Somatosensory Evoked Potential Model for Assessment of Nerve Regeneration

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Abstract

Electrophysiological studies are commonly used to evaluate nerve regeneration following repair or to simply determine the functional integrity of peripheral nerve and central neural structures in large animal models. Most often used large animals for nerve repair studies include cats, dogs, primates, gouts, and sheep. The vast majority of authors use electrophysiological methods to assess nerve regeneration including maximal nerve conduction velocity (NCV) and somatosensory evoked potential (SEPs), both spinal and cortical recorded. This chapter outlines a method to measure NCV and cortical SEPs in a sheep following median nerve stimulation. In addition, special anesthetic considerations, animal preparation, and common pitfalls that may be encounter during measurements are described.

Keywords

Assessment • Experimental model • Nerve Regeneration • Somatosensory evoked potential

Introduction

Evoked potentials is a useful technique to evaluate nerve regeneration following repair or to simply determine the functional integrity of peripheral nerve and central neural structures in large animal models. Most often used large animals for nerve repair studies include cats, dogs, primates, gouts, and sheep [1–15]. The vast majority of authors use electrophysiological methods to assess nerve regeneration including maximal nerve conduction velocity (NCV) and somatosensory evoked potential (SEPs), both spinal and cortical recorded. In this chapter we describe a method to measure NCV and cortical SEPs in a sheep following median nerve stimulation.

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M.Z. Siemionow (ed.), *Plastic and Reconstructive Surgery: Experimental Models and Research Designs*,
DOI 10.1007/978-1-4471-6335-0_52, © Springer-Verlag London 2015

Anesthetic Considerations for Intraoperative Measurements

Intraoperative SEPs can be significantly affected by different anesthetic regimens. Although cortical data is the most sensitive, all recordings may be affected in an anesthetized animal.

Drugs

In our model we use ketamine and xylazine for the induction, and isoflurane for the maintenance of anesthesia. Perioperative pain control is provided with either buprenorphine or fentanyl.

Halogenated anesthetics such as isoflurane are the most potent inhibitors of the SEPs. When these agents are use, it is often necessary to limit the dose to minimum to allow successful recordings. Typically, reducing the dose by half allows obtaining a reliable data.

Narcotics have lesser impact on the recording of evoked potentials and suppression of cortical data is not as severe. In the balanced anesthesia typically they are given with conjunction with other anesthetics, which allows using lower doses of other agents such as isoflurane. Some narcotics, i.e., ketamine, have even neuroexcitatory properties that can enhance signal recordings and facilitate data collection. These agents should be included to the anesthetic regimen whenever possible.

Finally, muscle relaxants are a routine part of the large animal anesthetic protocol. They do not adversely interfere with the SEPs measurements. Moreover, these agents are very useful in data collection since they eliminate potential movement of the animal during nerve stimulation. Limb jerking during repetitive nerve stimulation not only makes the procedure more difficult, but also may contaminate recorded data with artifacts.

Skin and Core Temperature

Peripheral nerve conduction velocities are affected by changes in limb temperature. Marked decrease in body temperature significantly decreases the absolute and interpeak latencies of all cortical potentials. Core temperature below 34 °C typically results in decrease of cortical data. All SEPs typically disappear at temperatures ranging from 20 to 25 °C.

Technique

In our model, we create a 6 cm median nerve defect and repair it with different conduits and techniques. 6 months following the repair we surgically expose the nerve and complete the maximal NCV measurements and cortical SEPs. After the neuroevaluation is completed, the nerve is harvested and the animal is euthanized.

Placing Stimulating Electrodes

The procedure is performed sequentially on both forelegs of the animal. With the sheep in lateral position, a forelimb and axilla are shaved and prepared with povidone iodine solution and then draped in the usual manner. Ten centimeter skin incision is made on the anteromedial aspect of the limb extending from the lateral chest wall proximally to the groove formed between the flexor carpi radialis muscle and radius distally. The fascia is incised and underlying fat tissue is dissected. The flexor carpi radialis muscle is identified and then detached from its point of insertion to improve the access to the median nerve. The nerve dissection needs to be done carefully with attention paid to the median artery, which runs close to the nerve.

Following the isolation of the 6 cm segment of the repaired median nerve, the dissection is continued 3 cm proximally and 3 cm distally. This is done to expose a naive nerve that can be further stimulated to measure NCV. Bipolar hook electrodes are placed proximally and distally to the nerve conduit (Fig. 52.1).

Placing Recording Electrodes

SEPs are recorded from the sensory cortex following stimulation of the peripheral nerve. After

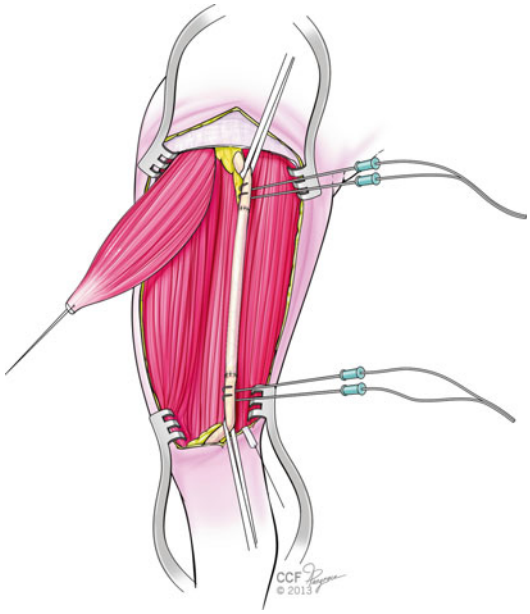

Fig. 52.1 Maximal nerve conduction velocity measurements. Stimulating hook electrodes are placed proximally and recording electrodes are placed distally to the repaired nerve segment

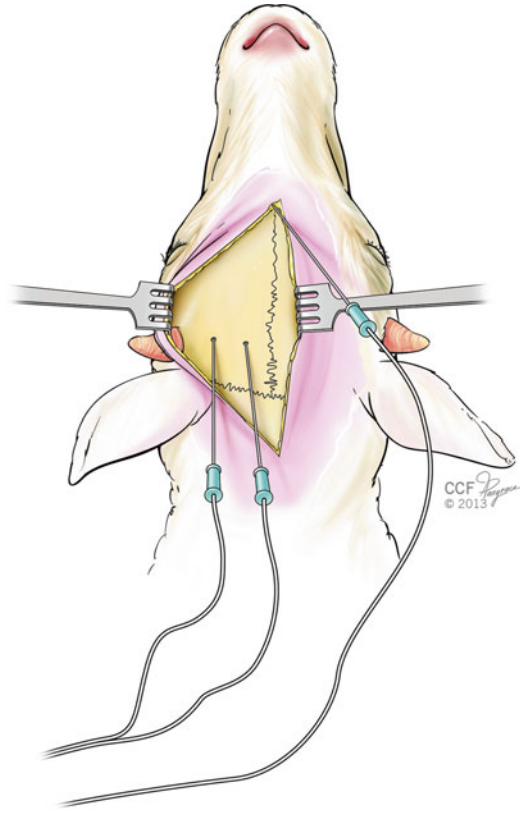

Fig. 52.2 Transcranial placement of the recording electrodes for somatosensory evoked potentials measurements

direct electrical stimulus to the median nerve, SEPs can be registered from the surface of the contralateral scalp, with the greatest signal obtained over the postcentral brain region. Based on our experience, however, the greater amplitude and more accurate measurements can be obtained transcranially with small point electrodes placed directly over the brain sensory cortex Kwiecien et al. [16].

With the sheep in lateral position, a scalp area is shaved and prepared with povidone iodine solution and then draped in the usual manner. A 6–8 cm skin incision over the midline of the scalp is made, widely exposing the skull bilaterally. Sagittal and coronal sutures should be easily visible. Next, an array of several burr holes is created in the parietotemporal location using a small 3 mm surgical drill. First two holes are drilled on the line connecting center points of each horn, 2 and 4 cm from the midline. Then, another sets of two holes are burred 1 and 2 cm anteriorly and posteriorly to the line (Fig. 52.2). Since the SEPs from both median nerves will be recorded, it may be preferable to drill the holes on the other side of the skull at the same time. Special attention needs to be paid not to penetrate the dura, which may result in injury to the brain and inability to record reliable data. Appearance of the clear, yellowish fluid at the burr side indicates injury to the dura and cerebrospinal fluid leakage.

The recording teflon-insulated monopolar needle electrodes are placed over the subdural space of sensory cortex.

Placing a Ground Electrode

The right position of the ground electrode is crucial for recording SEPs because of the electrical artifacts produced by the stimulation. To minimize these artifacts, the ground electrode should be

placed on the stimulated limb proximally to the stimulating electrodes. A flexible metal band covered with saline soaked gauze wrapped around the limb serves well as a ground electrode. In case of isolated direct nerve stimulation, the ground electrode may be also placed subdermally on the head, which adequately eliminates artifacts related to the electrical interference (Fig. 52.2).

SEPs Measurements

After connecting the stimulating electrodes with the stimulus box and plugging the cortical and ground electrodes into the recording unit, the system is ready for SEPs measurements.

Most commercially available SEPs systems allow to choose between stimulation with either constant voltage or constant current. For the direct median nerve stimulation we prefer to use a constant current in the range from 1 to 4 mA. For each measurement, 100 stimuli are delivered at a rate of 1–10 Hz. These rates are considered optimal in most textbooks. However, it is worth to mention that lower rates are recommended for SEPs peaking more than 25 ms after stimulation, which is the case in sheep median nerve evaluation. In our laboratory, we use a rate of 4.5 Hz. It is important not to choose frequency of stimulation a multiple of 60 Hz. This can result in AC current interference from electrical devices in the operating room. Duration of stimulus should be in range 0.2–0.5 ms.

Each examination consists of three sequential measurements. After completing the data collection, average latency and amplitude values are calculated.

Maximal Nerve Conduction Velocity Measurements

Bipolar hook stimulating and recording electrodes are placed 1 cm proximally and 1 cm distally to the repair sites and the exact distance between electrodes is measured to calculate the conduction velocity (Fig. 52.1). After placing and connecting the electrodes, the system is ready for measurements. The position of the stimulating electrodes can be confirmed by turning on the stimulation and observing hoof twitching as a result of flexor carpi radialis activity. Stimulus intensity is adjusted to obtain the lowest threshold for the muscle contraction.

Following stimulation, the signal latency and amplitude are recorded from the distal electrodes. The examination is repeated three times and average values are used to calculate maximal NCV using the following formula:

$$\text{maxNCV} = \frac{\text{distance between Es and Er}}{\text{L}} \left[\frac{\text{m}}{\text{s}} \right]$$

Where:

maxNCV = maximal nerve conduction velocity

Es = proximal stimulating electrode

Er = proximal recording electrode

L = average latency between stimulus and recorded action potential

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