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Original Article

Electrophysiological monitoring techniques for spinal cord function in a canine model

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Abstract: Electrophysiology recording has been utilized in a wide variety of spinal procedures. Until recently, electrophysiological techniques for assessing a canine model of spinal cord injuries have not been described in detail. The purpose of this study was to describe the technique of evoked potential in the canine species for evaluating spinal cord function. The dogs underwent either vertebrectomy with the spinal cord shortened or laminectomy without spinal cord shortening. The function of the spinal cord was evaluated by electromyography to detect somatosensory evoked potential, transcranial electric motor evoked potential, and spinal cord evoked potential at designated time points. Anesthetics, stimulation site, and stimulus intensity affected motor and somatosensory evoked potentials. Adequate analgesia easily abolished the elicited motor evoked potential. A lower amplitude stimulus (5-10 mA) was adequate to elicit spinal cord evoked potential in dogs and there was no advantage to using higher stimulation intensity. This study provides reference data of evoked potentials for evaluation of the function of the spinal cord. The combination of somatosensory evoked potential and spinal cord evoked potentials in intraoperative neurophysiologic monitoring is a reproducible and reliable modality.

Keywords: Somatosensory evoked potential, spinal cord evoked potential, motor-evoked potential, spinal cord function, canine model

Introduction

Intraoperative electrophysiology recording has been utilized in a wide variety of spinal procedures. Especially, evoked potentials provide information on the functional integrity of sensory and motor pathways and, therefore, are used extensively in neurophysiological monitoring during spinal surgery [1-3]. Clinically, spinal cord function is evaluated using both somatosensory evoked potential (SSEP) and motor-evoked potential (MEP). SSEP mainly examines dorsal column sensory function while MEP mainly reflects the function of descending motor tracts. These two evoked potentials can be used to study spinal cord function by electrical stimulation.

Studies on the utilization of SSEP and MEP have been reported in humans [4, 5], primates [6, 7], and cats [8] but not in dogs. Dogs are suitable for spinal cord injury (SCI) studies as they have very similar clinical signs and prognosis as their human counterparts [9]. Unfortunately, electrophysiological techniques for assessing the canine model of SCI such as SSEP, transcranial electric motor-evoked potential (TEMEP), and spinal cord evoked potential (SCEP) have not been described in detail in the literature.

In our present study, we investigated the use of evoked potentials in a canine model of SCI. By testing different stimuli, stimulus intensities, and stimulation sites, we attempted to find an adequate method for monitoring SCI.

Materials and methods

Animals

This study was approved by the Animal Experiment Committee of Xi'an Jiaotong University. Principles of laboratory animal care were followed and all procedures were conducted

Table 1. SSEP monitoring parameters

	Forelimb	Hindlimb
Stimulation site	Median nerve at the wrist	Tibial nerve proximal to the tarsus
Recording site	2 cm apart from the occipital protuberance	Occipital protuberance
Reference site	Midpoint between the superciliary arch	Midpoint between the superciliary arch
Ground site	Shoulder	Shoulder
Stimulation type	Square wave, monophasic	Square wave, monophasic
Stimulation intensity	10 mA	12 mA
Stimulation duration	0.2 ms	0.2 ms
Stimulation rate	4.0 Hz	4.0 Hz

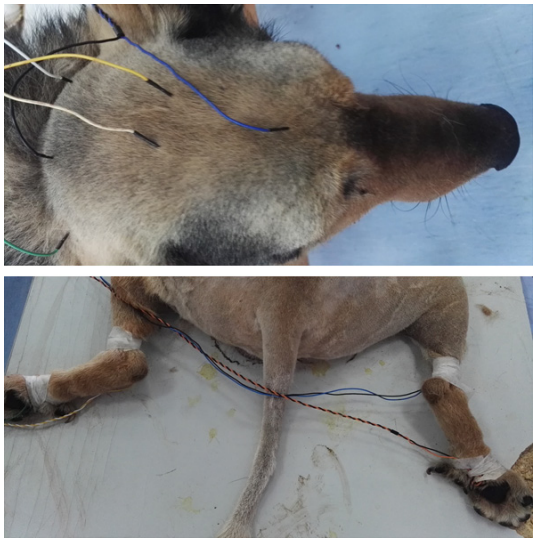

Figure 1. Needle electrode placement for evoked potential monitoring study.

according to guidelines established by the National Institutes of Health. Every effort was made to minimize suffering. Twelve adult mongrel dogs, weighing 12-15 kg, body length (anal-nasal) 60-80 cm, were used in our experiments. Prior to the experiments, an examination of their neurologic function was performed to exclude any gross abnormality. Each of the animals finally recruited had normal neurologic function including sensory, motor, and reflex function. The animals were anesthetized with sodium pentobarbital. An initial dose of 25 mg/kg was given by intraperitoneal injection and a supplemental dose of 5 mg/kg was given by intravenous injection as required. The tracheas of the dogs were intubated in the prone position and ventilated mechanically with a large animal anaesthesia machine (Shanghai Yuyan Instruments Co. Ltd, Shanghai, China). The lungs were ventilated using a volume-controlled time-cycled ventilator with the following settings: respiratory rate = 15 breaths/minute, tidal volume = 15 mL/kg, inspiratory time = 1 second, and peak inspiratory airway pressure < 20 cm H_2O. After the dogs were immobilized on an operating table with the head resting between the forelegs, a catheter was inserted into the radial vein for administration of drugs and fluid replacement. Their rectal temperature was monitored and maintained between 36°C and 38°C.

Surgical procedure

The dogs were randomly allocated into two groups with six dogs in each group. Group 1 was set as the control group, in which laminectomy was performed without shortening of the spinal cord. Group 2 was a shortening group, in which a laminectomy and vertebrectomy (two-thirds T13 vertebral bodies) was performed, followed with shortening of the spinal cord.

This procedure was similar to that described previously [10]. Briefly, after appropriate skin preparation, the thoracolumbar spine from T11 to L2 was exposed through a midline longitudinal incision and then T12 to L1 laminectomy was performed. Pedicle screws were inserted parallel in T12 and L1 and were fixed using a titanium rod. In the shortening group, vertebrectomy of T13 was performed. Proximal and distal bone surfaces were gradually contacted and fixed by titanium rods. All of the animals in the study were awakened from anesthesia. After surgery, the dogs were housed individually and allowed to recover. An intramuscular injection of cefazolin (20 mg/kg) was administered twice daily for three days, after which a neurological examination was performed. After the animals were sacrificed, their spinal cords were removed for histologic examination.

Table 2. SCEP monitoring parameters

	Parameter
Stimulation site	Posterior midline of the dura mater
Recording site	Muscle belly of the muscle of thenar
Reference site	Paravertebral muscles
Ground site	Shoulder
Stimulation type	Rectangular waveform
Stimulation intensity	10 mA
Stimulation duration	0.5 ms
Stimulation rate	4.0 Hz

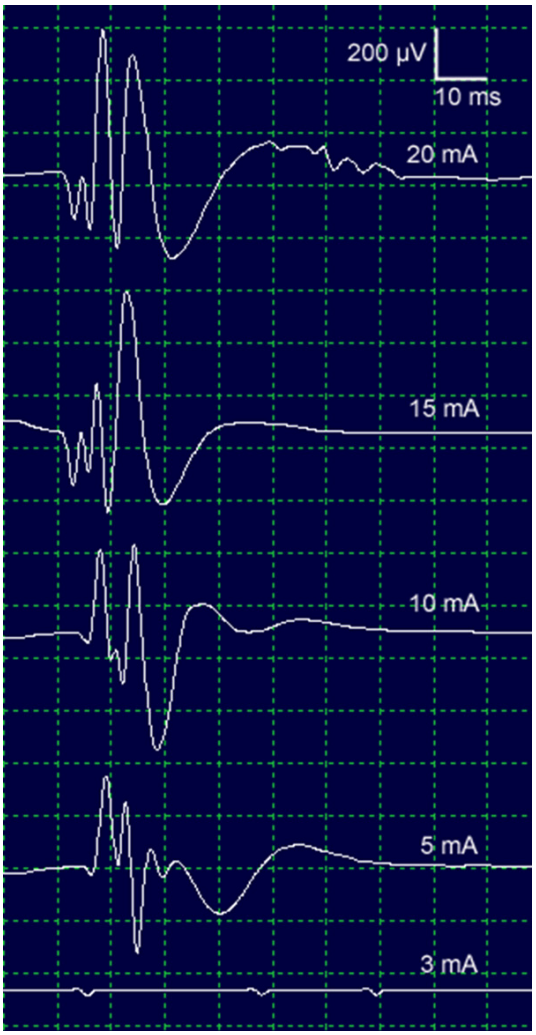

Figure 2. Spinal cord evoked potential in hindlimb at various stimulus intensities before injury.

Measurement of evoked potentials

Spinal cord function was evaluated via detection of SSEP, TEMEP, and SCEP produced by spinal cord stimulation. All evoked potentials were recorded using subdermal needle electrodes. The disposable stainless steel subdermal needle electrodes (13.0 × 0.4 mm, Xi'an Friendship Medical Electronics Co. Ltd., Xi'an, China), coated with medical grade polyvinyl chloride except for 13 mm at the distal tip, were fixated to the animal's skin using adhesive tape and connected to a neurophysiology workstation (Xltek, Natus Medical Incorporated Excel-Tech Ltd, Ontario, Cananda). Electrophysiology recording began one hour after anesthetic induction to allow time for sedation and establishment of steady-state condition. Evoked potentials were recorded at the time points of pre-operation (before skin incision), pre-injury (after exposure of the spine but before segmental instrumentation), and post-injury (after spinal column shortening and segmental instrumentation). Each recording was repeated twice. Evoked potential was then analyzed by visual inspection.

Measurement of SSEP

The stimulus and recording parameters of SSEP are outlined in **Table 1**. SSEP was recorded from needle electrodes placed subcutaneously on the head of the dog: the recording electrode over the occipital protuberance (caudal end of crista sagittalis) and the reference electrode at the site of midpoint between superciliary arches. The stimulating electrode was placed over tibial nerve immediately proximal to the tarsus in hindlimb and the ground electrode was placed in the shoulder ipsilateral to the side (**Figure 1**). Inter-electrode impedances were less than 2 kΩ.

Measurement of TEMEP

The stimulating electrode was placed 1 cm lateral to the sagittal suture on both anterior basal ears. The tip of the recording electrode was positioned percutaneously in the muscle belly of the muscle of thenar, just the palmar aspect of the claw (**Figure 1**). Stimulation parameters were 20 mA, 1 ms, 500 Hz. Intensity of the stimulation varied in order to capture the change in stimulation threshold at which TEMEP was not elicited.

Measurement of SCEP

To monitor SCEP, the stimulating electrode was placed on the posterior midline of the dura

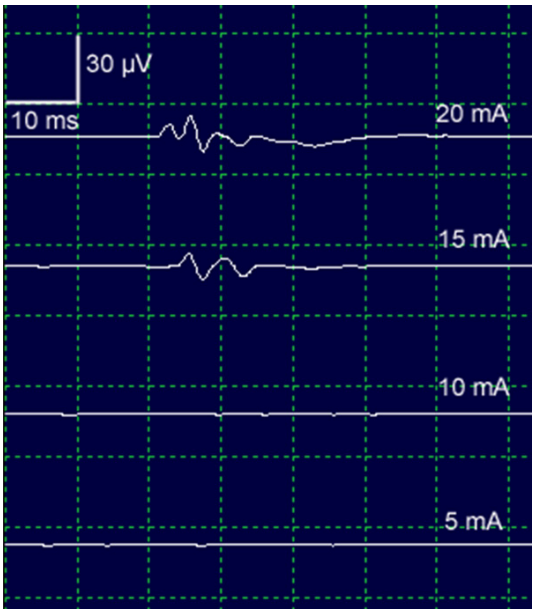

Figure 3. Spinal cord evoked potential in forelimb at various stimulus intensities after injury.

mater and the reference electrode was inserted into paravertebral muscles adjacent to the stimulating electrode. The recording electrode was then introduced into the muscle belly of the muscle of thenar (**Table 2**). A set-up mA with pulse duration of 0.5 ms rectangular waveform was applied between the stimulating electrode and recording electrode. Dura mater was stimulated rostrally and caudally from the laminectomy site. SCEP recording was performed twice, first before injury and then after injury. Intensity of the stimulus was varied, increasing gradually from 1 mA to 3, 5, 10, 15, 20, 25, 30, 35, and 40 mA, to detect the change in stimulation threshold under injury.

Histology

At the end of the experiment, each dog was perfused with paraformaldehyde and exposed spinal cord was removed. The spinal cord segment was fixed in paraformaldehyde for 24 hours and then the tissue was embedded in paraffin and sectioned for examination by light microscopy.

Results

Under a low dose of pentobarbital (25 mg/kg intraperitoneal injection), inadequate analgesia acted on SSEP without affecting TEMEP. In contrast, the conventional dose of pentobarbital (25 mg/kg intraperitoneal injection supplemented by 5 mg/kg intravenous injection) generated different results. In some dogs, a clearly identifiable TEMEP could not be obtained due to the presence of a large stimulus artifact, indicating that TEMEP was obliterated during adequate anaesthesia. SSEP did not change in magnitude before injury. Amplitude appeared widely varied between individual animals while latency was more constant.

It was essential to place electrodes within estimations of the site of stimulation. The location of electrode tip was far removed from the set site (more than 1 cm) and yet caused a substantial decrease in signal. That is to say that SSEP or TEMEP either greatly diminished in amplitude or was absent.

For stimulus levels in the range of 40 to 3 mA, waveforms of SCEP were usually detected in the responses. The waveform did not change notably under stimulation of less than 3 mA but then significantly decreased in amplitude with a decrease of stimulus current (**Figure 2**). When the evoked potential appeared to be blocked by an experimental lesion, stimulus current at the dura mater rostrally from the laminectomy site was raised to more than 15 mA. This elicited anti-dromically conducted potential in forelimbs where it was recorded through the same electrodes (**Figure 3**). A higher intensity of stimulation caused increased stimulus artifact but increased amplitude was small. In other words, within a stimulation intensity range of 15 to 40 mA, false positives were usually detected in the forelimbs. Recordings were obtained in all dogs without spinal cord shortening at stimulus intensity of 5 and 10 mA. In dogs with spinal cord shortening, hindlimb movements were not evoked by stimulation at rostrally from the laminectomy site. Therefore, upon shortening of the spinal cord, SSEP and SCEP evoked by stimulation at rostrally from the laminectomy site were abolished.

Loss of SSEP in the hind limbs was associated with a postoperative neurologic deficit in the models. Histologic examination of the spinal cord demonstrated central gray necrosis of the lumbar region in all dogs with shortening. All of the animals were paraplegic, which suggested lumbar cord necrosis. Histologic outcomes and neurologic examination results were closely conformed to electrophysiology monitoring.

Discussion

In this study, we developed an electrophysiological monitoring technique on a canine model in which SSEP and SCEP were proven to be sensitive and reliable for intraoperative monitoring of spinal cord sensory and motion function. To our knowledge, there are no reports on the methods of evoked potentials in dogs. Usage of the two techniques is desirable to detect intraoperative spinal cord dysfunction in a canine model. The present study demonstrates that monitoring SSEP and SCEP for spinal cord function in SCI dogs is a practical and sensitive procedure.

The depth of anesthesia has a significant effect upon SSEP and TEMEP in dogs. Effects of sedation and anaesthesia on evoked potentials in animals have been investigated [11-13]. In the present study, dogs were sedated with pentobarbital and SSEP, TEMEP, and SCEP were detected during pentobarbital to monitor neurological function. TEMEP may be more sensitive than SSEP to an anesthetic regimen. For TEMEP, in inadequate analgesia, animals often showed discomfort induced by evoked muscle contraction and the noise of stimulation. Since spinal surgical procedures in sedated conscious animals are ethically unacceptable, an adequate depth of anaesthesia is essential. In our experiment, TEMEP was observed in only some of the dogs under adequate anaesthesia, which could be due to an effect of anaesthesia. This inconstant presence excluded TEMEP as a means of monitoring spinal cord function. SSEP was found to remain the same before operation and before injury in all of the dogs. SCEP was stable in configuration and amplitude under adequate anesthesia, so SCEP is a choice of modality of monitoring motor pathways. Therefore, the combination of SSEP and SCEP is validated to provide valuable information during routine anaesthesia in animals.

The electrode providing stimulation needs to be carefully placed. It is important that the location of electrode tip is no more than 1 cm apart from the set site and near the skull. Otherwise, response to stimulation may diminish in amplitude or be absent.

It is essential to determine electrophysiological monitoring parameters in order to assess spinal cord function. In SCI, stimulation intensity resulted in a divergent response in the forelimbs and hindlimbs. In SCEP that we employed, with weak stimulus, it could reflect conduction of the spinal cord. With strong stimulus, conduction occurred antidromicly through the spinal cord. This permitted verification of a conduction block in SCEP after injury, through electrodes for stimulation of the dura mater rostrally and caudally from the laminectomy site. The signal at 5-10 mA was quite prominent. Upon stimulation intensity increasing to 15 mA, volume conducted stimulus artifact was seen in the forelimb records. Therefore, lower stimulus intensity could elicit reliable SCEP.

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Disclosure of conflict of interest

None.

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