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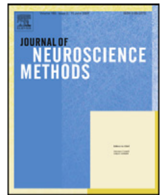

A method for unit recording in the lumbar spinal cord during locomotion of the conscious adult rat

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ABSTRACT

Extracellular recordings from single units in the brain, for example the neocortex, have proven feasible in moving, awake rats, but have not yet been possible in the spinal cord. Single-unit activity during locomotor-like activity in reduced preparations from adult cats and rats have provided valuable insights for the development of hypotheses about the organization of functional networks in the spinal cord. However, since reduced preparations could result in spurious conclusions, it is crucial to test these hypotheses in animals that are awake and behaving. Furthermore, unresolved issues such as how muscle force precision is achieved by motoneurons as well as how spinal neurons are spatio-temporally correlated are better to investigate in the conscious and behaving animal. We have therefore developed procedures to implant arrays of extracellular recording electrodes in the lumbar spinal cord of the adult rat for long-term studies. In addition, we implanted pairs of electromyographic electrodes in the hindlimbs for the purpose of monitoring locomotion. With our technique, we obtained stable long-term recordings of spinal units, even during locomotion. We suggest this as a novel method for investigating motor pattern-generating circuitry in the spinal cord.

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1. Introduction

It is well established that the local circuitry in the lumbar portion of the spinal cord generates rhythmic motor behaviors (Bizzi et al., 1995; Grillner, 2006) such as scratching (Berkinblit et al., 1978; Berkowitz, 2002; Stein, 2005; Stein et al., 1998), swimming (Berkowitz, 2005) and walking (Kiehn, 2006). The locomotor-related circuitry is distributed in the ventromedial region of the lumbar segments of the spinal cord (Cazalets et al., 1996; Cowley and Schmidt, 1997; Deliangina et al., 1983; Kjaerulff and Kiehn, 1996; Orlovsky et al., 2003; Tresch and Kiehn, 1999). Neural activity of rhythmic movements has been studied extensively in fictive locomotion in *in vitro* preparations and has been complemented with studies on functional electrical stimulation in the motor nuclei (Saigal et al., 2004; Tresch and Bizzi, 1999) and recordings from the dorsal root ganglia (Ayoyagi et al., 2003; Baker et al., 2006; Fetz et al., 2002; Weber et al., 2006) of monkey and cat. In spite of this, reports from awake and locomoting animals are missing (Orlovsky et al., 2003). In analogy with observations in the neocortex (Holt et al., 1996), fundamental differences most likely exist between pharmacologically induced activity in the developing/immature spinal cord *in vitro* compared with real movements in the adult, conscious animal *in vivo*. Furthermore, issues such as neural control of muscle force (Bizzi et al., 2008) and precision of force, as well as spatio-temporal correlations of activity (Tresch and Kiehn, 2002) are expected to be more pronounced in the awake animal. In spite of this and recent advances in large-scale recordings and neural prosthetics (Churchland et al., 2007; Hochberg et al., 2006; Schwartz et al., 2006), investigators of functional spinal circuitry have been reluctant to pursue these types of experiments since conscious animals generate movements that may interfere with the recording. We have therefore developed a technique that involves implanting

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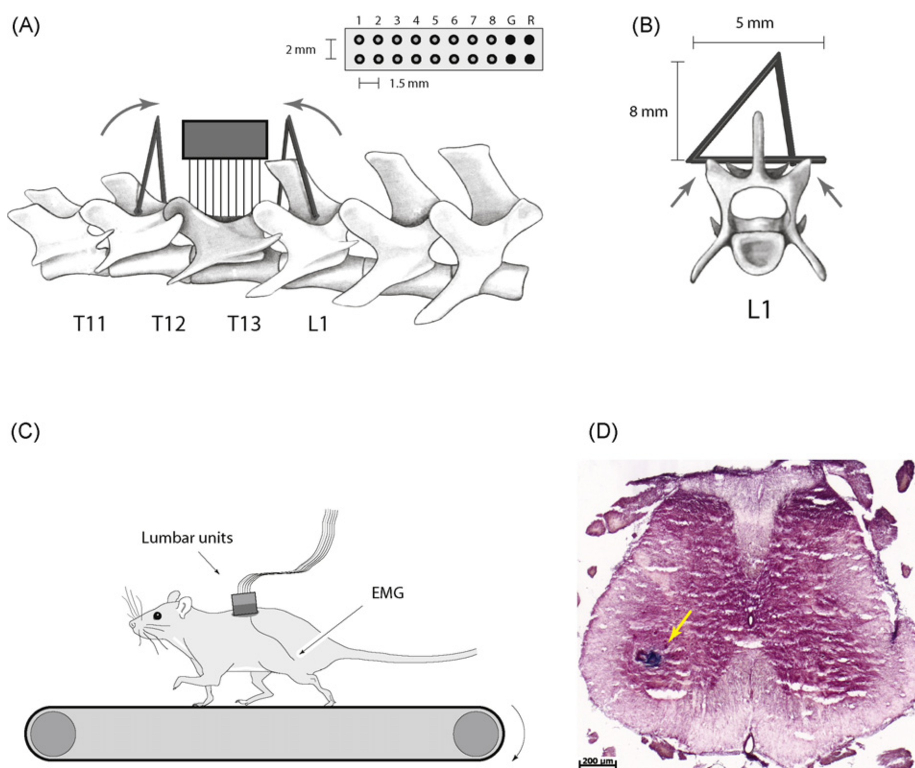

Fig. 1. Illustration of implantation and setup. (A) Sagittal view of lower thoracic and lumbar vertebrae. An adapted triangular steel wire was inserted in vertebrae T12 and L1, rotated in the direction of the arrows and glued with super glue. The 16-electrode array was inserted into the spinal segment of vertebra T13. The inset illustrates the 16-electrode array, including 2 ground and 2 reference electrodes. The electrode tips were at an equal depth of 1–2 mm from the surface. (B) Transverse view of the L1 vertebra illustrating the geometry and insertion of the steel triangle. The arrows show the lateral points of support for the steel wire. Vertebra figure adapted from (Popesko et al., 2003). (C) Illustration of the recording session in which the EMG output is combined with the multi-unit readings from the lumbar spinal cord. (D) Histological sample showing the lesion marks of an electrode in the ventral horn in L4 (yellow arrow, animal 322).

and anchoring extracellular multi-unit recording arrays in conjunction with electro-myographic (EMG) electrodes in the limb muscles. The term "multi-units" refers to multiple single units. A single unit is a representation of a putative neuron or a small group of neurons firing together in a point-like manner, i.e., a "single unit" (Buzsaki, 2004; Lewicki, 1998). We chose the lower lumbar spinal cord for the prospect of recording locomotor-related neural unit activity (Orlovsky et al., 2003; Puskar and Antal, 1997), but the technique should, in principle, work with most of the lumbar vertebrae with spinal processes.

2. Materials and methods

2.1. Animals

Six adult female rats (Sprague–Dawley, 280–290 g) were used for this study. All animals were kept in a ventilation-, humidity-, and temperature-controlled setting with a 12/12 h light/dark cycle. Rats were individually housed in clear polycarbonate cages with access to food pellets and water. The experimental procedures and treatment of rats were in accordance with Taiwanese laws governing the protection of animals used for experimental purposes.

2.2. Chronic microelectrode implantation

The animals were transferred from their cages to an air-tight container with a continuous flow of air mixed with isoflurane. When they were fully anesthetized, they were then placed on a heating pad. Anesthesia was maintained by inhalation of air mixed with isoflurane delivered through a nozzle, which was fitted to the nose. The amount of isoflurane was adjusted appropriately throughout the surgery to be between 0 and 3%, depending on the strength of the pinna reflex. The fur was shaved above vertebra T12, the area was cleaned carefully with surgical scrub and an incision was made along the length of the vertebrae. The last rib was identified as the index of T12, and the muscle tissue surrounding T11–L3 was removed with bone scissors until the vertebral joints were visible. The T11–L3 vertebral column was held in place with tightened transverse process clamps (Cunningham Spinal Adaptor, Stoelting Co.). Two transverse holes (outer diameter > 1 mm) were drilled with a dental drill (burr size 1/4 in.) through the dorsal spinal process of the T12 and L1 vertebrae so the stainless steel wire could fit through (Fig. 1A). Two pieces of stainless steel wire (3 cm in length, 1 mm in diameter, Ethicon steel wire monofilament (CrNi) non-absorbable suture (TR-55)) were bent into an open triangular shape. One end was inserted through the hole and the triangle was rotated towards the probe (see arrows, Fig. 1A) so the other open end rested on the vertebra. The triangles were then temporarily glued with superglue (cyanoacrylate, 405 Loctite). This positioning served the purpose of preventing rostro-caudal movement of the implantation, thereby stabilizing the recording of units. Similarly, lateral movement was prevented by letting the steel wires rest on the bone on both sides of the midline process (see arrows, Fig. 1B). After inserting the electrodes, the steel wires were further anchored with dental cement (see below) so that the stable positions of the wire triangles became permanent. Finally, the electrode implant, the stainless steel triangles and the exposed vertebrae were encapsulated in dental cement with connectors protruding.

The L4–L5 spinal segments were exposed after laminectomy. Due to differential development of the spinal cord and vertebrae, the L4–L5 segments of the spinal cord are located beneath the T13–L1 vertebrae (Gelderd and Chopin, 1977). Two sagittal incisions

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