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Laboratory Study

Evoked potential versus behavior to detect minor insult to the spinal cord in a rat model

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ABSTRACT

Reliable outcome measurement is needed for spinal cord injury research to critically evaluate the severity of injury and recovery thereafter. However, such measurements can sometimes be affected by minor, injury to the spinal cord during surgical procedures, including laminectomy. The open-field Basso, Beattie and Bresnahan (BBB) behavior motor scores are subjective and prone to human error. We investigated somatosensory evoked potential (SEP) as an electrophysiological measure to assess the integrity of the spinal cord after injury. In our experiment, control rats with a minor unintentional spinal cord insult during laminectomy showed a decrease in SEP amplitude by 16% to 18%, which recovered in around 7 days. However, there was no change in the BBB scores for the same animals over the same period. This highlights the sensitivity of SEP to minor insult as compared to BBB. These differences may be beneficial in accurate evaluation of the severity and progression of spinal cord injury, and subsequent recovery.

1. Introduction

In the area of spinal cord injury (SCI) research, there is a need for reliable tools to objectively assess the integrity of the spinal cord, and hence to evaluate the severity of the injury as well as to assess the efficacy of new therapeutic techniques.[1–5] Sometimes, however, wrong conclusions can be made due to small surgical errors that result in a minor insult to the spinal cord, for instance during experimental laminectomy. It is necessary to identify such errors at the right time to obtain correct assessment of the severity of injury or the efficacy of the therapy.[6]

The Basso, Beattie and Bresnahan (BBB) Locomotor Rating Scale, an open-field locomotor test, is one of the most widely used research tools to measure the outcome, progress, and/or recovery of SCI in rats.[7,8] It represents combinations of rat joint movements, hindlimb movements, stepping, forelimb and hindlimb coordination, trunk position and stability, paw placement, and tail position on a scale from 0 to 21.[7,8] Although it is a well-accepted tool, the BBB has certain shortcomings. One major issue is the accuracy/concurrence of two examiners. The other is the occasional unwillingness of the rodent to move around. In addition, the BBB score is an indirect measurement of the consequences of a primary injury to the dorsal (sensory) part of the spinal cord, which is most commonly what is achieved in most research studies.[9,10]

As an alternative, we postulate that electrophysiological measurement, specifically the somatosensory evoked potential (SEP), can serve as an objective and reliable measurement to study the integrity of the spinal cord.[11–15] SEPs are electrical signals in the somatosensory pathways obtained from the somatosensory cortex, in response to sensory stimuli. SEP is used commonly for clinical studies and provides valuable information regarding the functional integrity of the spinal cord. For example, injury to the spinal cord causes demyelination and manifests as a reduction of amplitude or an increase of latency in SEPs. It is a valuable monitoring and diagnostic tool in different phases, from the very acute to the long-term chronic, of SCI research. The SEP reflects the integrity and conductivity of sensory (dorsal) pathways through the spinal cord with high fidelity.[16–19]

2. Materials and methods

2.1. Animals

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

A total of 17 adult female Fischer rats, with an average body weight of 200 g to 230 g, were used. Rats were housed individually and had free access to food and water. Their bladders were expressed regularly with no complications or infections. One week prior to laminectomy, all rats were made familiar with the open field environment and specific research personnel at four different instances.

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2.2. Multi-limb SEP measurement

Rats were anesthetized 1 to 2 days prior to laminectomy, with intraperitoneal administration of 0.2 mL of a mixed solution containing 43 mg/mL of ketamine, 8.6 mg/mL of xylazine, and 1.4 mg/mL of acepromazine. An incision was made on the rat's head and the skull was cleaned by removing the tissue under the skin. Five transcranial screw electrodes (E363/20; Plastics One, Roanoke, VA, USA) were implanted on the somatosensory cortex in each hemisphere receiving input from sensory pathways originating in the hind and forelimbs (Fig. 1). The electrodes made light contact with the dura mater, but did not compress the dura or brain structures. Subcutaneous needle electrodes (Safelead F-E3-48; Grass-Telefactor, West Warwick, RI, USA) were used to electrically stimulate the median and tibial nerves of both left and right limbs (Fig. 2), without direct contact with the nerve bundle.

An isolated constant current stimulator (DS3; Digitimer, Hertfordshire, UK) was used for the electrical stimulation of the limbs. Custom intraoperative neurological monitoring (INM) software (Infinite Biomedical Technologies, Baltimore, MD, USA) was used to set the stimulation parameters and trigger the stimulator. Positive current pulses of 3.5 mA magnitude and 200 μs duration at a frequency of 1 Hz were used for limb stimulation, which sequentially stimulated each of the four limbs at a frequency of 0.25 Hz using an advanced demultiplexer circuit. Cortical SEPs from the transcranial electrodes were amplified by an optically isolated biopotential amplifier (Opti-Amp 8002; Intelligent Hearing Systems, Miami, FL, USA) with a gain of 30,000 and the signals were sampled at 5 kHz.

2.3. Study design

The animals were divided into a control group with laminectomy only ($n = 5$) and four different injury groups ($n = 3$ each) with 6.25 mm, 12.5 mm, 25 mm and 50 mm height contusion injury.

Prior to laminectomy, motor functions of all animals were assessed using the open-field BBB locomotor rating scale. In order to correlate BBB with SEP, the SEP recording was done immediately following the BBB test. Prior to each recording, rats were anesthetized for about 30 min as described in Section 2.2. Body temperature was maintained at 37 ± 0.5 °C using a heating pad. The anesthesia level was kept uniform throughout all experiments. After injury (for control rats, laminectomy only), behavioral tests were performed after day 1, day 4, and then once per week for up to 7 weeks, followed by SEP recordings.

2.4. Laminectomy and injury

A laminectomy was performed between thoracic vertebrae T7 and T9 to expose the dorsal surface of the spinal cord. In two of

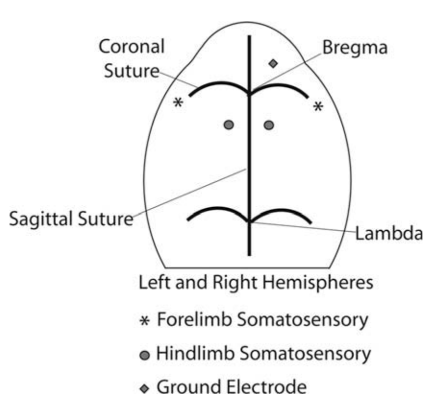

Fig. 1. Rat skull landmarks and positions for 5 recording screw electrodes.

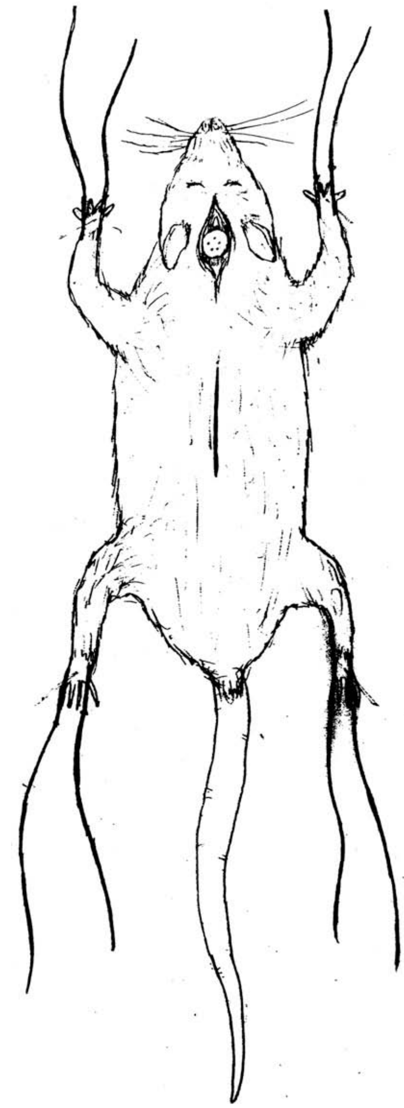

Fig. 2. The position of four pairs of skin needle electrodes for limb stimulation, the cranium somatosensory evoked potential (SEP) recording electrodes pack, and approximate position of the laminectomy incision (dorsal thoracic to lumbar, T5-L3).

the control rats, the surgical tools unintentionally touched and lightly scratched the dorsal spinal cord. Hence we decided to group these two rats separately from the rest of the control rats.

In the injury-group rats, the exposed spinal cord was then injured using the well-known and standardized New York University (NYU)-impactor,[2,9] by dropping a 10 g rod with a flat circular impact surface from graded height levels. The NYU impactor stabilizer clamps were used to immobilize the T6 and T12 vertebrae for supporting the column during impact. The computer recorded the dynamics of the impact trajectory, which was closely monitored for all the injuries.

2.5. SEP analysis

Contralateral SEP recordings were used for analysis. All signal processing was performed using Matlab 7.0.0.19920 (The Mathworks, Natick, MA, USA). The signal to noise ratio was improved by ensemble averaging of 100 stimulus-locked sweeps, with the averaging window shifting by 20 sweeps each time. To account for interanimal variability, the post-surgical SEP amplitudes were expressed as a percentage of the baseline amplitude for each rat.

3. Results

Since the injury was at T8, the analysis was based on the variation of SEP signals from injured pathways (tibial nerves through the spinal cord and cortex) from the hindlimbs. The signals from the forelimbs were used for internal control purposes to ensure the integrity of our recordings, which may be affected by the health of the rat, the quality of the electrodes, and the consistency of measurement at each time point. The SEP signals from the forelimb non-injured pathways (median nerves through the spinal cord and cortex) were expected to be constant at any time during the survival measurement period. A change in SEP signals from the forelimbs would indicate that variables other than the injury were affecting the SEP recordings. However, in our studies, analysis of SEP recordings from the forelimbs showed no change (data not shown).

The hindlimb SEP amplitudes for the control group with laminectomy only are shown in Fig. 3A, B. They show no change in amplitude throughout the entire 7 weeks. However, there is a slight reduction in amplitude by 16% to 18% on day 1 and day 4 in the 2 rats with minor spinal cord insult during the laminectomy. Their SEP signals became almost identical to the baseline recording after the first week of laminectomy, indicating recovery. In comparison, the BBB scores for the same animals remained constant at 21 and showed no decline during the 7 weeks for both the groups, as shown in Fig. 3C, D.

Differences are also seen in the progression of SEP amplitude abnormalities for the different impactor intensities (Fig. 3A, B) along with corresponding BBB scores (Fig. 3C, D). In the 6.25 mm injury group, there is an initial decrease of amplitude followed by a gradual recovery. The amplitude recovers from around 40% of the baseline to around 70%. However, the BBB score shows very slight recovery after the injury. In the 12.5 mm injury group, we see a similar pattern with around 70% reduction in amplitude accompanied by recovery to around 50%. The pattern of motor recovery as indicated by the BBB is definitely not as robust. In the 25 mm injury group, we see a marked depression in SEP amplitude to below 10% of the baseline, followed by a feeble recovery. The BBB shows a large reduction followed by quick resumption of function. A final injury level is achieved in the 50 mm impactor intensity setting, in which the SEP amplitude again drops to very low amplitude followed by almost no recovery. However, the BBB score recovers appreciably even in the rats that are very severely injured.

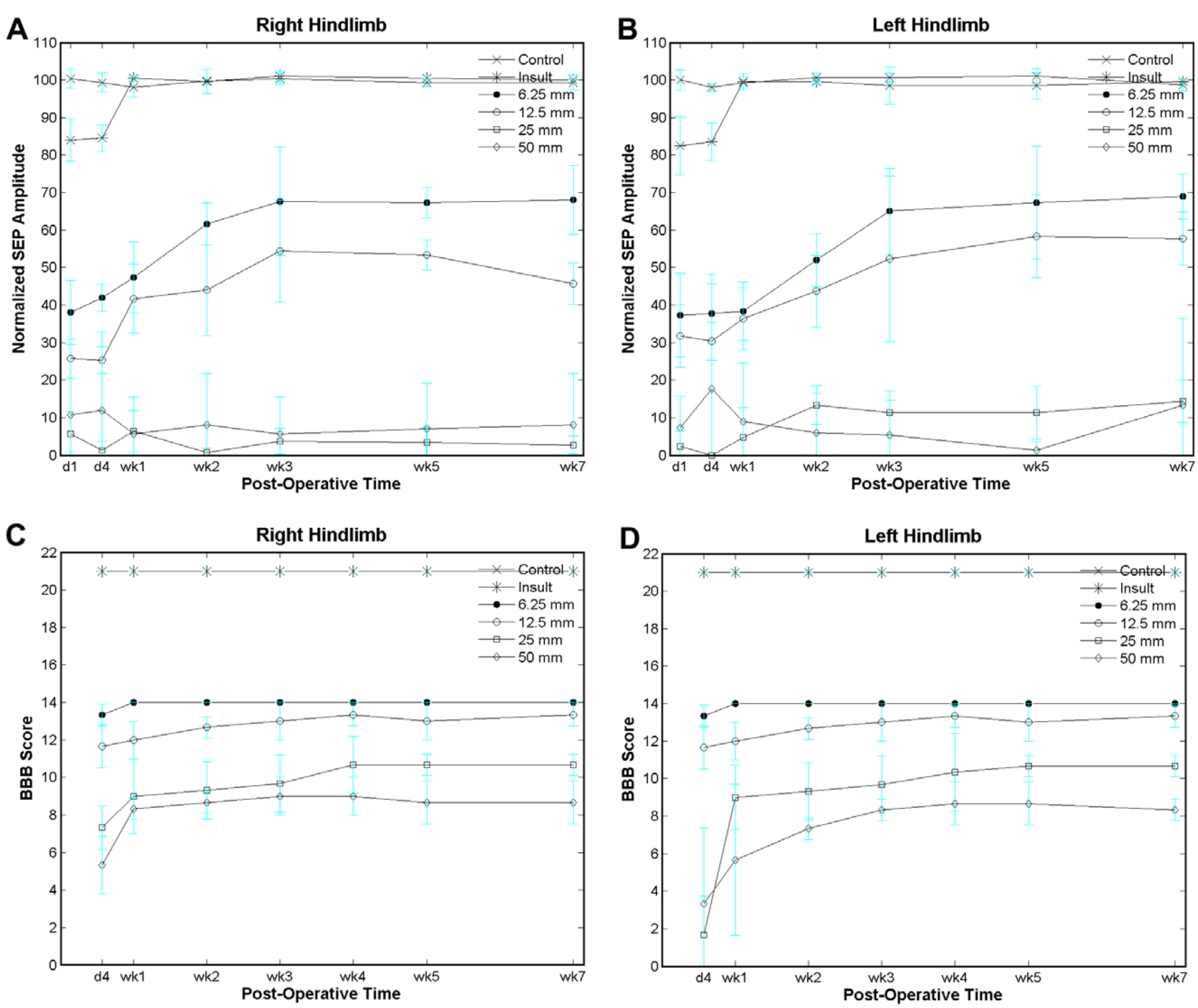

Fig. 3. (A, B) Contralateral normalized somatosensory evoked potential (SEP) amplitudes for the right and left hindlimbs, respectively. (C, D) Basso, Beattie and Bresnahan (BBB) Locomotor Rating Scale scores for the right and left hindlimbs, respectively. X = control (laminectomy only), ∗ = insult, ● = 6.25 mm, ○ = 12.5 mm, □ = 25 mm, ◇ = 50 mm. Bars indicate means ± SD. d = day, wk = weak.

4. Discussion

During laminectomy, two of the rats had minor injuries on the dorsal spinal cord. These were caused unintentionally when the surgical tools softly touched the spinal cord. The result of this phenomenon (Fig. 3A, B) was interesting and led us to compare the two outcome measurement methods, SEP signal analysis and BBB scores, more precisely. The BBB outcome did not show any decline in these animals during the 7 week survival period. However the SEP outcome measurements reflected the element of minor injury in the spinal cord for a few days after the insult, consistently. Interestingly, this situation is comparable to conditions that might occasionally occur in operative rooms during spinal cord surgeries. Our observation validates the need for intraoperative SEP monitoring.[20–22]

Since the results of any therapy in SCI research are based on the assumption that all injuries in the same severity category should have an acceptable range of outcomes and/or decline in central nervous system function, we suggest that injury to the sensory and motor pathways should be distinguished. It is critical to be able to verify the injury to avoid false positives and false negatives. Motor behavior measurements (BBB) score identify the secondary injury caused by injury to sensory pathways on the dorsal spinal cord, and hence are relatively ineffective in evaluating the extent of SCI either immediately after or on the same day as injury. In addition, the BBB may not estimate SCI well during the very acute phase of injury, because of inflammation, muscle and skin incision, and pain. However, our data show that the neuroelectrophysiological assessment using SEP monitoring is responsive under acute circumstances.

SEP analysis also identifies and clearly distinguishes between the recovery and non-recovery groups. No significant recovery in SEP amplitudes was observed in the severe and very severe injury groups, while the mild and moderate injury groups differed significantly in their recovery pattern from the non-recovery groups. BBB recordings showed graded difference among the various injury groups, but did not distinguish between recovery and non-recovery groups.

5. Conclusion

We compared two important outcomes: electrophysiology (SEP) and motor behavior (BBB) measurements in rats with SCI. Our study elucidates certain strengths and weaknesses of each method, and suggests that electrophysiology measurements using SEP provide more detailed information and complement the BBB evaluations.

We showed that SEP monitoring distinguishes very mild insults to the spinal cord such as minor injury during laminectomy, whereas BBB does not. Furthermore, SEP is also an excellent indicator of early changes that BBB is unable to detect. In addition, electrophysiological monitoring shows a greater variability for mild and moderate injury, while the BBB score is similar in all animals from the same group. Lastly, the possibility to record SEP for very severe injury in temporarily paralyzed animals allows us to monitor recovery or effect of therapy. BBB, however, is not feasible as the animals cannot move. Although BBB is relatively easy to execute and takes only 4 minutes, it also has an element of subjectivity, whereas SEP is a more objective, measurable parameter with reliable technology. The weakness of SEP monitoring is that it requires additional electrode preparation, instrumentation, and biosignal processing. Nevertheless, these techniques can be easily replicated and adopted, and lend themselves to standardization. We conclude that SEP should be integrated into SCI research, when possible, and be used to supplement current motor behavior assessment.

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