., 1991).

Ascending pathways can activate the MLR via the pontomedullary locomotor region (PLR) (Mori et al., 1977, 1978; Shik and Yagodnitsyn, 1977; Kazennikov et al., 1979, 1983a; Budakova and Shik, 1980; Selionov and Shik, 1981, 1984). The MLR also can be stimulated via the fibers in the dorsolateral funiculus (DLF) from C1 to L1. These fibers have been shown to be functionally connected with the PLR and can induce quadrupedal stepping-like movements perhaps via brainstem locomotor areas (Kazennikov and Shik, 1988; Kazennikov et al., 1980, 1983a, 1985). On the other hand, local activation of spinal circuits responsible for hindlimb stepping-like movements has been achieved by epidural stimulation (ES) at the midline of the lumbar spinal cord enlargement in decerebrated cats (Iwahara et al., 1992; Gerasimenko et al., 2003, 2009, Musienko et al., 2012) and chronic spinal rats (Ichiyama et al., 2005; Lavrov et al., 2006,2088b) or by intraspinal stimulation (IS) in the caudal region of the lumbosacral enlargement in decerebrated and spinal cats (Mushahwar et al., 2002, 2004; Guevremont et al., 2006). Combined, these studies demonstrate that multiple pathways in the brainstem and spinal cord can influence the spinal networks to generate stepping-like movements. These findings raise the question as to whether the ES and IS circuitries generate similar movements and reflect the different neuronal mechanisms. We studied the functional differences and interactions between two approaches that generate stepping-like movements with IS or ES and when both were used simultaneously. While there appeared to be some functional convergence of pathways that activate locomotor-related networks during ES vs. IS, there were clear differences between these two approaches.

2. Experimental procedures

Twenty-five adult cats (2.5–3.0 kg body weight) were used in this study: 18 cats were tested with ES and 7 with IS. All animal procedures were conducted according to the European Community Council Directive (24 November 1986, 86/609/EEC) in accordance with a protocol approved by the Animal Care Committee of the Pavlov Institute of Physiology, St. Petersburg, Russia.

2.1. *Surgical procedures*

The cats were anesthetized deeply with a mixture of xylazine (1 mg/kg) and ketamine (40 mg/kg) administered intramuscularly and all surgical procedures were performed under aseptic conditions.

2.1.1. *Decerebration procedures*

Following tracheal intubation and ligation of the carotid arteries, a precollicular post-mammillary decerebration was performed as described previously (Gerasimenko et al., 2003, 2009; Musienko et al., 2007). Anesthesia was discontinued after the decerebration and the experiments were started 2 to 3 h later. Once the surgery was completed, the cat was fixed in a stereotaxic frame and suspended in a harness with all four limbs pendant. Systemic arterial pressure was monitored continuously and maintained above 80 mm Hg by fluid infusion via a carotid artery cannula. Rectal temperature was monitored and kept constant at 37 ± 0.5 °C using a heating blanket and heat irradiation. The electrocardiogram (ECG) was amplified and monitored via a loudspeaker to continuously assess the status of the animal throughout the experiment.

2.2. *Laminectomy and EMG implants*

A mid-dorsal skin incision was made between T10 and S4 and the paravertebral muscles retracted as needed. A partial laminectomy was performed between T12 and S2 to expose the dorsal surface of the spinal cord. The exposed lumbosacral spinal cord was covered with warm paraffin oil that was maintained at 35–37 °C. Intramuscular electrodes were implanted bilaterally into the following muscles: vastus lateralis (VL), semitendinosus (St), tibialis anterior (TA), and lateral gastrocnemius (LG). Skin and fascial incisions were made to expose each muscle. Two teflon-coated stainless steel wires were routed subcutaneously from a low back skin incision to each muscle. The wires were inserted intramuscularly after ~3 mm of Teflon was removed, and secured into the mid-belly of each muscle as described previously (Roy et al., 1991). The EMG wires were coiled near each implant site to provide stress relief.

2.3. *Spinal cord stimulation procedures*

Monopolar stimulation with a silver ball electrode (2 mm diameter) located epidurally on the dorsal surface of the spinal cord and a ground electrode inserted into the paravertebral muscles were used for ES as described previously (Gerasimenko et al., 2003). Spinal cord segments L1 to L7 were stimulated individually by moving the monopolar electrode to each of the spinal cord segments. For IS, tungsten needle microelectrodes (tip diameter of 15 μm at a 0.5 to 5.0 mm depth) were used to stimulate spinal segments L1 to L7. To compare the effects of IS at the DLF and the surrounding