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Hemisection spinal cord injury in rat: The value of intraoperative somatosensory evoked potential monitoring

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HIGHLIGHTS

- ► Somatosensory evoked potentials to assess hemisection spinal cord injury in rats.
- ► Intraoperative somatosensory evoked potentials relate to post-mortem tissue integrity.
- ► Amount of normal tissue after injury correlates with motor function outcomes.

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ABSTRACT

Techniques used to produce partial spinal cord injuries in animal models have the potential for creating variability in lesions. The amount of tissue affected may influence the functional outcomes assessed in the animals. The recording of somatosensory evoked potentials (SSEPs) may be a valuable tool for assessing the extent of lesion applied in animal models of traumatic spinal cord injury (SCI). Intraoperative tibial SSEP recordings were assessed during surgically induced lateral thoracic hemisection SCI in Sprague-Dawley rats. The transmission of SSEPs, or lack thereof, was determined and compared against the integrity of the dorsal funiculi on each side of the spinal cord upon histological sectioning. An association was found between the presence of an SSEP signal and presence of intact dorsal funiculus tissue. The relative risk is 4.50 (95% confidence interval: 1.83–11.08) for having an intact dorsal funiculus when the ipsilateral SSEP was present compared to when it was absent. Additionally, the amount of spared spinal cord tissue correlates with final functional assessments at nine weeks post injury: BBB (linear regression, $R^2 = 0.618$, $p < 0.001$) and treadmill test (linear regression, $R^2 = 0.369$, $p = 0.016$). Therefore, we propose intraoperative SSEP monitoring as a valuable tool to assess extent of lesion and reduce variability between animals in experimental studies of SCI.

1. Introduction

Traumatic spinal cord injury (SCI) results from local, abrupt damage to the spinal cord. Of the estimated 12,000 new SCI cases per year in the United States a majority, approximately 61%, of the injuries are classified as incomplete (NSCIS Center, 2011). Individuals with incomplete injuries maintain some connections between the areas above and below the point of damage. The amount of tissue spared for these connections will influence the affected individual's ability to recover volitional control below the level of injury (Rossignol and Frigon, 2011). Understanding the mechanisms of an incomplete injury is critical to determine appropriate interventions and future treatments to enhance recovery.

Various animal models of SCI have been developed to study mechanisms of injury and recovery associated with the condition. The most commonly used animal models of SCI are the result of transection, compression, or contusion injury directly to the spinal cord (Rosenzweig and McDonald, 2004; Talac et al., 2004). All of these approaches can be used to produce an anatomically and functionally incomplete injury. The transection injury model utilizes a precise, clean approach by incision to render a lesion in specific target areas (Talac et al., 2004). This incision can be used to apply either complete transection or incomplete transection (hemisection). To

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date a variety of hemisection-type injuries have been used including the lateral hemisection and dorsal hemisection (Brechtel et al., 2006; Fouad et al., 2000; Goldshmit et al., 2008; Ying et al., 2005). However, due to the technical difficulty of a partial cut, there is the possibility of inconsistencies in the injuries from one animal to the next. It has been noted that the amount of injured cord is related to functional recovery (Basso et al., 1995; Brechtel et al., 2006). Therefore, to ensure consistent injuries and homogenous groups to study, it would be valuable to designate a tool to monitor the extent of injury when it occurs.

Electrophysiology is a functional means to assess the integrity of various aspects of the nervous system, including the spinal cord. Clinical applications of electrophysiology, particularly evoked potentials, include diagnosis of peripheral or central nervous system damage and monitoring central nervous system integrity during surgical procedures (Cruccu et al., 2008; Malhotra and Shaffrey, 2010; Wang et al., 2008). Particularly, somatosensory evoked potentials (SSEPs) use a peripherally applied stimulus that is monitored in the central nervous system and can be used to detect the integrity of the dorsal columns in the spinal cord (Cruccu et al., 2008). In the rat spinal cord, the dorsal columns and the corticospinal tract comprise the dorsal funiculi, an area of white matter that lies medial to the dorsal horns (Paxinos, 1995). Stimulation applied to a limb is transmitted via the dorsal root to the spinal cord. Light touch, vibratory, and proprioceptive sensations are localized to the dorsal column tract. This sensory information travels via fast, myelinated fibers in the dorsal column ipsilateral to the side of sensation until decussating at the level of the medulla. The pathway then travels to thalamus and continues via the internal capsule to synapse in the somatosensory cortex contralateral to the side of stimulation. A disruption in the pathway between location of applied stimulus and recording in the cortex would be detectable as an abnormal cortical reading. SSEP monitoring has been applied to detect minor damage, assess ultrastructural damage, and functional recovery in various rat models of peripheral nerve injury and SCI (Agrawal et al., 2009; Hu et al., 2011; Nashmi et al., 1997; Onifer et al., 2005, 2007; Schlag et al., 2001; Sen and Moller, 1991; Wang et al., 2008; Zhang et al., 2007). Additionally, electrophysiology parameters have found to be associated with function and extent of lesion in both humans and rats (Metz et al., 2000). Based on its prior use, SSEP monitoring may be a useful approach for monitoring the extent of an injury at the time of insult as well.

The purpose of this study was to determine the value of using intraoperative SSEPs for monitoring the extent of tissue damage in a lateral hemisection model of thoracic spinal cord injury in rat. We propose that absence or presence of SSEP will correspond to the integrity of the dorsal funiculi on post-mortem tissue analysis. Here we describe the use of SSEP monitoring in the context of an intervention study assessing the effect of exercise on functional recovery in rats following a right lateral hemisection SCI.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats ($n = 19$, Harlan Laboratories, Indianapolis, IN) weighing 250–300 g underwent the lateral hemisection spinal cord injury procedure. Seventeen survived and were randomized to either the exercise ($n = 9$) or control ($n = 8$) group. Of the two rats that did not survive, one was lost during the procedure and one was euthanized four days following the procedure due to complications. One of the 17 surviving rats was excluded after review of histology due to having a nearly complete transection injury. Thus the data from 16 rats (exercise: $n = 9$ and control: $n = 7$) were considered suitable for final functional analysis. An additional animal's tissue was damaged during the sectioning process. Thus the data from 15 rats (exercise: $n = 8$ and control: $n = 7$) were used for histological analysis. All animal care, handling, and surgical procedures were approved by and conducted in accordance with the guidelines of the institutional animal care committee (Institutional Animal Use and Care Committee, Mayo Clinic).

2.2. Surgical procedure

2.2.1. Lateral hemisection injury

Animals were deeply anesthetized by intraperitoneal injection of ketamine (80 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (5 mg/kg, Lloyd Laboratories, Shenandoah, IA). This maintained deep anesthesia for at least 2 h, allowing all electrophysiological monitoring to be performed while the rats were under deep anesthesia. Before surgery, animals were weighed and given intramuscular Buprenex (0.05 mg/kg, Reckitt Benckiser Pharmaceuticals Inc, Richmond, VA), intraperitoneal Baytril (65 mg/kg) and 5 mL subcutaneous 0.9% Saline (Baxter Healthcare Corporation, Deerfield, IL). Lacrilube gel was applied to both eyes and each animal was maintained on a $37 \pm 0.5\,^{\circ}C$ heating pad prior to surgery until waking up after surgery.

The rat's back was shaved and aseptically prepared using a povidone-iodine swabstick (Professional Disposables Inc, Orangeburg, NY). A skin incision was made along the midline of the back and the musculature was bluntly dissected to the spinous processes. The laminae were exposed and a laminectomy was performed at the level of T9 or T10. The midline of the spinal cord was identified and a transverse cut was made laterally from midline to create a right hemisection injury. The accuracy of the lesion was assessed by somatosensory evoked potential (SSEP). SSEP measurements were made between 30 and 70 min after induction of anesthesia. After loss of right SSEP was determined the wound was closed in muscle and skin layers using simple interrupted stitches with absorbable vicryl suture.

2.2.2. Intraoperative somatosensory evoked potential (SSEP) monitoring

During the surgical procedure tibial SSEPs were monitored bilaterally (Nicolet Viking IV; Viasys Healthcare, Madison, WI). The tibial nerve was stimulated proximal to the ankle with a dual tip electrode (Fig. 1). The stimulation parameters were as follows: 0.05 ms duration, 2.1 Hz frequency, and intensity to elicit a mild muscle twitch (3.9–10.2 mA). Recordings were made via two needle electrodes placed over the somatosensory cortex through the scalp. Waves were odd-even averaged for analysis. SSEP technique has been used and published by our lab (Wang et al., 2008) and was validated via monitoring SSEPs in normal, anesthetized rats. SSEP signals were consistently acquired in the normal animals using the procedure described. These signals had a consistent early negative followed by a positive deflection as shown in Fig. 2b.

Both the right and left SSEP were assessed pre- and post-hemisection cut. At the time of the post-operative recordings, the waves were determined to be absent or present. The response was considered absent when neither negative nor positive deflection was observed. If the right SSEP was considered present following the cut, an additional cut was made to the right side of the spinal cord in the same position as the first. This was done up to three times, until the signal was deemed absent. Five of the rats received these additional cuts. Final assessments used for data analysis of the signal integrity (absent or present) were made by three neurologists blinded to the source of each wave.

2.2.3. Post-operative care

For one week following surgery the animals were kept in low-sided cages. Acetaminophen (Mapap™ Major Pharmaceuticals,