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Laboratory Study

Slope analysis of somatosensory evoked potentials in spinal cord injury for detecting contusion injury and focal demyelination

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ABSTRACT

In spinal cord injury (SCI) research there is a need for reliable measures to determine the extent of injury and assess progress due to natural recovery, drug therapy, surgical intervention or rehabilitation. Somatosensory evoked potentials (SEP) can be used to quantitatively examine the functionality of the ascending sensory pathways in the spinal cord. A reduction of more than 50% in peak amplitude or an increase of more than 10% in latency are threshold indicators of injury. However, in the context of injury, SEP peaks are often obscured by noise. We have developed a new technique to investigate the morphology of the SEP waveform, rather than focusing on a small number of peaks. In this study, we compare SEP signals before and after SCI using two rat models: a contusion injury model and a focal experimental autoimmune encephalomyelitis model. Based on mean slope changes over the signal, we were able to effectively differentiate pre-injury and post-injury SEP values with high levels of sensitivity (83.3%) and specificity (79.2%).

1. Introduction

Based on an average annual incidence of approximately 40 cases per million people in the United States, it is estimated that more than 12 000 people survive a spinal cord injury (SCI) each year.[1] Around 258 000 people in the United States are reportedly living with the devastating effects of an SCI.[1] On the basis of etiology, there are two general types of SCI: traumatic (due to blunt mechanical impact) and non-traumatic (due to vascular, ischemic or neoplastic causes, or immunological disorders). Traumatic SCI accounts for nearly 60% of all injuries to the spinal cord.[2]

The number of spared axonal fibers and the degree to which they are demyelinated play important roles in determining the residual functionality present after SCI. "Anatomically incomplete" injuries are those in which a number of spared but demyelinated axons remain intact across the lesion, without electrophysiological responses.[3–6] Even a small number of spared fibers remaining after SCI can greatly improve the quality of life of SCI patients. Development of therapeutic strategies to reduce secondary injury, and to remyelinate spared, demyelinated axons has generated considerable interest in the past.[7–9] When evaluating any therapeutic approach for SCI, a suitable SCI animal model and reliable monitoring measures are essential, to allow calibration of the severity of the SCI and monitoring of the progress of injury and extent of recovery.[10,11]

A popular animal model of SCI for blunt contusion injuries is a rat model with injury induced using the New York University (NYU) impactor,[12] which is known to reliably emulate the pathophysiology seen in humans after SCI.[13] In this model, some neuronal tissue remains intact along the periphery of the primary site of injury,[13] similar to the situation in humans after blunt injury.[3]

A chemically mediated SCI model is a targeted approach to simulate specific aspects of SCI-like demyelination, inflammation, ischemia or immunological disorders.[14] A focal demyelinating lesion can be induced in the spinal cord of the rat experimental autoimmune encephalomyelitis (EAE) model by administering inflammatory factors directly into the spinal cord of the immunized rat.[15] This model is analogous to the human paralyzing disorder transverse myelitis, which often arises idiopathically or in association with multiple sclerosis.

Various outcome measures for animal models can be used to assess changes due to endogenous recovery, drug therapy, surgical intervention or rehabilitation. Behavioral tests can be used to examine functional recovery in laboratory animals after SCI; however, such tests are often subjective. In contrast, electrophysiological techniques present an objective means for quantitative, non-invasive, accurate assessment of the integrity of neural pathways. Somatosensory evoked potential (SEP) is the electrophysiological

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response of the nervous system to electrical stimulation of a peripheral nerve. SEPs can be used to examine the functionality of the ascending sensory pathways, and allow longitudinal measurements. In a number of SCI studies, SEP values have been correlated with neurological deficit scores.[16–21] Unfortunately, no detection standards exist for SEPs; however, a general rule for indicating possible tissue damage has been widely adopted: a reduction of more than 50% in peak and inter-peak amplitudes or an increase of more than 10% in latency with respect to baseline are considered indicative of significant likelihood of injury.[22,23] However, peaks in the SEP waveform are often obscured by noise and may even be indistinguishable in some injuries. This necessitates human intervention for the detection of peaks, rendering the process subject to error and inter-observer variability. This problem prompted us to develop a new technique for quantifying the morphology of the SEP waveform as a whole, rather than just focusing on a few salient peaks.[24] The technique is largely borrowed from shape analysis tools in the field of image processing.[25]

In this paper, we introduce the slope analysis technique for SEPs and present the results of our studies in two rodent SCI models (a contusion model and a focal EAE model).

2. Materials and methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University. Principles of laboratory animal care as outlined in the National Institutes of Health publication no. 85–23 (revised 1985) were followed.

2.1. Animals

Adult female Fischer rats (n = 12; 200–220 g; Charles River Laboratories, Raleigh, NC, USA) were used for the contusion model, and adult female Lewis rats (n = 12; 200–220 g; Charles River Laboratories) were used for the focal EAE model. The rats were housed individually in cages and had free access to food and water.

2.2. Anesthesia

Anesthesia for all surgical procedures, except SEP recording, was established using a mixture of 45 mg/mL ketamine and 5 mg/mL xylazine administered via intraperitoneal injection.

For SEP recording, anesthesia was established by placing the rat in a transparent chamber with 3% isoflurane gas in room air until the onset of drowsiness. The rat's mouth and nose were then covered with an anesthesia mask with a close-fitting rodent-size diaphragm. A mixture of 1.5% isoflurane, 80% oxygen and room air was delivered to the mask at a rate of 2 L/min to maintain anesthesia. The mask was connected to a C-Pram circuit designed to deliver and evacuate the gas through one tube.

That the level of anesthesia was adequate was confirmed by monitoring the corneal reflex and hindlimb withdrawal to painful stimuli. Rats continued spontaneous breathing and the depth of anesthesia was maintained throughout the experiment. Throughout the entire experiment, rats were placed on a homeothermic blanket system (Harvard Apparatus; Edenbridge, Kent, UK) to maintain their body temperature at 37 ± 0.5 °C, measured using a rectal probe. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals; Irvine, CA, USA) was applied to the rats' eyes to prevent drying.

2.3. Injury

2.3.1. Contusion injury

Following anesthesia, each rat's dorsal region was shaved and aseptically prepared with chlorhexidine (Phoenix Pharmaceuticals; St. Joseph, MO, USA). A midline incision was made along the thoracic vertebrae and the skin was opened. The paravertebral muscles in the region of interest (T6–T12) were retracted. A laminectomy was performed at thoracic vertebra T8 to expose the dorsal surface of the spinal cord underneath, without opening the dura mater. The spinous processes of the vertebrae at T6 and T12 were secured in stabilization clamps to reduce movement of the spinal column during the impact. The impact rod was centered above the exposed part of the spinal cord at the T8 level. The rod was slowly lowered until it came into contact with the dura. This event was detected using an alarm triggered by the completion of an electrical circuit. The exposed dorsal surface of the spinal cord at the T8 level was then contused with the NYU device by dropping the 10 g rod with a flat circular cross-section from a predetermined height (6.25, 12.5, 25.0 or 50.0 mm). The control group underwent only laminectomy with no contusion. Various biomechanical parameters, such as the impact velocity of the rod, the distance of cord compression, the cord compression rate, and the dynamic force applied to the cord were precisely monitored using a computer, and there was <0.05% variation in these values.

2.3.2. Focal autoimmune encephalomyelitis lesion

Recombinant myelin oligodendrocyte glycoprotein (rMOG; Biogen-Idec; Cambridge, MA, USA) corresponding to the N-terminal sequence of rat MOG (amino acids 1 to 125) was emulsified in Freund's incomplete adjuvant (IFA) as a 1:1 mixture (Inject IFA; Pierce, Rockford, IL, USA). Then 100 μL (50 μg per rat, diluted in saline) of this emulsified MOG-IFA mixture was injected subcutaneously at two sites near the base of the tail, one on each side (50 μL per injection, to minimize backflow). After 18 days of MOG sensitization, the rats were anesthetized, then their dorsal region was shaved and aseptically prepared with chlorhexidine. A midline incision was made along the thoracic vertebrae and the skin was opened. The paravertebral muscles in the region of interest (T6–T12) were retracted. A laminectomy was performed at thoracic vertebrae T7–T9 to expose the dorsal surface of the spinal cord underneath without opening the dura mater. The T6–T12 segments of the spinal column were stabilized in a stereotaxic frame, then a Hamilton needle (31G) was used to bilaterally inject cytokines (2 × 2 μL; 250 ng of tumor necrosis factor α, 150 U of interferon γ and 40 ng of interleukin 6) and ethidium bromide (1 μg) into the dorsal white matter at T8.

2.3.3. Post-injury procedure

After injury, the muscles were sutured in layers using an absorbable 2-0 suture, and the skin was closed with a 4-0 suture. All rats were allowed to recover in a warmed cage and food and water were easily accessible. Intramuscular gentamicin (5 mg/kg) (Abbott Laboratories; Abbott Park, IL, USA) was administered immediately post-surgery and then daily for 4 days. Intramuscular buprenorphine (0.01 mg/kg using a 0.3 mg/mL preparation; Buprenex, Reckitt Benckiser Pharmaceuticals; Richmond, VA, USA) was administered post-surgery and daily thereafter for 3 days. After surgery, the rats' bladders were expressed twice per day for the first 4 days or until the rats regained control of urination. There were no complications or other infections. No sign of autotomy or autophagy were observed. The rats were maintained for 8 weeks after injury, and thereafter anesthetized and killed by transcardial perfusion with formaldehyde.

2.4. Somatosensory evoked potential recording

2.4.1. Electrode implantation

One week prior to injury, the rats were anesthetized and their head regions were shaved and aseptically prepared with chlorhexidine. Lidocaine HCl (2%) (Abbott Laboratories; North Chicago, IL,