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A Comparative Study of Recording Procedures for Motor Evoked Potential Signals

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***Abstract*—Motor evoked potential (MEP) signals serve as an objective measure of the functional integrity of motor pathways in the spinal cord. Hence, they provide a reliable assessment of the extent of spinal cord injury (SCI). There are two methods currently being used for serial MEP recordings in rats: a low-frequency and a high-frequency method. In this paper, we compared the two methods and determined the better method for MEP recordings. We also compared the effect of two anesthetic agents – inhalational isoflurane and intraperitoneal ketamine – on the MEP signals. We found that under ketamine anesthesia, low-frequency stimulation led to more consistent results, while high-frequency stimulation required greater stimulation intensity and was prone to unwanted side-effects including excessive head twitches. We further found that isoflurane anesthesia severely depressed the MEP response for both low-frequency and high-frequency stimulation which rendered the resulting signal unusable.**

I. Introduction

SPINAL cord injury (SCI) is a leading cause of sensory and motor impairment [1]. The development of objective methods that analyze the extent of injury to the spinal cord is, therefore, essential to track the progress of various therapies that attempt to control and treat spinal cord injury. Motor evoked potentials provide such an objective measure to analyze the health of the spinal cord with reliability [2].

Motor evoked potential (MEP) is the electrophysiological response of the nervous system to stimulation of the motor system. It was first described in 1980 by Merton and Morton [3]. As a response to the electrical stimulation of the motor cortex, MEP signals can be recorded at the peripheral muscle through electromyographic (EMG) recording of the muscle contraction [4], [5].

Unlike subjective behavioral studies which are prone to variability and lack of rigor, recording and analysis of MEP signals provides a quantifiable and repeatable measure of integrity and functionality of motor pathways through the spinal cord [6], [7]. The determination of a consistent and repeatable method for the recording of MEPs is, therefore, critical for the use of these MEP recordings in SCI research, and is, as a result, an investigation worth pursuing.

Two major methods have been outlined in the literature for serial MEP recordings: 1) a low-frequency stimulation method proposed by Schlag, *et al.* [8], and 2) a high-frequency stimulation method proposed by Kakinohana, *et al.* [9]. While both methods are being used for MEP recording, there exists, to the extent of our knowledge, no literature regarding which method provides for a clearer and more reliable MEP signal. This lack of comparative information is compounded by the degree of difference between the two methods in terms of stimulation parameters, particularly the frequency of the pulse trains used for stimulation. Method 1 uses low-frequency impulse trains of approximately 15.1 Hz, while method 2 uses high-frequency impulse trains that are at 500 Hz [8], [9].

While isoflurane has been previously shown to depress MEP signals [10], its ease of use within a laboratory setting indicates that further investigation into its viability in MEP recording is justified. Further, maintenance of a constant anesthesia depth is easily possible with isoflurane as it is an inhalational anesthetic. Therefore, a greater degree of consistency with regard to recording parameters is possible. Ketamine, on the other hand, must be injected into a rat, which makes the maintenance of a constant depth of anesthesia difficult. However, ketamine has been shown to not significantly affect MEP signals [11], and therefore provides for a 'best case' comparison of the two stimulation methodologies.

In this paper, we present a comparison of the two methods of stimulation under two different types of anesthesia – inhalational isoflurane and intraperitoneal ketamine. In evaluating the success of a particular method, we attempt to maximize the consistency and the amplitude of the MEP signal. We also attempt to minimize the stimulation intensity required to produce an observable limb twitch as well as any side effects of stimulation such as excessive twitching of the head and ears.

II. Materials and Methods

All experimental procedures were in accordance with the guidelines provided in the Rodent Survival Surgery manual and were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University.

A single adult female Lewis rat (Charles River Laboratories, Inc.), with a body weight of 380 gm, was used for this study. The rat was housed individually in a cage and had free access to food and water. Multiple experiments were performed on the rat to test repeatability and consistency.

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A. Anesthesia

An adequate level of anesthesia was determined by monitoring the corneal reflex and limb withdrawal to painful stimuli.

1) Ketamine Anesthesia: A mixture of 45 mg/ml of ketamine and 5 mg/ml of xylazine was administered via intra-peritoneal injection.

2) Isoflurane Anesthesia: The rat was held in a transparent chamber with 3% isoflurane and room air flow until the onset of drowsiness. Its mouth and nose was then placed within an anesthesia mask with a well-fitting rodent size diaphragm, which was connected to a C-Pram circuit designed to deliver and evacuate the gas through one tube. A mixture of 1.5% isoflurane, 80% oxygen and room air was delivered to the mask at the rate of 2 L/min for anesthesia.

B. Electrode Implantation

The rat was anesthetized and its head region was shaved. An incision was then made along the midline of the skull. The cranium bone was cleaned by removing the tissue under the skin.

Five burr holes were drilled into the exposed part of the cranium, using a standard dental drill (Fine Science Tools, North Vancouver, BC, Canada). Four of these holes corresponded to the stimulation sites for hindlimbs and forelimbs on each hemisphere, as shown in Fig. 1. Forelimb sites were located 0.2 mm posterior to bregma and 3.8 mm laterally from the bregma, and hindlimb sites were located 2.5 mm posterior to bregma, and 2.8 mm laterally from the bregma. The fifth hole was drilled on the right frontal bone, situated 2 mm from both the sagittal and coronal sutures, to serve as an intracranial reference, as shown in Fig. 1. Transcranial screw electrodes (E363/20, Plastics One, Inc., Roanoke, VA) were then screwed into the holes. Care was taken to ensure that they made very light contact with the dura mater, and did not put pressure on the brain tissue.

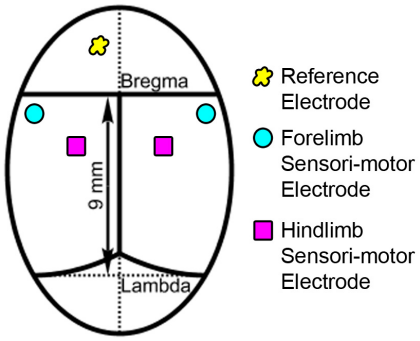

Fig. 1. Illustration of screw electrode placement on the rat's cranium

The distal end of each electrode was inserted into a slot of an electrode pedestal (MS363, Plastics One Inc., Roanoke, VA). Carboxylate dental cement (Durelon Carboxylate Cement, 3M ESPE, St. Paul, MN) was used to hold the screw electrodes and the electrode pedestal in place; and to secure them for long-term stimulation for cortical MEPs. After hardening of the cement, the skin incision was closed with a 4-0 suture.

C. Stimulation

An isolated constant current stimulator (DS3, Digitimer Ltd., Hertfordshire, England) was used for electrical stimulation of the cortex. The stimulator was triggered using an RP2.1 Real-Time Processor (Tucker-Davis Technologies, Alachua, FL) with the OpenEx Suite (Tucker-Davis Technologies, Alachua, FL).

Stimulation was performed with a cortical screw as the anode and the frontal screw as the cathode. In the present experiment, we used the forelimb sensori-motor screw electrode in the left hemisphere, which corresponded to the right forelimb; though the procedure outlined is equally applicable to any limb. The overall inter-stimulus frequency was set to be 0.2 Hz.

1) Low-Frequency Stimulation: The pattern of stimulation used was that proposed by Schlag, *et al.* [8]. We used short trains of low-intensity impulses (100 µsec duration) at a low frequency of 15.1 Hz. The number of impulses in each train and the stimulation intensity was varied in order to determine the parameters for stimulation. The stimulation intensity was increased in 1 mA increments within a range of 5-12 mA, until a train of 14 impulses elicited an observable twitch in the right forelimb. At this point, the intensity was recorded as the threshold stimulation intensity [8]. The recording was performed under supra-maximal stimulation, with intensity 10% above the threshold intensity. Our experiments suggested a stimulation intensity of 5 mA for ketamine, and 12 mA for isoflurane. The number of impulses was then reduced to the minimum necessary to produce an observable twitch. For stimulation under ketamine anesthesia, this was found to be 6 pulses, while under isoflurane anesthesia, this was found to be 14 pulses.

2) High-Frequency Stimulation: The pattern of stimulation used was that proposed by Kakinohana, *et al.* [9]. We used short trains of low-intensity impulses (50 µsec duration) at a high frequency of 500 Hz. Each train consisted of 5 impulses. The stimulation intensity was increased in 1 mA increments within a range of 5-12 mA, until a twitch was observed in the right forelimb. This was observed at 7 mA for recording using ketamine, and at 12 mA for recording using isoflurane.

D. Recording

Subdermal needle electrodes (Safelead F-E3-48, Grass Technologies, West Warwick, RI) were used to record the MEP signals. The recording electrode was inserted into the belly of *extensor digitorum communis* muscle in the right forelimb of the rat, with reference electrode inserted into the footpad. The ground electrode was inserted subdermally over the dorsum of the neck.

The signals were recorded using an RA4LI Low-Impedance Headstage (Tucker-Davis Technologies, Alachua, FL) with a gain of 20, which connected to an RA4PA Medusa PreAmp (Tucker-Davis Technologies, Alachua, FL) via a standard 25-pin connector. The signal was digitized at 4882.8125 Hz on the preamplifier and sent over a fiber optic link to an RA16 Medusa Base Station (Tucker-Davis Technologies, Alachua, FL). The recording was performed for a total of 2 minutes.

All signal processing was performed using Matlab 7.0 (The Mathworks, Inc., Natick, MA). The signal to noise ratio was improved using moving average of 10 stimulus-locked sweeps (time-locked with respect to stimulation time), advancing the moving average window by 2 sweeps every time. This is commonly used to smooth out short-term fluctuations in time-series data, and has been previously utilized for somatosensory evoked potentials (SEPs) [12].

E. Coefficient of Variation

The peak-to-peak amplitudes of the MEP responses for all the methodologies were calculated. The variability in the response for each methodology was quantified using the coefficient of variation c_v, which is defined as the ratio of the standard deviation σ to the mean μ:

$$c_v = \frac{\sigma}{\mu} \tag{1}$$

This parameter is a normalized measure of the dispersion.

III. Results

The results for low-frequency and high-frequency stimulation methods are shown in Fig. 2 and 3 respectively, with 7 averaged signals and a grand average plotted upon each other, and the stimulus artifacts marked.

A. Low-Frequency Stimulation

1) Ketamine Anesthesia: Low-frequency stimulation under ketamine anesthesia produced excellent MEP signals, as shown in Fig. 2a. Stimulation parameters were reduced to 5 mA and 6 pulses without degrading the quality of the MEP signal or the strength of the limb twitch observed. Moreover, there was no twitching of the head or the ears. The characteristic polyphasic shape of the MEP signal was consistent throughout the recordings. The peak-to-peak amplitude of the MEP signal was found to be 384.60 ± 13.84 µV with very low variability of 3.60% (coefficient of variation = 0.0360).

2) Isoflurane Anesthesia: The use of isoflurane anesthesia severely diminished the MEP response, as shown in Fig. 2b. Even at maximum stimulation parameters of 12 mA and 14 pulses, only a faint twitch was observed and the characteristic polyphasic shape of the MEP signals was highly disturbed. The peak-to-peak amplitude of the response was found to be 31.68 ± 14.56 µV with high variability of 45.96% (coefficient of variation = 0.4596).

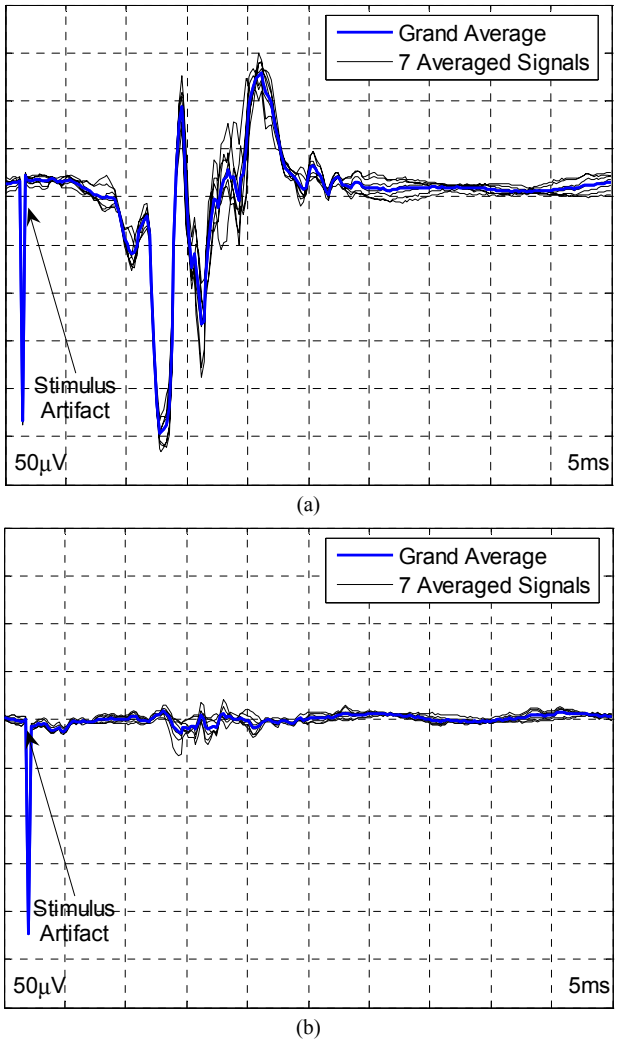

Fig. 2. Signals recorded in response to short trains of (a) 6 low-frequency impulses (100µsec, 15.1Hz, 5mA) using ketamine anesthesia; (b) 14 low-frequency impulses (100µsec, 15.1Hz, 12mA) using isoflurane anesthesia. Since the stimulation was at a low frequency, the response to only the last impulse (6^{th} in ketamine and 14^{th} in isoflurane) is shown. x-axis: 5ms/division; y-axis: 50µV/division.

B. High-Frequency Stimulation

1) Ketamine Anesthesia: High-frequency stimulation under ketamine produced an adequate MEP signal as shown in Fig. 3a. The characteristic polyphasic shape of the MEP signal was present with high distortion. As compared to the low-frequency stimulation method, the peak-to-peak amplitude of the MEP signal was reduced to 215.62 ± 17.67 µV with a variability of 8.19% (coefficient of variation = 0.0819). Further, a higher stimulation intensity of 7 mA was necessitated. This stimulation method also caused significant side-effects including severe head and ear twitches.

2) Isoflurane Anesthesia: Using the high-frequency stimulation method, isoflurane completely removed all traces of the MEP response, as shown in Fig. 3b. No twitch was observed, even at high stimulation intensity of 12 mA, and the only observable effect on the rat was excessive head twitching.

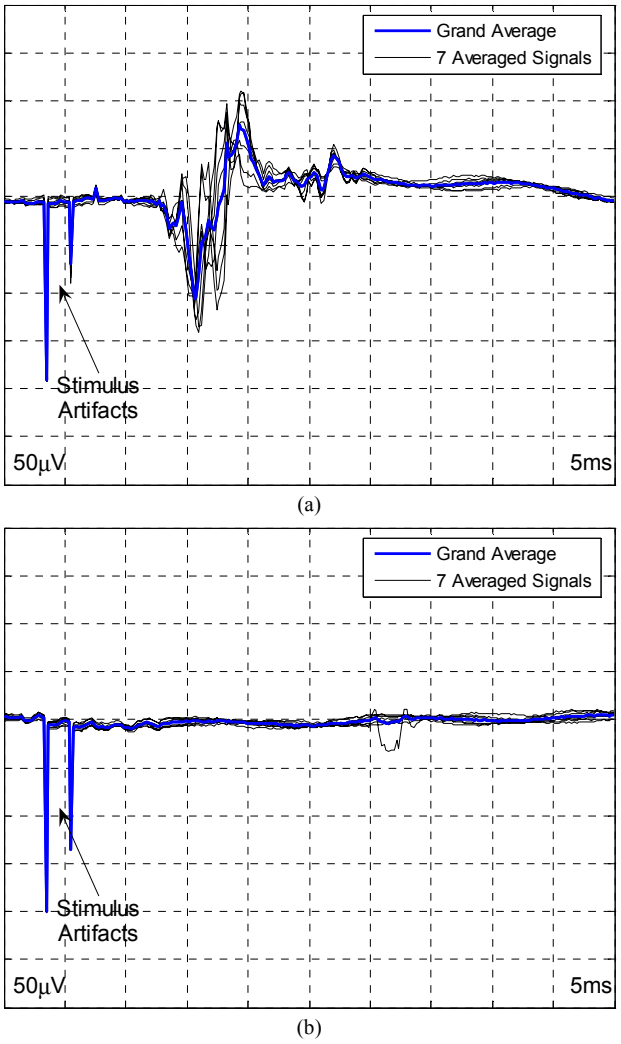

Fig. 3. Signals recorded in response to short trains of (a) 5 high-frequency impulses (50µsec, 500Hz, 7mA) using ketamine anesthesia; (b) 5 high-frequency impulses (50µsec, 500Hz, 12mA) using isoflurane anesthesia. Since the stimulation was at a high frequency, the response to the entire sequence of pulse is shown. x-axis: 5ms/division; y-axis: 50µV/division.

IV. Conclusion & Discussion

The aim of this paper was to present a comparative study of the different recordings procedures for MEP signals. The low-frequency stimulation method under ketamine anesthesia was found to perform the best. This method was reasonably consistent, and did not suffer from side-effects like excessive twitches of the head or the ear. We therefore plan to use this method to investigate the effect of contusion spinal cord injuries on the motor pathways of the spinal cord, and to also examine any possible recovery due to therapeutic interventions such as stem cell therapy.

This method can be easily adapted to allow for multi-limb MEP recording using current screw electrode placements on the rat's cranium. This would allow for measurements and comparison of physiological integrity of motor pathways on both sides of the spinal cord. It could also be used to examine the effect of unilateral spinal cord injury. Further, this potentially has repercussions in allowing for accurate analysis of thoracic spinal cord injury, which is most commonly used in experimental SCI research. In such a model, the rat forelimbs would serve as a control, as their functioning should not be affected by an injury at the thoracic level. MEP recording from the hindlimbs would then allow for nuanced analysis that would not be affected by other factors such as the anesthesia level.

Further, the placement of cranial screws used for stimulation in this paper is identical to our placement of screws required for recording of somatosensory evoked potentials (SEPs) [12]. As a result, it would be possible now to track the SEP and MEP responses due to spinal cord injury on the same rat over a period of 8 weeks or more to assess the integrity of both the sensory and motor pathways in the spinal cord.

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