



Multi-limb acquisition of motor evoked potentials and its application in spinal cord injury

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ABSTRACT

The motor evoked potential (MEP) is an electrical response of peripheral neuro-muscular pathways to stimulation of the motor cortex. MEPs provide objective assessment of electrical conduction through the associated neural pathways, and therefore detect disruption due to a nervous system injury such as spinal cord injury (SCI).

In our studies of SCI, we developed a novel, multi-channel set-up for MEP acquisition in rat models. Unlike existing electrophysiological systems for SCI assessment, the set-up allows for multi-channel MEP acquisition from all limbs of rats and enables longitudinal monitoring of injury and treatment for *in vivo* models of experimental SCI.

The article describes the development of the set-up and discusses its capabilities to acquire MEPs in rat models of SCI. We demonstrate its use for MEP acquisition under two types of anesthesia as well as a range of cortical stimulation parameters, identifying parameters yielding consistent and reliable MEPs.

To validate our set-up, MEPs were recorded from a group of 10 rats before and after contusive SCI. Upon contusion with moderate severity (12.5 mm impact height), MEP amplitude decreased by $91.36 \pm 6.03\%$. A corresponding decline of $93.8 \pm 11.4\%$ was seen in the motor behavioral score (BBB), a gold standard in rodent models of SCI.

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1. Introduction

The motor evoked potential (MEP) is the peripheral nervous system's response to an external electrical or magnetic stimulus in the motor cortex (Nashmi et al., 1997; Haan and Kalkman, 2001). Changes in MEP waveform shape and amplitude have been shown in many studies to exhibit significant correlation with spinal cord injury, neurological deficit (Patil et al., 1985; Fehlings et al., 1989), and recovery (Nashmi et al., 1997; Onifer et al., 2007b). In two early studies, Patil et al. showed that transection of the spinal cord caused disappearance of the MEP, while Fehlings et al. showed a relationship between the severity of the spinal cord injury induced and the amplitude of the MEP.

Over time, MEP recording has been applied to various clinical situations in order to increase understanding of spinal cord motor pathways. In two such recent studies, Diehl et al. assessed facilitation of lower limb MEPs to improve diagnosis of incomplete spinal cord injury, and Gagliardo et al. showed that MEPs were sig-

nificantly different in patients with multiple sclerosis as opposed to controls (Diehl et al., 2006; Gagliardo et al., 2007). However, such application is constrained by the difficulties in obtaining a consistent MEP signal, and by the lack of a standardized protocol for recording of MEPs in *in vivo* experimental research studies. In experimental or animal-based MEP research, many different approaches have been used for MEP recording. For example, Schlag et al. have used protocols with low-frequency cortical stimulation, while Kakinohana et al. used comparatively higher-frequency stimulation (Schlag et al., 2001; García-Alías et al., 2006; Kakinohana et al., 2007).

In the context of spinal cord injury (SCI), MEP monitoring is important since it can provide direct assessment of the electrical function of the spinal cord motor pathways (Nashmi et al., 1997; Gruner and Yee, 1999; Cao et al., 2005; Lee et al., 2007). This may be used to examine the effects of graded spinal cord injury, as in studies by Nashmi et al. or by Cao et al. or to examine the efficacy of various therapeutics as in studies by Gruner et al. and Lee et al. Moderate and severe injuries to the spinal cord do not always destroy the entire neuro-pathways at and around the epicenter of impact. After injury, a small number of axons may retain their anatomical continuity, although they may not be functional. MR-Imaging is used for identification of this "functional incompleteness" and "anatomical completeness", allowing treatment to be designed; its

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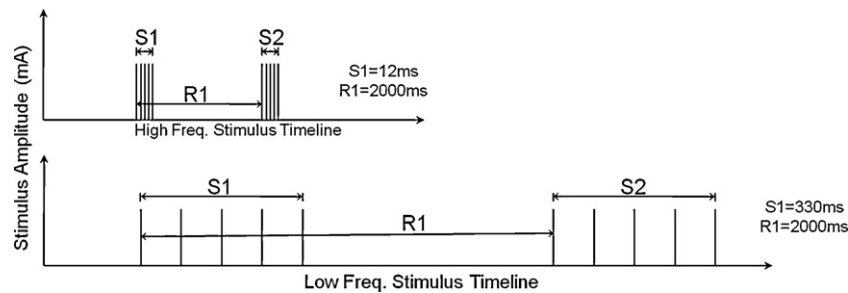


Fig. 1. The figure shows a schematic time course of the two stimulation protocols used in this study, high-frequency (500 Hz; top) and low-frequency (15.1 Hz, bottom). S denotes the stimulus interval and R denotes response interval, or the 'sweep time' during which an MEP signal can be recorded. It should be noted that successive pulse trains were used to stimulate each of the four limbs so that each fourth stimulus evokes a response from the same limb again.

effectiveness can be monitored using MEPs (Bunge et al., 1993; Kakulas, 1999, 2004; Fawcett, 2002).

Similar electrophysiological monitoring is performed using somatosensory evoked potentials (SSEPs), which are a measure of the functional integrity of the sensory pathways of the spinal cord. Such evoked potential studies, as performed by García-Álías et al., Lee et al., Al-Nashash et al. and others are generally longitudinal; with evoked potential monitoring being performed both before and after the rat is injured (García-Álías et al., 2006; Lee et al., 2007; Onifer et al., 2007b; Agrawal et al., 2009a; Al-Nashash et al., 2009; All et al., 2009). However, these studies generally do not record evoked potentials from all four limbs and as a result do not provide a complete picture of spinal cord integrity. The development of a multi-channel set-up for evoked potential recording is therefore important. The multi-channel system would be particularly applicable to models of SCI that induce paraplegia. In these models, only hindlimb function is affected. However, there is need for quality control of the MEP changes and unless forelimb MEP signals can be recorded in conjunction with hindlimb MEP signals, it is difficult to determine whether any change in hindlimb MEP signals is directly correlated with the injury, or with other injury parameters that may not be related to contusion.

In this article we describe a set-up to record multi-channel MEPs in rats from all the four limbs. This method uses a set of cranial electrode positions, which allows for concurrent recording of MEPs and SSEPs from the same rat. In developing this method, we draw upon two previous methods for electrical MEP stimulation (1) a low-frequency stimulation method (15 Hz) (Schlag et al., 2001), and (2) a high-frequency stimulation (500 Hz) (Kakinohana et al., 2007). Fig. 1 shows the stimulus pulse-trains used in both methods. After attempting both methods, we determine a set of stimulation parameters that allows for consistent MEP recording. We then validate our MEP set-up in detecting injury to the spinal cord. To do this, we record MEP signals and perform behavioral assessments using the Basso, Beattie and Bresnahan (BBB) locomotor test for a group of 10 rats. The BBB test is a motor behavioral assessment method, initially presented by Basso et al. that subjectively quantifies locomotive deficit in rats; it is widely used in rodent models of spinal cord injury basic research as discussed by Brösamle et al. and Onifer et al. in two reviews of the use of animal models to study spinal cord injury (Basso et al., 1995, 1996; Brösamle and Huber, 2006; Onifer et al., 2007b). We then examine the effect of contusive injury generated by the NYU-MASCIS impactor on MEP signal amplitude and BBB score by comparing baseline and day 1 post-injury values.

2. 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 (IACUC) at the Johns Hopkins University.

A total of 10 adult female Fischer rats, with a mean body weight of 220 ± 20 g, were used. Rats were housed individually and had free access to food and water. After injury, their bladders were expressed regularly for up to 5 days with no complications or infections observed. During the survival period, no sign of autotomy or autophagy was observed. The rats were also placed on a homeothermic blanket system (Harvard Apparatus Ltd., Kent, UK) to maintain their body temperature at 37 ± 0.5 °C, as measured by a rectal probe, throughout the entire experiment. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA) was applied to the rats' eyes to prevent drying.

Electrode implantation: MEP electrode implantation was performed 2 days prior to contusive injury and was performed using a mixture of Ketamine (75 mg/kg), Xylazine (10 mg/kg) and Atropine (0.3 mg/kg), 0.14 ml of which was administered via intra-peritoneal injection 10–15 min prior to surgery. Following anesthesia, a midline incision was made on the rat's head skin, the tissues underneath were cleaned and the cranium exposed. Five holes were drilled in the skull, as shown in Fig. 2, using a standard dental drill (Fine Science Tools, North Vancouver, BC, Canada).

Screw electrodes (E363/20, Plastics One, Inc, Roanoke, VA) were implanted to a depth of 0.75 mm. Care was taken to ensure that the electrodes made light contact with, but did not put pressure on or puncture the dura mater. Forelimb screw electrodes were inserted 0.2 mm posterior to bregma, and 3.8 mm lateral to the midline. Hindlimb screw electrodes were inserted 2 mm posterior to the bregma and 2 mm lateral to the midline. During determination of the stimulation parameters, a reference electrode was inserted in one hemisphere, in the right frontal bone, 2 mm from the coronal and sagittal sutures. After determination of appropriate stimulation parameters, the position of this reference electrode was changed in order to allow for concurrent recording of SSEPs and MEPs using the same electrode arrangement. This new reference electrode position was then used in validation of the recording procedure. The new reference electrode was inserted 4 mm lateral to the midline and 6 mm posterior to the bregma, in the occipital bone. The distal end of each electrode was then inserted into a slot on an electrode pedestal (MS363, Plastics One, Inc, Roanoke, VA). Carboxylate dental cement (Durelon Carboxylate Cement, 3M, ESPE, St. Paul, MN) was then used to hold the screw electrodes and the pedestal in place, securing them for long term stimulation of MEPs.

The electrode implantation was done with high level of precision by an expert micro-surgeon with many years of experience of rat surgery with many publications (Dr. All) (Agrawal et al., 2009b, 2010a,b). Although the positional accuracy with respect to anatomy is hard to characterize, the reproducibility of our MEP signals across

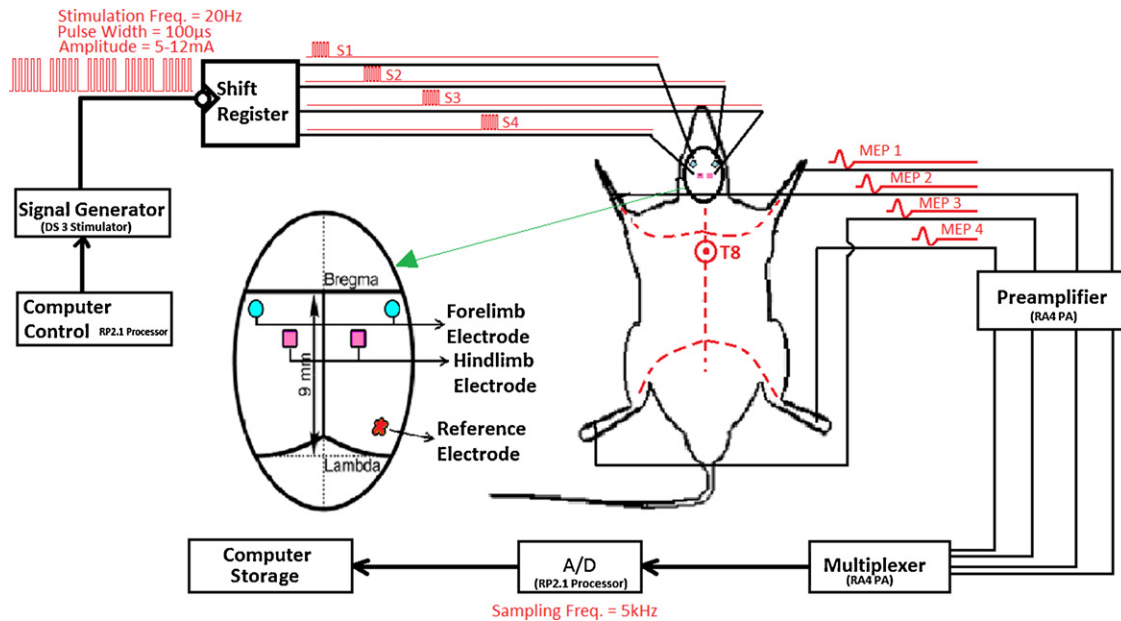


Fig. 2. A schematic of the experimental set-up for the acquisition of the multi-limb motor evoked potentials (MEP). The rat skull was implanted with a set of 5 screw electrodes making light touch with the dura. The stimulation was generated with the help of a computer controlled signal generator and delivered in the form of 4 successive pulse-trains (S1–S4; freq. 20 Hz, pulse width 100 μ s, amplitude 5–12 mA). The four-channel bipolar MEPs were recorded with 4 sub-dermal electrodes, amplified and digitally sampled at 5 kHz and stored for further analysis. The signals were acquired before and after the contusive spinal cord injury (at the thoracic vertebra T8) induced using the NYU impactor with an impact height of 12.5 mm.

different animals and across different trials in a single animal, is indicative of this precision.

The contusive injury: Following intra-peritoneal anesthesia, the back region of the rat was shaved and aseptically prepared with Chlorhexidine (Phoenix Pharmaceuticals, Inc., St. Joseph, MO). A midline incision was made along the thoracic vertebrae and the skin was opened. The paravertebral muscles at 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, without opening the dura mater. The spinous processes of the vertebrae at T6 and T12 were secured in stabilization clamps to reduce the motion of the spinal column during 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 in contact with the dura. This contact was detected by the completion of a circuit, activating an alarm. The exposed dorsal surface of the spinal cord at the T8 level was then contused with the NYU weight-drop device by dropping a 10 g rod with a flat circular impact surface from a height of 12.5 mm. This was a moderate contusion injury, similar to more than 50% of SCI cases observed in humans. 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 the computer, and there was <0.05% variation between these values, which was statistically insignificant.

Post-injury care: After injury, the muscles were sutured in layers using absorbable 2-0 suture, and the skin was closed with 4-0 suture. All rats were allowed to recover in a warmed cage and food and water was kept easily accessible. The antibiotic Gentamicin (5 mg/kg, intramuscular; Abbott Laboratories, Abbott Park, IL) was administered immediately post-surgery and then daily for 4 days. The analgesic, Buprenex (0.01 mg/kg of 0.3 mg/ml, intramuscular; Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA) was delivered post-surgery and daily for 3 days. After surgery, the rat's bladders were expressed 2 times a day either for the first 4 days or until they regained control of their urination.

Multi-limb MEP: MEP signals were acquired 15 min after induction of anesthesia. During determination of MEP stimulation parameters, two types of anesthesia were tried.

Ketamine anesthesia: A mixture of 45 mg/ml of Ketamine and 5 mg/ml of Xylazine was administered via intra-peritoneal injection.

After determination of stimulation parameters, it was found that the addition of Atropine to the anesthesia cocktail improved MEP amplitude. This is consistent with previous research which indicates that the addition of Atropine to a Ketamine/Xylazine anesthesia cocktail helps reduce the incidence of cardiac arrhythmia and excessive salivation (Green et al., 1981).

Implemented anesthesia: MEP signal validation was therefore performed under a mixture of Ketamine (75 mg/kg), Xylazine (10 mg/kg) and Atropine (0.3 mg/kg), which was administered via intra-peritoneal injection.

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.

During recording, the rat's mouth and nose was then placed within a gas mask with a well-fitting rodent size diaphragm. The rat continued spontaneous breathing throughout the course of the recording.

MEP acquisition: An isolated constant current stimulator (DS3, Digitimer Ltd., Hertfordshire, England) was used for the electrical stimulation of the MEP. This stimulator was controlled by an RP2.1 Real-Time Processor (Tucker-Davis Technologies, Alachua, FL), using the OpenEx software suite (Tucker-Davis Technologies, Alachua, FL), installed on a PC running the Windows XP operating system. MEPs were recorded from each of the limbs using sub-dermal needle electrode pairs (Safelead F-E3-48, Grass Technologies, West Warwick, RI). For each limb, one needle electrode was inserted as a recording electrode into the belly of the *tib-*

ialis anterior (TA) muscle in the case of the hindlimbs and the *extensor digitorum communis* muscle in the case of the forelimbs, and one needle electrode was inserted as a reference electrode into the footpad of the limb. These needle electrodes were connected to an RA16LI headstage, with a gain of 10,000, which was connected to an RA4PA pre-amplifier (both from Tucker-Davis Technologies, Alachua, FL). This pre-amplifier interfaced with an RA16BA Medusa Base Station (Tucker-Davis Technologies, Alachua, FL), which performed the data acquisition at a sampling frequency of 5000 Hz. The Medusa Base Station was also controlled using the OpenEx software suite. MEP stimulation and recording was done separately for each limb, moving clockwise from the right forelimb to the right hindlimb. Fig. 2 shows a schematic of the entire set-up.

Stimulation parameters: Trains of electrical stimulation were used to induce MEP signals. In order to determine MEP stimulation parameters, two stimulus frequencies were used: low-frequency stimulation at 15.1 Hz and high-frequency stimulation at 500 Hz. These stimulation frequencies were chosen through reference to papers by Schlag et al. (2001) and Kakinohana et al. (2007). In both cases, stimulus intensity varied between 5 and 12 mA.

High frequency: A pulse train of 6 pulses was used; this was the lowest number found to produce adequate MEP signals. Pulse width was 100 μ s with an inter-train frequency of 0.5 Hz.

Low frequency: A pulse train of 5 pulses was used, with pulse width of 50 μ s.

After comparing these two approaches, we determined that low-frequency stimulation produced more consistent MEP signals, of higher amplitude. A range of frequencies \sim 15 Hz (10 Hz, 15 Hz, 20 Hz) and pulse numbers \sim 6 (4–10) were then tried. A compromise set of stimulation parameters was determined, which was used for validation of the MEP signal in an SCI model.

Implemented parameters: A pulse train of 5 pulses was used, with pulse width 100 μ s, intra-pulse period of 50 ms, and inter-train frequency of 0.5 Hz. Stimulation intensity was varied between 3.5 and 12 mA.

The MEP signal was recorded for 500 ms after the initiation of each pulse-train, but only the portion of the MEP signal located within 50 ms of the final stimulus pulse was analyzed. 500 ms of recording was performed in order to also allow for analysis of any high-latency changes, no such changes were however observed, which is why 50 ms of the MEP signal was analyzed. Due to the high signal-to-noise ratio, 5 min of MEP recording, or 150 sweeps were found to be sufficient, both for baseline and post-injury recordings. No sweeps were rejected. In order to verify the ability of MEP to detect injury to the spinal cord, MEP recordings were performed on the first day after the contusion injury was performed. Baseline (pre-injury) MEP recordings were also performed.

A notch filter was then used to remove 60 Hz noise from the signals. Running averages, each containing 10 sweeps of the signal were then obtained and analyzed to ensure consistent behavior of the signal throughout the recording cycle. The grand average of all time-locked sweeps was then taken. This average was used for all further analysis.

Behavioral assessment: The BBB motor behavioral test was performed to validate the MEP set-up. One week prior to surgery, all rats were made familiar with the open field area and research assistant personnel at four different instances. Two days prior to the injury, the locomotor function of all animals was assessed using the 21-point open-field (BBB) locomotor rating scale (Basso et al., 1995, 1996). Behavioral assessments were then performed on the first day after the injury. All observations were made by two independent observers, who were unaware of the extent or nature of the injury. Behavioral assessment was performed within a few hours of MEP recording. In our studies, Matlab 7.0 (The Mathworks, Inc.) was used for the statistical analysis.

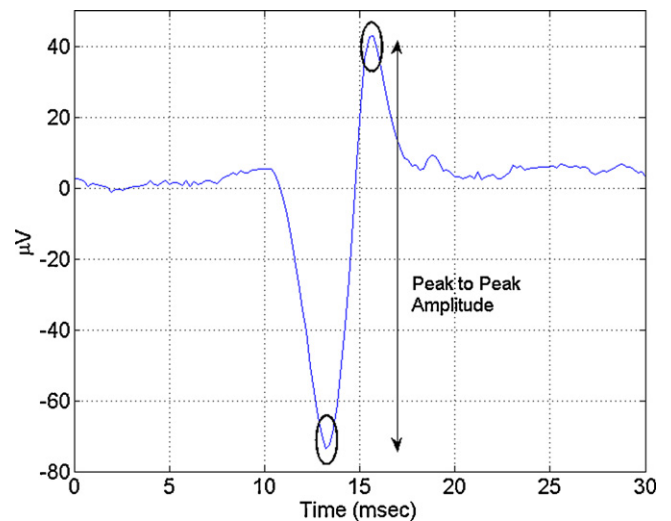


Fig. 3. The figure shows a biphasic MEP signal. The peaks of the signal are circled. Peak-to-peak amplitude obtained from such time-domain analysis is a commonly used measure for MEPs.

Mean peak-to-peak amplitude was calculated for each of the MEP signals, as shown in Fig. 3 by taking the difference in amplitude between the first negative and first positive peak. Percentage change in amplitude from baseline recordings after injury to the spinal cord was then determined and used to validate the ability of MEP signals obtained to detect SCI.

3. Results

Ketamine anesthesia and high-frequency stimulation: High-frequency stimulation produced an MEP signal with amplitude of $215.62 \pm 17.67 \mu\text{V}$ and variability of 8.19% (coefficient of variation = 0.0819). High stimulation intensities of 12 mA were required to obtain MEPs. A representative MEP signal is shown in Fig. 4a.

Isoflurane anesthesia and high-frequency stimulation: In the high-frequency stimulation method, no MEP signal could be seen. A representative MEP figure is shown in Fig. 4b.

Ketamine anesthesia and low-frequency stimulation: Low-frequency stimulation produced MEP signals with amplitude of $384.60 \pm 13.84 \mu\text{V}$ and very low variability between sweeps of 3.60% (coefficient of variation = 0.0360). A representative MEP figure is shown in Fig. 4c.

Isoflurane anesthesia and low-frequency stimulation: The use of Isoflurane anesthesia severely diminished the MEP response, as shown in Fig. 4d. 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 $31.68 \pm 14.56 \mu\text{V}$ with high variability of 45.96% (coefficient of variation = 0.4596). A representative MEP figure is shown in Fig. 4d.

Validation: Time-domain analysis of MEPs obtained at baseline and post-injury show dramatic differences, validating the ability of MEPs obtained through this system to detect SCI. Fig. 5a and b shows representative MEP signals obtained from the right and left hindlimb of a rat. Note the flat nature of the MEP signal after injury, as compared to the polyphasic shape of the baseline MEP signal.

Fig. 6 shows the percentage change in the amplitude of MEPs observed. MEP peak-to-peak amplitude dropped $91.36 \pm 6.03\%$. This is normalized with respect to the baseline amplitude observed for each rat. A paired *t*-test was then performed, to validate that the reduction in MEP amplitude was significant with *p*-value < 0.05 for the hindlimbs.

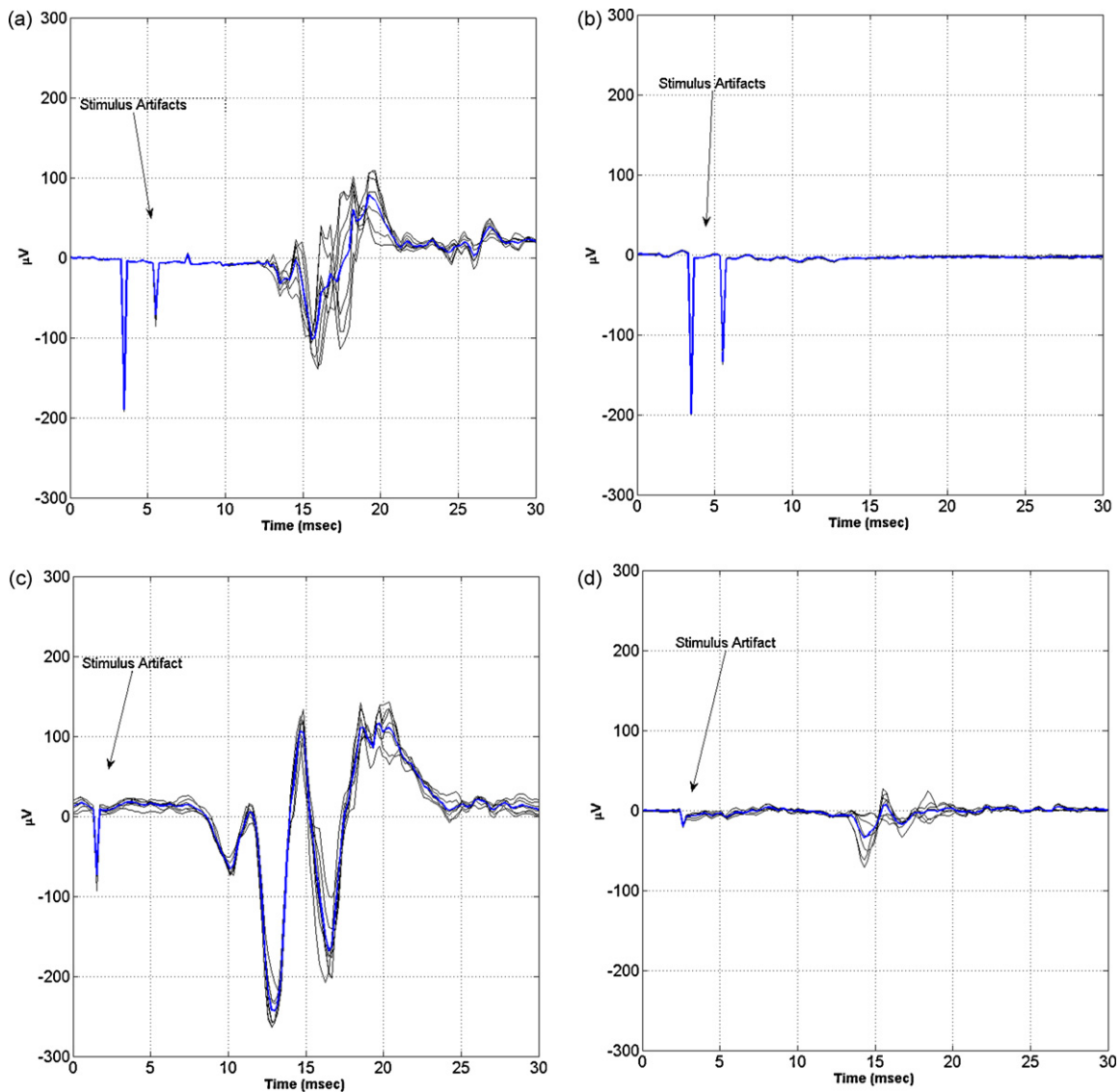


Fig. 4. The figure shows MEP signals obtained using (a) Ketamine anesthesia and high-frequency stimulation, (b) Isoflurane anesthesia and high-frequency stimulation, (c) Ketamine anesthesia and low-frequency stimulation and (d) Isoflurane anesthesia and low-frequency stimulation. Note that low-frequency stimulation under Ketamine anesthesia produces the most consistent and high-amplitude MEP.

Behavioral analysis: Evaluation of open-field locomotor performance using BBB scoring directly correlated with the results obtained with the amplitude analysis. The injury group showed dramatic reduction in locomotion immediately following spinal cord injury. Fig. 6 clearly shows this agreement.

BBB baseline motor behavioral scores were 21 in all rats indicating no impairments or injuries. After the contusion, the average of the BBB scores was 1.3 ± 2.4 , confirming the results obtained with the MEPs recordings.

However, no significant correlation was observed between MEP amplitude variations at the injured stage and BBB score variations at the injured stage. This is possibly due to the narrow nature of the BBB score in the assessment of injured rats. BBB scores varied between 0 and 1 for all but 1 of the rats considered, while MEP percentage amplitudes varied from 1.2% to 15.6% of baseline values. An absence of such correlation is therefore not unexpected.

4. Discussion

The main aim of this study was to determine an experimental protocol that would consistently and successfully record multi-

channel MEPs. As seen in Fig. 5a and b using our multi-channel set-up and Ketamine cocktail anesthesia with low-frequency (20 Hz) stimulation pulses for cortical stimulation, we were able to obtain stable MEPs in the baseline, injury and control groups of our study. Further, the MEP signals obtained decline dramatically after injury.

Some details of our MEP stimulation protocol merit further discussion. Our results suggest that high-frequency stimulation does not produce reliable MEP signals, a result that contrasts with that of Kakinohana et al. (2007). We also found that Isoflurane severely depresses MEP signals, to the point of eradicating them in high-frequency recording. This might be due to the fact that Isoflurane has been shown to depress I-waves of the MEP in previous clinical and experimental studies (Calancie et al., 1991; Hicks et al., 1992; Russell et al., 1994; Ubags et al., 1998; Zhou and Zhu, 2000). Further, we found that a combination Ketamine/Xylazine/Atropine cocktail allows for recording of high-amplitude MEPs during light anesthesia. This is consistent with other findings for a Ketamine/Xylazine cocktail (Green et al., 1981; Zandieh et al., 2003).

Following the comparison of these two methods, we attempted to explore low-frequency stimulation, to determine useful stimula-

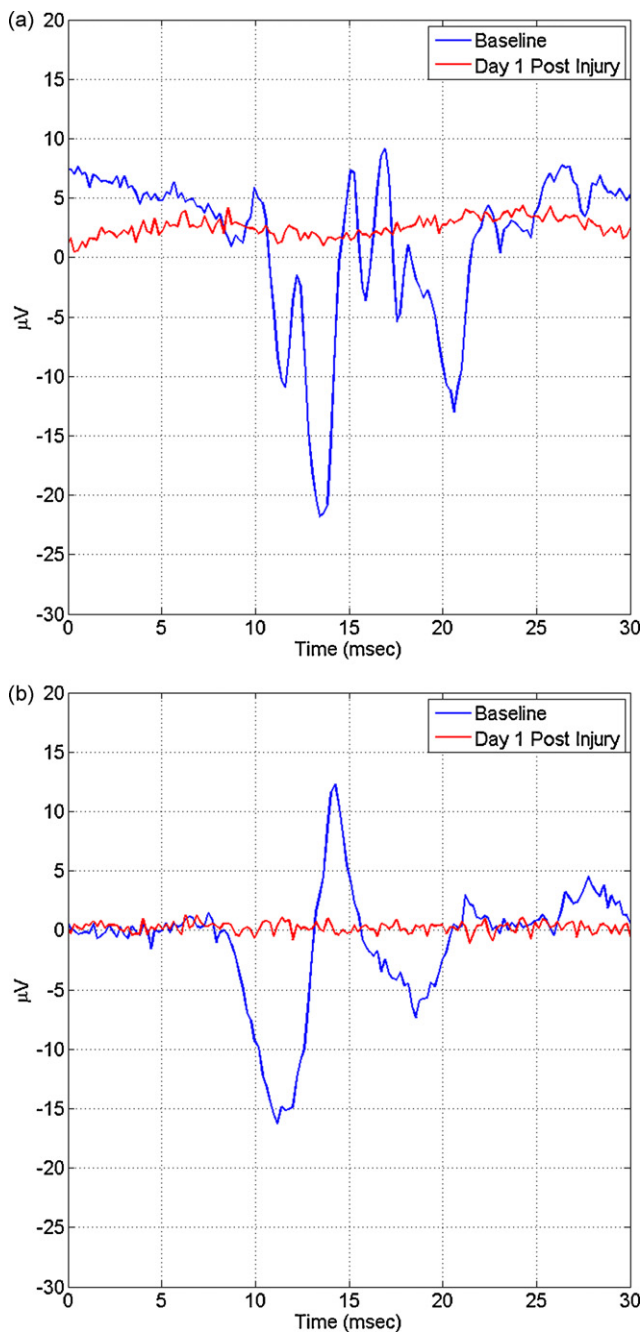


Fig. 5. The figure shows representative MEP signal before and after injury for the (a) left hindlimb and (b) right hindlimb. Note the dramatic reduction in amplitude of the MEP signal following spinal cord injury. These MEP signals were obtained using Ketamine anesthesia.

tion parameters. We observed that stimulus intensity could not be standardized, except insofar as a determination of the correct range of stimulation was possible (5–12 mA). Hindlimb MEP stimulation generally required higher MEP stimulus intensity than Forelimb MEP stimulation, but rat-to-rat variation was common. We therefore did not attempt to standardize this parameter. We however decided that pulse width of 100 μ s was the appropriate parameter to use. Higher pulse width of stimulation was observed to cause excessive twitching of limbs in the rats, during stimulation, and lower stimulus width failed to produce a usable signal. We then attempted to select appropriate stimulation intensities. In our experience, stimulation frequencies >20 Hz produced high levels of twitch in the rat during recording and therefore affected replicabil-

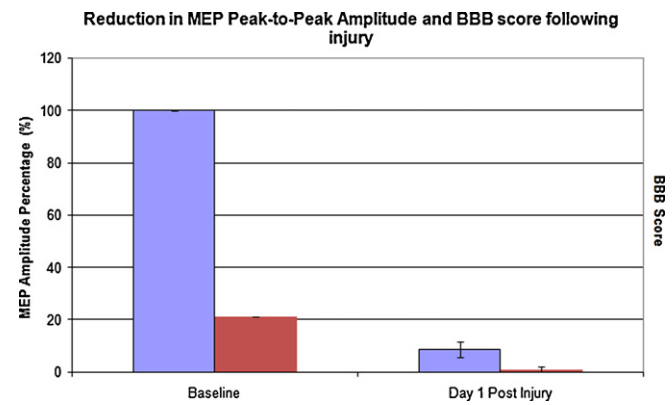


Fig. 6. The figure shows the percentage change in MEP amplitude and the change in BBB score following injury. The error bar indicates the standard deviation of the change. Note the dramatic reduction in MEP amplitude following injury.

ity of the MEP signal. Stimulation frequencies <10 Hz produced an excessive delay between each twitch, preventing improvement in the MEP signal shape within each pulse train, thereby negating the effect of using a pulse train. In our experience, both 15 Hz stimulation and 20 Hz stimulation produced consistent MEP recordings. However, to improve MEP signal development, we decided to use 20 Hz as our stimulation frequency, as such use appeared to provide just enough time for the MEP signal to completely develop in between each stimulus of the pulse train.

The inter-stimulation interval was also varied. No effect of such variation on either experimental quality or MEP signal-to-noise ratio was observed. It should also be noted that as presented, our multi-limb set up can record all four peripheral channels simultaneously. This is important, since the set up could then allow characterization of any electrical “leakage” across neural pathways – especially in the case of injured pathways. Note that the so called “sprouting” of neuronal axons after contusive spinal cord injury – equivalent to electrical shorting of a circuit – is a documented anatomical fact (Hill et al., 2001; Weaver et al., 2001). The sprouting could lead to partial leakage of a single cortical electrical stimulus, through all four peripheral pathways. Our set up will allow characterization of such a leakage due to its ability to record simultaneously from all four limbs.

The second aim of the study was to validate this MEP protocol, and apply it to spinal cord injury. As seen in the results, the maximal MEP amplitude (recorded at around 230 ms after the last stimulus of the train) dropped approximately 90% one day after contusion, indicating the high sensitivity of the MEP signal to injury to the spinal cord.

The BBB open-field motor behavioral test was applied to all the rats before and after the contusion, in order to corroborate and reinforce the results obtained by analyzing the MEPs in the time and time-frequency domain. These scores were in agreement with the MEPs. The rats had a score of 21 (no hindlimb impairment) before the injury and a score in the range of 1 following the contusion.

Note that the BBB scores and electrophysiology with MEP should be viewed as complementary assessments of efficacy of various treatment regimens. The objectivity of MEP recording allows for the confirmation of BBB scores obtained, and ensures that any improvement seen in the functional ability of the rat has an electrophysiological basis and not as a result of the subjective components of the BBB tests.

It is also important to note that the placement of skull electrodes used in this study allows for concurrent measurement of SSEP signals from the rat. While there have been many studies which use either MEPs (Cao et al., 2005; García-Alías et al., 2006; Beaumont et al., 2009) or SSEPs (Nagai et al., 2006; Onifer et

al., 2007a; Nakashima et al., 2008; Saadoun et al., 2008; Agrawal et al., 2009b) to characterize SCI, fewer studies use both methods and record MEPs and SSEPs from the same rat before and after SCI. Concurrent measurement of SSEPs and MEPs is possible using the set-up described in this paper. This provides electrophysiological determination of the functional integrity of both the sensory and motor pathways of the spinal cord in a single rat, and therefore is a more complete view of spinal cord neuro-pathway functionality.

In summary, we have developed and implemented a successful protocol for recording robust MEPs. From our comparison of various anesthesia and stimulation parameters, we have found that cortical current stimulation with a 3.5–12 mA pulse train with pulse frequency of 20 Hz and pulse width of 100 μ s under Ketamine anesthesia yields the best MEP signal in a rat. Use of such a protocol would improve the objectivity of current animal models research with MEP.

Acknowledgments

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References

- Agrawal G, Iyer S, All AH. A comparative study of recording procedures for motor evoked potential signals. Conference Proceedings - IEEE Engineering in Medicine and Biology Society 2009a.
- Agrawal G, Kerr C, Thakor NV, All AH. Characterization of graded multicenter animal spinal cord injury study contusion spinal cord injury using somatosensory-evoked potentials. *Spine (Phila Pa 1976)* 2010a;35(11):1122–7.
- Agrawal G, Sherman D, Maybhate A, Gorelik M, Kerr DA, Thakor NV, et al. Slope analysis of somatosensory evoked potentials in spinal cord injury for detecting contusion injury and focal demyelination. *Journal of Clinical Neuroscience* 2010b.
- Agrawal G, Thakor NV, All AH. Evoked potential versus behavior to detect minor insult to the spinal cord in a rat model. *Journal of Clinical Neuroscience* 2009b;16(8):1052–5.
- Al-Nashash H, Fatoo N, Mirza NN, Ahmed RI, Agrawal G, Thakor NV, et al. Spinal cord injury detection and monitoring using spectral coherence. *IEEE Transactions on Biomedical Engineering* 2009;56(8):1971.
- All A, Walczak P, Agrawal G, Gorelik M, Lee C, Thakor NV, et al. Effect of MOG sensitization on somatosensory evoked potential in Lewis rats. *Journal of the Neurological Sciences* 2009;284(1–2):81–9.
- Basso D, Beattie M, Bresnahan JC. A sensitive and reliable locomotor scale for open field testing in rats. *Journal of Neurotrauma* 1995;12:1–21.
- Basso D, Beattie M, Bresnahan JC. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight drop device versus transection. *Experimental Neurology* 1996;139:244–56.
- Beaumont E, Whitaker CM, Burke DA, Hetman M, Onifer SM. Effects of rolipram on adult rat oligodendrocytes and functional recovery after contusive cervical spinal cord injury. *Neuroscience* 2009;163(4):985–90.
- Brösamle C, Huber AB. Cracking the black-box and putting it together again: animal models of spinal cord injury. *Drug Discovery Today: Disease Models* 2006;3:341–7.
- Bunge RP, Puckett WR, Becerra JL, Marcillo A, Quencer RM. Observations on the pathology of human spinal cord injury: a review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. *Advances in Neurology* 1993;59:75–89.
- Calancie B, Klose K, Baier S, Green BA. Isoflurane-induced attenuation of motor evoked potentials caused by electrical motor cortex stimulation during surgery. *Journal of Neurosurgery* 1991;74(6):897–904.
- Cao Q, Zhang YP, Iannotti C, DeVries WH, Xu XM, Shields CB, et al. Functional and electrophysiological changes after graded traumatic spinal cord injury in adult rat. *Experimental Neurology* 2005;191:3–16.
- Diehl P, Kliesch U, Dietz V, Curt A. Impaired facilitation of motor evoked potentials in incomplete spinal cord injury. *Journal of Neurology* 2006;253(1):51–7.
- Fawcett J. Repair of spinal cord injuries: where are we, where are we going. *Spinal Cord* 2002;40:615–23.
- Fehlings MG, Tator CH. The relationships among the severity of spinal cord injury, motor and somatosensory evoked potentials and spinal cord blood flow. *Electroencephalography and Clinical Neurophysiology* 1989;74:241–59.
- Gagliardo A, Galli F, Grippo A, Amantini A, Martinelli C, Amato MP, et al. Motor evoked potentials in multiple sclerosis patients without walking limitation: amplitude vs. conduction time abnormalities. *Journal of Neurology* 2007;254(2):220–7.
- García-Álías G, Valero-Cabré A, López-Vales R, Forés J, Verdú E, Navarro X. Differential motor and electrophysiological outcome in rats with mid-thoracic or high lumbar incomplete spinal cord injuries. *Brain Research* 2006;1108(1):195–204.
- Green C, Knight J, Precious S, Simpkin S. Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Laboratory Animals* 1981;15(2):163.
- Gruner JA, Yee AK. 4-Aminopyridine enhances motor evoked potentials following graded spinal cord compression injury in rats. *Brain Research* 1999;816(2):446–56.
- Haan Pd, Kalkman CJ. Spinal cord monitoring: somatosensory- and motor-evoked potentials. *Anesthesiology Clinics of North America* 2001;19(4):923–45.
- Hicks R, Woodforth IJ, Crawford MR, Stephen JPH, Burke DJ. Some effects of isoflurane on I waves of the motor evoked potential. *British Journal of Anaesthesia* 1992;69(2):130.
- Hill C, Beattie M, Bresnahan JC. Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. *Experimental Neurology* 2001;171(1):153–69.
- Kakinohana M, Nakamura S, Fuchigami T, Sugahara K. Transcranial motor-evoked potentials monitoring can detect spinal cord ischemia more rapidly than spinal cord-evoked potentials monitoring during aortic occlusion in rats. *European Spine Journal* 2007;16(6):787–93.
- Kakulas BA. A review of the neuropathology of human spinal cord injury with emphasis on special features. *Journal of Spinal Cord Medicine* 1999;22:119–24.
- Kakulas BA. Neuropathology: the foundation for new treatments in spinal cord injury. *Spinal Cord* 2004;42:549–63.
- Lee K, Suh-Kim H, Choi JS, Jeun SS, Kim EJ, K SS, et al. Human mesenchymal stem cell transplantation promotes functional recovery following acute spinal cord injury in rats. *Acta Neurobiologica Experimentalis* 2007;67(1):13.
- Nagai MK, Dabney K, Ehrenshteyn M, Willis D, Twiss J. Experimental distraction spinal cord injury and recovery of motor function. *The Spine Journal* 2006;16(5S):51.
- Nakashima S, Arnold SA, Mahoney ET, Sithu SD, Zhang YP, D'Souza SE, et al. Small-molecule protein tyrosine phosphatase inhibition as a neuroprotective treatment after spinal cord injury in adult rats. *Soc Neuroscience* 2008;28(29):7293–303.
- Nashmi R, Imamura H, Tator CH, Fehlings MG. Serial recording of somatosensory and myoelectric motor evoked potentials: role in assessing functional recovery after graded spinal cord injury in the rat. *Journal of Neurotrauma* 1997;14(3):151–9.
- Onifer SM, Nunn CD, Decker JA, Payne BN, Wagoner MR, Puckett AH, et al. Loss and spontaneous recovery of forelimb evoked potentials in both the adult rat cuneate nucleus and somatosensory cortex following contusive cervical spinal cord injury. *Experimental Neurology* 2007a;207(2):238–47.
- Onifer SM, Rabchevsky AG, Scheff SW. Rat models of traumatic spinal cord injury to assess motor recovery. *ILAR Journal* 2007b;48:385–95.
- Patil A, Nagaraj M, Mehta R. Cortically evoked motor action potential in spinal cord injury research. *Neurosurgery* 1985;16(4):473.
- Russell G, Schwentker M, Graybeal JM. Preservation of neurogenic motor-evoked potentials during isoflurane electroencephalographic burst suppression in rats. *Spine* 1994;19(23):2632.
- Saadoun S, Bell BA, Verkman AS, Papadopoulos MC. Greatly improved neurological outcome after spinal cord compression injury in AQP4-deficient mice. *Brain Research* 2008.
- Schlag MG, Hopf R, Heinz R. Serial recording of sensory, corticomotor, and brainstem-derived motor evoked potentials in the rat. *Somatosensory and Motor Research* 2001;18(2):106–16.
- Ubags L, Kalkman C, Been HD. Influence of isoflurane on myogenic motor evoked potentials to single and multiple transcranial stimuli during nitrous oxide/opioid anesthesia. *Neurosurgery* 1998;43(1):90.
- Weaver L, Verghese P, Bruce JC, Fehlings MG, Krenz NR, Marsh DR. Autonomic dysreflexia and primary afferent sprouting after clip-compression injury of the rat spinal cord. *Journal of Neurotrauma* 2001;18(10):1107–19.
- Zandieh S, Hopf R, Redl H, Schlag MG. The effect of ketamine/xylazine anesthesia on sensory and motor evoked potentials in the rat. *Spinal Cord* 2003;41(1):16–22.
- Zhou H, Zhu C. Comparison of isoflurane effects on motor evoked potential and F wave. *Anesthesiology* 2000;93(1):32.