Spinal Cord Injury Detection and Monitoring Using Spectral Coherence

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***Abstract*—In this paper, spectral coherence (SC) is used to study the somatosensory evoked potential (SEP) signals in rodent model before and after spinal cord injury (SCI). The SC technique is complemented with the Basso, Beattie, and Bresnahan (BBB) behavior analysis method to help us assess the status of the motor recovery. SC can be used to follow the effects of SCI without any preinjury baseline information. In this study, adult female Fischer rats received contusion injury at T8 level with varying impact heights using the standard New York University impactor. The results show that the average SC between forelimb and hindlimb SEP signals before injury was relatively high ($\geq$0.7). Following injury, the SC between the forelimb and hindlimb SEP signals dropped to various levels ($\leq$0.7) corresponding to the severity of SCI. The SC analysis gave normalized quantifiable results for the evaluation of SCI and recovery thereafter using the forelimb signals as an effective control, without the need of any baseline data. This technique solves the problems associated with the commonly used time-domain analysis like the need of a trained neurophysiologist to interpret the data and the need for baseline data. We believe that both SC and BBB may provide a comprehensive and complementary picture of the health status of the spinal cord after injury. The presented method is applicable to SCIs not affecting the forelimb SEP signals.**

***Index Terms*—Basso, Beattie and Bresnahan (BBB) score, New York University (NYU) impactor, somatosensory evoked potentials (SEPs), spectral coherence (SC), spinal cord injury (SCI).**

I. Introduction

THE spinal cord serves as the transmission pathway for all electrical activities, including efferent or motor information and afferent or sensory information, between the central nervous system (CNS) and the peripheral nervous system (PNS). Any injury to the spinal cord, partially or completely, impairs the transmission via these pathways, leading to sensory and/or motor function's loss. Many patients worldwide are living with the devastating effects of spinal cord injuries (SCIs). There are approximately 400 000 SCI patients in the United States alone, with nearly 15 000 new injuries each year [1]. Clearly, there is a need to diagnose SCI [2]–[6] and develop novel therapeutic approaches [7]–[14]. Current approaches to SCI detection include electrophysiological [2]–[4] and imaging [5], [6]. Many therapeutic approaches have been proposed including functional electrical stimulation [7], [8], pharmacologic or genetic therapies [9], cellular and tissue engineering [10]–[12], and stem cell therapy [13], [14].

The existing approaches, like MRI, only give information about the location of the injury and the anatomical damage, and not the functional integrity of the spinal cord [5], [6]. It is important to identify if there are any functional fibers after incomplete SCI. Even a small number of spared fibers, with immediate treatment, can greatly improve the quality of life of patients with SCI. There are other techniques that are more applicable to animal SCI models. These methods include histological analysis and behavioral analysis [15]. Histological analysis may provide detailed information about the injury; however, it is possible only after sacrificing the animal, which does not give any idea about the progress of injury/recovery. The most common behavioral approach in rodent models is developed by Basso, Beattie, and Bresnahan (called the BBB method) [15], [16]. It involves a 4-min observation of a rat in an open field, in which a score from a scale of 0 to 21 is given. The BBB score is an observational measure that reflects the animal's locomotor capabilities. It is well accepted and easy to execute; nevertheless, it is subjective, assesses only motor function, and does not account for the unwillingness of the rodent to move. A complementary objective quantitative assessment method is required to enable researchers in the area of SCI recovery and rehabilitation to accurately and objectively evaluate possible therapeutic mechanisms to reverse and prevent the effects of SCI.

One powerful technique used in SCI studies is the evoked potential, which reflects the electrophysiological response of the neural system to an external stimulus. Of particular interest are the cortical signals recorded in response to sensory stimulation, namely somatosensory evoked potentials (SEP), which have the potential to provide complementary measure to the BBB-based behavioral scoring of the motor function recovery. SEPs are obtained by electrical stimulation of the median or the tibial nerve [17]. This technique, together with other motor pathways testing methods such as the BBB, is used by researchers to evaluate the ongoing neurophysiological changes throughout the recovery period after SCI. Previous studies using SEP data for

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SCI detection have used changes in latency and peak amplitude of SEP signals [17]. The inherent disadvantage of the time-domain analysis of SEPs is that complex signal shape changes make it difficult to locate signal peaks and obtain amplitude and latency trends. Hence, various signal processing methods have been developed to analyze SEP signals including autoregressive algorithms, adaptive latency measurement, and kinematic measures [18]–[20]. However, these methods do not allow a critical comparison between cortical response recordings made through stimulation of different limbs.

In this paper, we propose the use of spectral coherence (SC) to not only allow analysis of varying signal shapes but also comparison between multiple SEP signals before and after SCI [21], [22]. SC is the normalized cross-power spectrum computed between two signals. This method has many advantages such as it: 1) is a normalized quantitative measure; 2) does not require a trained examiner; 3) does not necessarily require the baseline or preinjury signals; and 4) is an objective method. It is also programmable for real-time analysis. This paper presents the SC method, the experimental setup, and the results of our studies in a rodent SCI model. Contusion SCIs were induced in adult female Fischer rats using standardized New York University (NYU) impactor with varying degrees of injury severity. This injury mainly affects the dorsal (sensory) pathways of the spinal cord. The progress of this primary injury may result in further damage to the gray matter and the central part of the spinal cord, and even ventral (motor) pathways in case of severe injuries. This rodent model was used because the injury is reproducible and the evolution of injury is similar to that in humans. The SC method was used to evaluate the sensory function of the spinal pathways, complemented with BBB to assess the locomotor capabilities.

II. Protocol and Data Collection

All experimental procedures were carried out in accordance with the guidance provided in the Rodent Survival Surgery manual and were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University. Fifteen female adult Fischer rats, with an average body weight of 200 g, were used in this investigation. Rats were housed individually per cage and had free access to food and water. Their bladders were expressed regularly and there were no complications or infections. All the surgical procedures were performed under anesthesia (45 mg/kg ketamine and 5 mg/kg xylazine) via intraperitoneal injection (0.1 mL/100 g weight).

Prior to laminectomy, an incision was made on anesthetized rat's head and the cranium bone was cleaned. Four transcranial screw electrodes (E363/20, Plastics One, Inc.) were implanted on the cranium at the forelimb and hindlimb somatosensory cortex area on the right and left hemispheres, making very light contact with the dura mater. A fifth screw electrode was implanted on the frontal bone of the cranium, serving as a reference electrode. Subcutaneous needle electrodes (Safelead F-E3-48, Grass-Telefactor, West Warwick, RI) were used to electrically stimulate the median and tibial nerves of both left and right limbs, without direct contact with the nerve bundle. 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. Each SEP recording was performed on anesthetized rats (uniform anesthesia level), with their body temperature maintained at 37 $\pm$ 0.5 °C using a heating pad. The cortical SEPs were amplified with a gain of 30 000 and sampled at 5000 Hz.

TABLE I
List of Rats and Corresponding Injury Levels

Rat #	Injury Level	Injury Severity
1, 2, 3	Control	No Injury
4, 5, 6	50 mm	Very Severe
7, 8, 9	25 mm	Severe
10, 11, 12	12.5 mm	Moderate
13, 14, 15	6.25 mm	Mild

After implanting the skull SEP electrodes, a 30–60 min "baseline" SEP was recorded. This was followed by laminectomy (exposing the dorsal part of T7–T9 vertebrae). Right after that, "postlamin baseline" SEP was recorded for 30–60 min. The rat was then removed from SEP setup, and SCI was administered using the well-known and standardized NYU impactor [23]. The injury was administered at T8, and graded levels of SCI were produced by dropping a 10.0-g rod with a flat circular impact surface from heights of 6.25, 12.5, 25, or 50 mm to cause mild, moderate, severe, and very severe injuries, as listed in Table I, with three animals in each group. The contusion impact height, velocity, and compression were precisely monitored.

The first 250 SEP responses of each raw experimental data were not included in signal analysis to ensure a stable steady-state measurement. In addition, the first 4 ms of data containing the stimulus artifact was excluded from the SC analysis. To improve the signal-to-noise ratio of the SEP signal, 100 responses were averaged with the window shifted by 20 responses every time.

In this paper, the SC is found between right forelimb SEP and right hindlimb SEPs for different stages. Hence, this method would not suffer from the problem of a common reference signal, and consequently, there would no problem of any false increase in SC.

III. Methods

A. Basso, Beattie, and Bresnahan Score

The BBB test involves observations of a rat in an open field over a time period of 4 min, which are translated into a number on a scale of 0–21 [15], [16]. The BBB score is an observational measure that reflects the animal's locomotor capabilities. It classifies the recovery after SCI into three phases-–early (isolated joint movements), intermediate (gradual improvement of hindlimb movement and limb coordination), and late (fine paw coordination with the tail balancing off the ground) [24]. Fig. 1 illustrates the BBB score over time for a rat from both control group and 50 mm injury group. Only two rats are included in

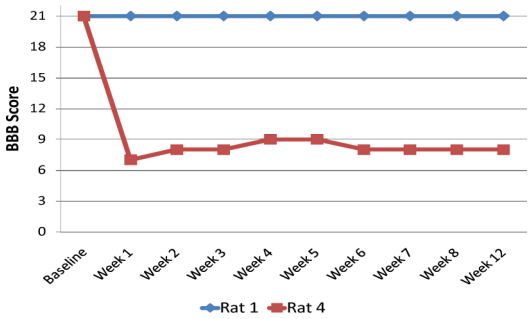


Fig. 1. BBB scores for a rat from control group and 50 mm injury group.

this figure for illustration. A clear drop in the BBB score below 21 is observed for 50 mm injury case indicating severe injury.

B. Somatosensory Evoked Potentials

The SEP signals can reflect the integrity and conductivity of the sensory pathways with high fidelity [17], [25]–[27]. Previous studies have shown that SCI causes a reduction in SEP amplitudes and an increase in latencies. Unfortunately, SEP signals do not always show detectable peaks, particularly following severe SCI. Fig. 2 shows a sequence of (a) baseline time-domain SEP signals obtained from a rat in the 25 mm injury group and (b) week 3 postinjury time-domain SEP signals obtained from the same rat. The figure demonstrates that the hindlimb SEP signals have been severely affected by injury while the forelimb SEPs are similar to those obtained during the baseline period. The signal has no detectable latency and peak amplitude. This example demonstrates the difficulty in measuring amplitude and latency trends and the disadvantage of time-domain analysis in the cases of severe injury. Similar results are obtained in all 6.25, 12.5, 25, and 50 mm injury groups. Such a disadvantage prompted us to find an alternative to amplitude and time latency measure to quantify spinal injury cord injury.

C. Spectral Coherence

SC is the normalized cross-power spectrum computed between two signals [28]. The coherence function gives a measure of similarity between signals and is related to cross-correlation function. The magnitude-squared SC $\gamma_{xy}^2(e^{j\omega})$ function of any two arbitrary signals $x(n)$ and $y(n)$ is a normalized version of the cross-power spectral density between them and is defined as [28], [29]

$$\gamma_{xy}^2(e^{j\omega}) = \frac{\left|P_{xy}(e^{j\omega})\right|^2}{P_{xx}(e^{j\omega})P_{yy}(e^{j\omega})} \tag{1}$$

where $P_{xy}(e^{j\omega})$ is the cross-power spectrum between $x(n)$ and $y(n)$ signals, $P_{xx}(e^{j\omega})$ is the power spectrum of $x(n)$ signal, and $P_{yy}(e^{j\omega})$ is the power spectrum of the $y(n)$ signal.

To examine how magnitude-squared SC function may be used in SCI studies, consider Fig. 3. Assume that a 1-Hz stimulus pulse signal $S(n)$ is applied to the forelimbs or hindlimbs. The repetitive stimulus signal $S(n)$ is portioned into contiguous

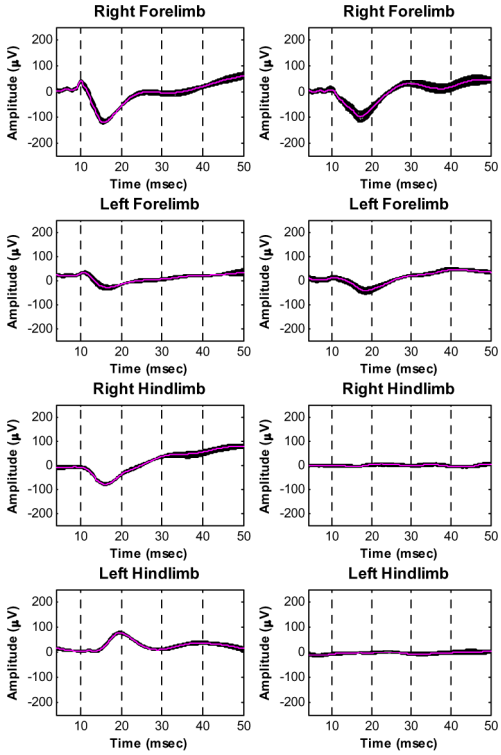


Fig. 2. SEP signals recorded from a rat (rat 8) in the 25 mm injury group (a) before and (b) three weeks after injury.

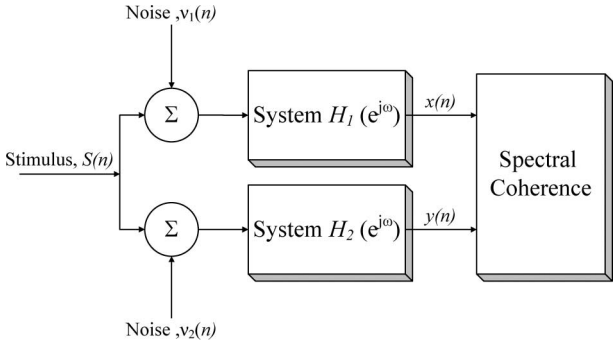


Fig. 3. Stimulus passing through two systems H_1 and H_2, which represent the biological system from the simulation site to the recording site.

frames of N-samples long. The ith frame of $S(n)$ is

$$S_i(m) = S(iN + m), \qquad \text{where } 0 \le m \le N - 1. \tag{2}$$

Assume that $x(n)$ and $y(n)$ are the SEP signals recorded at the cortex as a result of stimulating the right forelimb and right hindlimb, respectively. The signals $x(n)$ and $y(n)$ can also be obtained from stimulating other limb combinations of right forelimb, right hindlimb, left forelimb, and left hindlimb. The SEP signals $x(n)$ and $y(n)$ are repetitive like the stimulus signal $S(n)$ and portioned into contiguous frames of N-samples long. The ith frames of $x(n)$ and y(n) are

$$x_i(m) = x(iN + m) \tag{3}$$

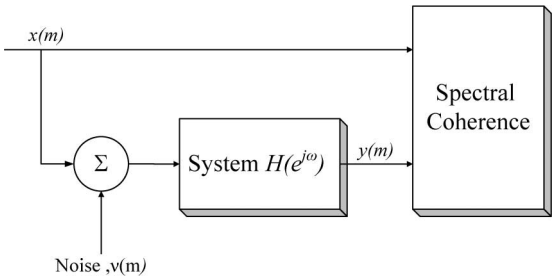


Fig. 4. Equivalent model of the biological system from the simulation site to the recording site.

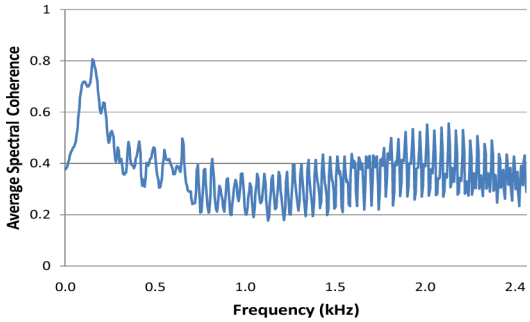


Fig. 5. Average of the SC for the right hindlimb baseline with the right forelimb baseline for all rats.

and

$$y_i(m) = y(iN + m). \tag{4}$$

We assume that the SEP signals $x(n)$ and $y(n)$ are related to the stimulus signal $S(n)$ through linear systems $H_1(e^{j\omega})$ and $H_2(e^{j\omega})$, respectively, but they also contain additive independent noise $v_1(n)$ and $v_2(n)$. $H_1(e^{j\omega})$ and $H_2(e^{j\omega})$ are used to model the transfer function of the biological system from the stimulation site to the recording site at the cortex. To improve the signal-to-noise ratio (SNR) of the SEP signals, ensemble averaging is performed on M frames. In humans, M would have to be much larger than what we would normally use in the animal model to achieve a relatively high SNR. Since $S(n)$, $H_1(e^{j\omega})$, and $H_2(e^{j\omega})$ are assumed to be unaltered during averaging, only noise power is reduced. The frequency spectrum of the averaged estimates $x(m)$ and $y(m)$ are

$$X(e^{j\omega}) = H_1(e^{j\omega})\left[S(e^{j\omega}) + \tilde{v}_1(e^{j\omega})\right] \tag{5}$$

and

$$Y(e^{j\omega}) = H_2(e^{j\omega})\left[S(e^{j\omega}) + \tilde{v}_2(e^{j\omega})\right]. \tag{6}$$

Substituting (5) into (6) yields

$$Y(e^{j\omega}) = \frac{H_2(e^{j\omega})}{H_1(e^{j\omega})}\left[X(e^{j\omega}) + H_1(e^{j\omega})\left(\tilde{v}_2(e^{j\omega}) - \tilde{v}_1(e^{j\omega})\right)\right]. \tag{7}$$

Assume that both $H_1(e^{j\omega})$ and $H_2(e^{j\omega})$ have no zeros on the unit circle with $H(e^{j\omega}) = H_2(e^{j\omega})/H_1(e^{j\omega})$ and the total noise spectrum $\tilde{v}(e^{j\omega}) = H_1(e^{j\omega})\left(\tilde{v}_2(e^{j\omega}) - \tilde{v}_1(e^{j\omega})\right)$. Hence, the model described in Fig. 3 can now be reduced to the equivalent model shown in Fig. 4 where the power spectral densities $P_{yy}(e^{j\omega})$ and $P_{xy}(e^{j\omega})$ are

$$P_{yy}(e^{j\omega}) = \left[P_{xx}(e^{j\omega}) + P_{vv}(e^{j\omega})\right]\left|H(e^{j\omega})\right|^2 \tag{8}$$

and

$$P_{xy}(e^{j\omega}) = H(e^{j\omega})P_{xx}(e^{j\omega}). \tag{9}$$

Hence, the magnitude-squared SC $\gamma^2_{xy}(e^{j\omega})$ function is

$$\gamma^2_{xy}(e^{j\omega}) = \frac{\left|H(e^{j\omega})P_{xx}(e^{j\omega})\right|^2}{P_{xx}(e^{j\omega})\left|H(e^{j\omega})\right|^2\left[P_{xx}(e^{j\omega}) + P_{vv}(e^{j\omega})\right]}. \tag{10}$$

It can be hypothesized that for a normal healthy spinal cord transmission system, $H(e^{j\omega})$ is expected to have a finite- and fixed-frequency transfer characteristics [21], [22]. With a finite $H(e^{j\omega})$ and low noise power density, which is the case after ensemble averaging, we expect $\gamma^2_{xy}(e^{j\omega})$ to approach unity. However, following SCI, $H(e^{j\omega})$ will be modified and the signal power $P_{yy}(e^{j\omega})$ will be primarily due to uncorrelated noise power. Consequently, $\gamma^2_{xy}(e^{j\omega})$ will decrease and may reach zero under severe SCI conditions. In conclusion, $\gamma^2_{xy}(e^{j\omega})$ will indicate whether the signals $x(m)$ and $y(m)$ are really from the same source $S(m)$ or just from the residual noise originating from other sources.

In this paper, $\gamma^2(e^{j\omega})$ is computed for M epochs averaged, with an SC given by

$$\bar{\gamma}^2(e^{j\omega}) = \frac{1}{M}\sum_{i=1}^{M}\gamma_i^2(e^{j\omega}). \tag{11}$$

The SEP data size controls the selection of M, which was taken as 20 in our study for all the average SC values shown in Fig. 5. This number is selected because higher values of M did not show any visual improvement in the SC. The average coherence was performed for a band concentrating on the region with the highest baseline SC. This was called the global SC. According to literature, the frequency band containing most of the power spectral density of SEP signals is 0–200 Hz [27]. Choosing the right frequency band of interest for the SC between the baseline right forelimb and other right hindlimb signals is very important. This frequency band was chosen to maximize SC before injury. Closer observation of the average of the SC in Fig. 5 revealed that SC peak value was at 150 Hz. The frequency band of interest was chosen to be -3 dB (or half-power) bandwidth, which was calculated to be 75–225 Hz.

IV. Results

The results were obtained for a cohort of 15 rats with 3 rats in each group. A larger number of animals would normally be required in the case of clinical or electrophysiological studies. Nevertheless, this number of rats is adequate to investigate the application of SC method to SCI. Data analysis of left versus right side responses is not considered in this paper. The SC of the cortical signals of each rat, recorded in response to sensory electrical stimulation, namely SEPs, is computed.

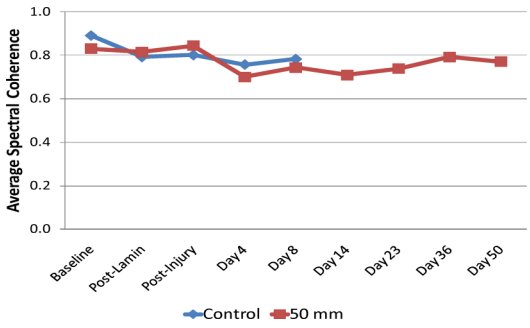
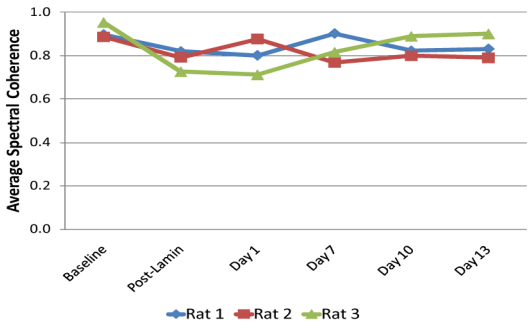
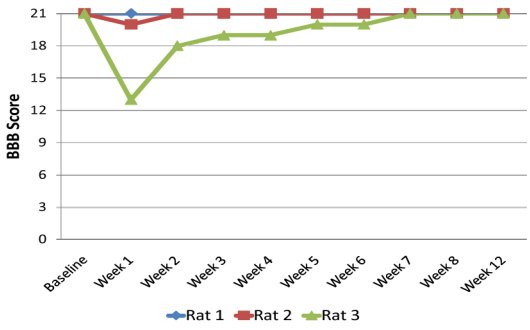


Fig. 6. Average of the global SC of forelimbs for all the rats from both control group and 50 mm injury group.



(a)

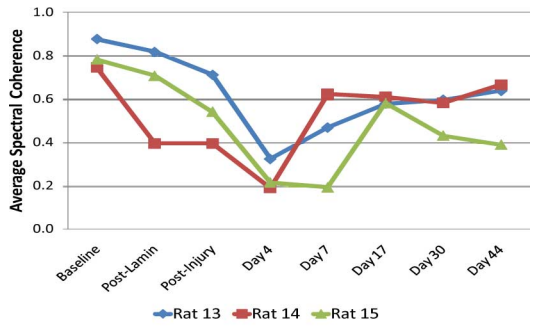


(b)

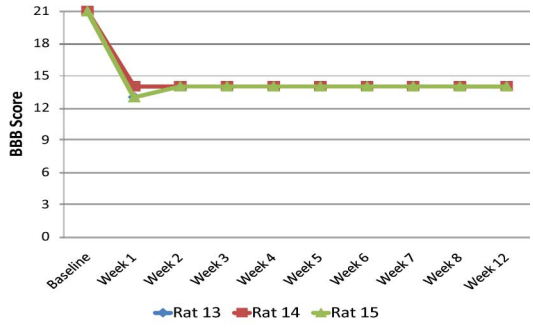
Fig. 7. (a) Global SC between the right hindlimb and the baseline right forelimb signals. (b) BBB scores for the rats from the control group.

A. Coherence of Baseline Right Forelimb With Other Forelimb Signals

Observing the SC changes over time before and after injury helps us to understand the effect of injury on the limbs. Since the SCI is at the thoracic level, it is expected that the injury would affect mostly the hindlimbs and not the forelimbs. Therefore, the coherence associated with forelimb signals should be high. Fig. 6 compares the averaged global SC between the forelimb signals for all the rats from both control group and very severe injury group. The global SC obtained from the forelimb signals was affected slightly by the hindlimb injury or the experimental conditions, and was always relatively high ($\geq$0.7). Hence, any right forelimb signal can be used, instead of its baseline, for the analysis of SEPs of injured rats.



(a)



(b)

Fig. 8. (a) Global SC between the right hindlimb and the baseline right forelimb signals. (b) BBB scores for the rats from the 6.25 mm injury group.

B. Coherence of Baseline Right Forelimb With Hindlimb Signals

In a typical clinical situation, baseline hindlimb information would not be available when monitoring is done following SCI. Therefore, using the baseline hindlimb as a control signal is of no practical importance. Hence, after injury, forelimb signals can be used as a control signal for the hindlimb.

Fig. 7 shows (a) the global SC between the right hindlimb and baseline right forelimb signals and (b) the BBB scores for the control rats. Control 1 and control 2 rats show consistent high SC for all days indicating no injury. Nevertheless, control 3 rat did not respond the same way to the surgery. The existence of insult demonstrated by both BBB and SC indicates that the spine was touched involuntarily during the laminectomy process. These changes in BBB and SC scores were only temporary and disappeared after few days. Our SC results for the control rats are in harmony with the results attained by professionals using the BBB technique on the control rats.

Fig. 8 shows (a) the global SC and (b) the BBB scores for the rats from the mild injury group. Although ideally SC close to 1 is anticipated prior to injury, SC values as low as 0.75 may be obtained depending on factors including some very light insult to the spinal cord during the surgical procedure. It can be observed that all the rats recover to different degrees relative to the same level of coherence before injury. The complementary BBB results do not show any variations of motor recovery with time. Fig. 9 shows the averaged time-domain SEP signals obtained from rat 13 before injury and on days 4 and 44 after injury. We note that on day 4, the SEP signal has lost all normal

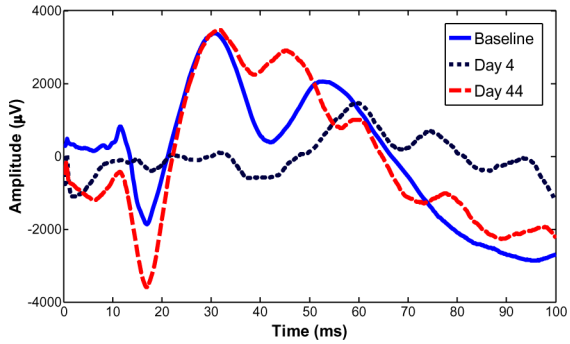


Fig. 9. Averaged right hindlimb SEP signals recorded from a rat (rat 13) in the 6.25 mm injury group before, 4 days, and 44 days after injury.

features. This is concurrent with a low SC (≈0.32). On day 44, the similarity of the SEP's morphology to that of the baseline has increased, corresponding to the increased SC (≈0.69). This example demonstrates how the SC of the cortical signals in response to sensory electrical stimulation shows some recovery while the BBB complement shows no motor function recovery. A recovery in the shape of the waveform of the SEP produced by stimulating the right hindlimb reflects the health of the right hindlimb spinal pathway. While not all results are included due to space constraints, similar results were obtained from the left hindlimb spinal pathway.

Fig. 10 shows (a) the global SC and (b) the BBB scores for the rats from the moderate injury group. It is interesting to note that the right hindlimb sensory pathways of rat 10 seem to have recovered with SC of 0.3 on day 4 after injury to SC of 0.79 on day 82. Rats 11 and 12 do not show any right hindlimb recovery. The complementary BBB results do not show any variations of motor recovery with time. The time-domain SEP signals shown in Fig. 11 obtained from rat 10 before injury and on days 4 and 82 after injury support the SC results. This is another example where the SC of the sensory signals of one of the rats shows recovery while the BBB complement shows no motor function recovery.

The aforementioned two examples presented in Figs. 8 and 10 demonstrate that neither SC nor BBB reflects the complete status of the SCI. The SC may be used to reflect the health of the sensory function while the BBB helps to assess the status of the motor recovery. Hence, it shows that due to their complementarities, SC and BBB together can provide a more comprehensive picture of the health status of the spinal cord after injury.

C. Statistical Analysis

Fig. 12(a)–(c) shows the box-and-whisker plots of the SC at baseline, two weeks postinjury, and eight weeks postinjury, respectively. The figures depict the range of extreme values and the median of SC with values always ≥0.7 during the baseline.

The Jarque–Bera test was also performed on all SC values obtained from the 15 animals to test the null hypothesis that the SC sample comes from a normal distribution. The test returns the logical value $h = 1$ if it rejects the null hypothesis at the 5% significance level, and $h = 0$ if it cannot. The Jarque–Bera

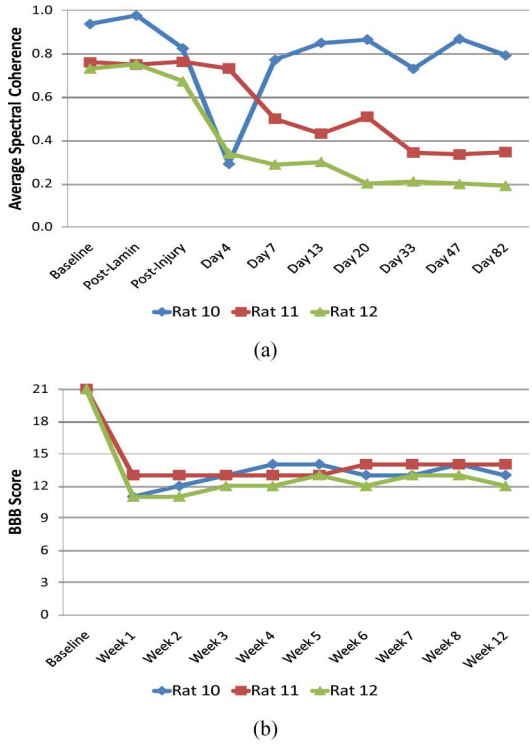


Fig. 10. (a) Global SC between the right hindlimb and the baseline right forelimb signals. (b) BBB scores for the rats from the 12.5 mm injury group.

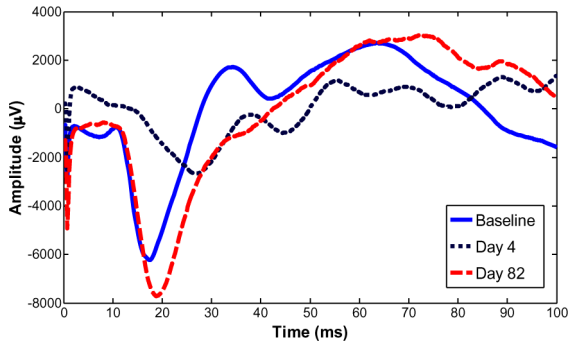


Fig. 11. Averaged right hindlimb SEP signals recorded from a rat (rat 10) in the 12.5 mm injury group before, 4 days, and 82 days after injury.

test results on our SC data sample are $h = 0$ with $p = 0.0503$, which increases confidence that our SC data sample comes from a normal distribution. The Jarque–Bera test was also performed on SC data samples during the baseline, two weeks postinjury, and eight weeks postinjury. The results are $h = 0$ with $p = 0.2196$, $h = 0$ with $p = 0.1406$, and $h = 0$ with $p = 0.1571$, respectively.

Analysis of variance (ANOVA) was then conducted on SC values obtained during the three time points to test the hypothesis

$$H_0 : \mu_{\text{no injury}} = \mu_{\text{Mild}} = \mu_{\text{Medium}} = \mu_{\text{Severe}} = \mu_{\text{Very severe}}$$

$$H_1 : \text{At least 2 of the means are not equal.}$$

The null hypothesis, that all the SC means are equal, was tested against the alternative hypothesis that at least two of them are different. The p-values were found to be 0.443

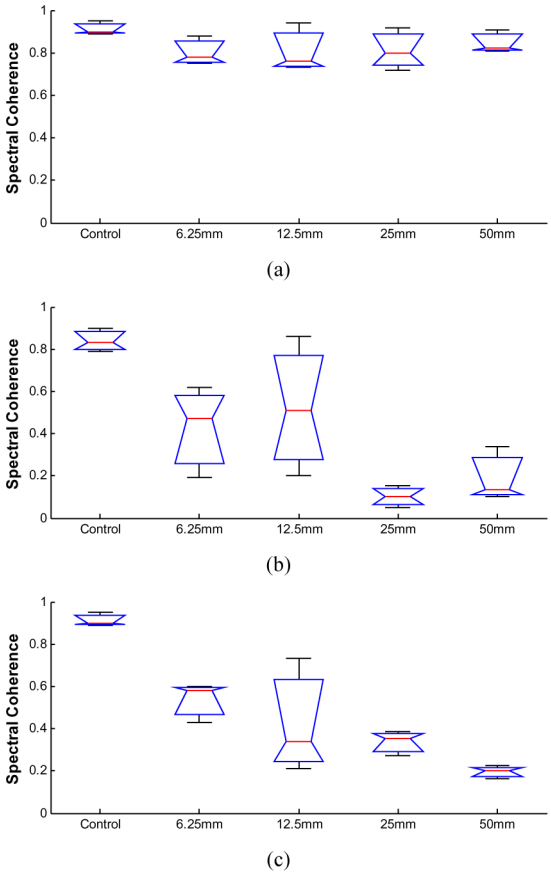


Fig. 12. Box-and-whisker plots of the SC at the (a) baseline, (b) two weeks after injury, and (c) eight weeks after injury.

(statistical power = 47%), 0.005 (statistical power = 60%), and 0.0006 (statistical power = 60%) for baseline, two weeks postinjury, and eight weeks postinjury, respectively. At 5% significance level, we conclude that the null hypothesis that the mean SC is the same for all rats during baseline cannot be rejected. However, the null hypothesis that the mean SC is the same for all rats two weeks and eight weeks postinjury can be rejected. The box plots in Fig. 12(a)–(c) support the ANOVA results.

The Tukey-honestly significantly different (HSD) test was also performed on the SC data eight weeks postinjury (Table II). The p-values obtained from the different animal groups show that after eight weeks, there was a significant difference between control and all other groups at 5% significance level. The Tukey-HSD statistical test was also performed on the available SC data where we compared the baseline SC values with those obtained at different times within the same injury level group, and the results of which are shown in Table III. The statistical power was found to be 40% for control data, 80% for 6.25 mm, 50% for 12.5 mm, 60% for 25 mm, and 73% for 50 mm injury levels.

V. Conclusion

The main objective of this paper is to provide a low-cost quantitative method for the detection and assessment of SCI. The proposed method of SC analysis of SEP signals meets the aforementioned requirements. The results obtained using SC tested on 15 rats show that the proposed method has many advantages such as it: 1) is a normalized quantitative measure; 2) does not require a trained examiner to interpret waveform shapes; 3) does not necessarily require the baseline signals; and 4) can be easily programmed for real-time use. However, SC also has some weaknesses, which are also addressable. First, every rat has a different frequency band of interest because of the natural differences that occur in biological nonlinear systems. Therefore, SC requires careful tuning of the frequency band of interest. Moreover, it is also very susceptible to random noise introduced during the acquiring of SEP signals. This problem can be ameliorated by sufficient averaging. The power line interference is not included because it was reduced by using state-of-the-art low-noise amplifiers with high common-mode rejection ratio and notch filtering. The power spectrum density of the acquired SEP signals showed that the 120 Hz harmonic was not higher than the signal power at that frequency.

The SC assumes that we would always have a reference signal. In this study, the forelimbs acted as the reference signal. If the injury is at a higher level on the spinal cord and affects the forelimb, then this technique may not be used. In this research, we have assumed that averaged peak coherence before injury coincides for all rats and have used a constant frequency band of interest to compute the global SC. Using this approach, one of our first and most important results is that the global coherence between the baseline right forelimb signals and other forelimb signals is always relatively high. Therefore, it can be assumed that the effect of injury on the forelimb is insignificant. Hence, any right forelimb signal can be used instead of its baseline for the analysis of rats injured by the NYU impactor. Our results confirm the usability of forelimb signals, even after SCI, as control signals for SC computation. Furthermore, the SC

TABLE II
Tukey-HSD Test Results Performed on SC Data Eight Weeks After Injury

	Control	6.25mm	12.5mm	25mm	50mm
Control					
6.25mm	0.0364				
12.5mm	0.0077	0.8404			
25mm	0.0023	0.3985	0.9148		
50mm	0.0004	0.0608	0.2745	0.6901	

TABLE III
Tukey-HSD Test Results Performed on Baseline SC Data With Those Obtained at Different Time Points Within Same Injury Level Group

Baseline with	Control	6.25mm	12.5mm	25mm	50mm
Post-Lamin	0.1194	0.8563	1.0000	1.0000	0.0190
Post-Injury	N/A	0.3984	1.0000	0.0305	0.1544
Week 1	0.2060	0.0035	0.7680	0.2824	0.0074
Week 2	0.5180	0.0762	0.9116	0.1915	0.0019
Week 3	0.6389	0.6100	0.9219	0.0050	0.0109
Week 4	N/A	0.3552	0.6943	0.0161	0.0040
Week 6	N/A	0.4924	0.8102	0.0366	0.0293
Week 8	N/A	N/A	0.7411	0.1206	0.0020
Week 13	N/A	N/A	N/A	0.0521	0.0032

analysis of the SEPs, obtained as a response to sensory electrical stimulation, complements the BBB scoring technique, which reflects the motor function recovery. SC and BBB together can provide a more comprehensive picture of the health status of the spinal cord after injury.

The SC also reveals small but specific experimental differences, and such dissimilar left- and right-side responses are missing in conventional methods of assessing SCI. Similarly, the progression of improvement in global SC over the recovery period differs among the rats from the same injury group. This could be due to several reasons such as the differences in every individual's recovery pattern or the exact location of the injury. SC analysis allows us to map individual recovery in somatosensory pathways, hence permitting us to deduce the conditions of the spinal cord in particular. The statistical analysis results demonstrate that all rats show similar and relatively high SC ($\geq$0.7) before injury and relatively low SC ($\leq$0.6) two weeks after injury.

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REFERENCES

[1] L. Sekhon and M. G. Fehlings, "Epidemiology, demographics, and pathophysiology of acute spinal cord injury," *Spine*, vol. 26, pp. S2–S12, 2001.

[2] W. A. Truccolo, M. Ding, K. H. Knuth, R. Nakamura, and S. L. Bressler, "Trial-to-trial variability of cortical evoked responses: Implications for the analysis of functional connectivity," *Clin. Neurophysiol.*, vol. 113, pp. 206–226, 2002.

[3] N. B. Finnerup, C. Gyldensted, A. F. Frederiksen, F. W. Bach, and T. S. Jensen, "Sensory perception in complete spinal cord injury," *Acta Neurol. Scand.*, vol. 109, pp. 194–199, 2004.

[4] M. Nuwer, "Spinal cord monitoring," *Muscle Nerve*, vol. 22, pp. 1620–1630, 1999.

[5] M. Shepard and M. Bracken, "Magnetic resonance imaging and neurological recovery in acute spinal cord injury: Observations from the National Acute Spinal Cord Injury Study 3," *Spinal Cord*, vol. 37, pp. 833–837, 1999.

[6] O. A. Glenn and J. Barkovich, "Magnetic resonance imaging of the fetal brain and spine: An increasingly important tool in prenatal diagnosis: Part 2," *Amer. J. Neuroradiol.*, vol. 27, pp. 1807–1814, 2006.

[7] P. Peckham, M. Keith, and A. Freehafer, "Restoration of functional control by electrical stimulation in the upper extremity of the quadriplegic patient," *J. Bone Joint Surg.*, vol. 70A, pp. 144–148, 1988.

[8] P. Peckham, E. Marsolais, and J. Mortimer, "Restoration of key grip and release in the C6 tetraplegic patient through functional electrical stimulation," *J. Hand Surg.*, vol. 5, pp. 462–469, 1980.

[9] A. Blesch and M. H. Tuszynski, "Gene therapy and cell transplantation for Alzheimer's disease and spinal cord injury," *Yonsei Med. J.*, vol. 45, pp. 28–31, 2004.

[10] D. A. Kerr, J. Llado, M. J. Shamblott, N. J. Maragakis, D. N. Irani, T. O. Crawford, C. Krishnan, S. Dike, J. D. Gearhart, and J. D. Rothstein, "Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury," *J. Neurosci.*, vol. 23, pp. 5131–5140, 2003.

[11] D. A. Kerr, T. Larsen, S. H. Cook, Y. R. Fannjiang, E. Choi, D. E. Griffin, J. M. Hardwick, and D. N. Irani, "BCL-2 and BAX protect adult mice from lethal Sindbis virus infection but do not protect spinal cord motor neurons or prevent paralysis," *J. Virol.*, vol. 76, pp. 10393–10400, 2002.

[12] L. Shi, G. P. Tang, S. J. Gao, Y. X. Ma, B. H. Liu, Y. Li, J. M. Zeng, Y. K. Ng, K. W. Leong, and S. Wang, "Repeated intrathecal administration of plasmid DNA complexed with polyethylene glycol-grafted polyethylenimine led to prolonged transgene expression in the spinal cord," *Gene Ther.*, vol. 10, pp. 1179–1188, 2003.

[13] N. S. Roya, T. Nakanoa, L. Xuingb, J. Kangb, M. Nedergaardb, and S. Goldmana, "Enhancer-specified GFP-based FACS purification of human spinal motor neurons from embryonic stem cells," *Exp. Neurol.*, vol. 196, pp. 224–234, 2005.

[14] H. Wichterle, I. Lieberam, J. Porter, and T. Jessell, "Directed differentiation of embryonic stem cells into motor neurons," *Cell*, vol. 110, pp. 385–397, 2002.

[15] D. Basso, M. Beattie, and J. Bresnahan, "A sensitive and reliable locomotor rating scale for open field testing in rats," *J. Neurotrauma*, vol. 12, pp. 1–21, 1995.

[16] D. Basso, M. Beattie, and J. Bresnahan, "Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection," *Exp. Neurol.*, vol. 139, pp. 244–256, 1996.

[17] M. Nuwer, "Fundamentals of evoked potentials and common clinical applications today," *Electroencephalogr. Clin. Neurophysiol.*, vol. 106, pp. 142–148, 1998.

[18] S. Cerutti, G. Chiarenza, D. Liberati, P. Mascellani, and G. Pavesi, "A parametric method of identification of single-trial-event-related potentials in the brain," *IEEE Trans. Biomed. Eng.*, vol. 35, no. 9, pp. 701–711, Sep. 1988.

[19] K. Xuan and N. V. Thakor, "Adaptive estimation of latency changes in evoked potentials," *IEEE Trans. Biomed. Eng.*, vol. 43, no. 2, pp. 189–197, Feb. 1996.

[20] R. Jung, E. A. Knapp, A. K. Thota, B. T. Thompson, S. Mulligan, N. Ravi, and A. Quick, "Quantitative outcome measures for assessing motor control in a rodent model of spinal contusion injury," in *Proc. Joint IEEE EMBS/BMES Conf.*, 2002, vol. 3, pp. 2556–2557.

[21] N. Fatoo, N. Mirza, R. Ahmed, H. Al-Nashash, N. Thakor, and A. All, "Detection and assessment of spinal cord injury using spectral coherence," in *Proc. IEEE Conf. Eng. Med. Biol. Soc.*, 2007, pp. 1426–1429.

[22] A. All, N. Fatoo, N. Mirza, R. Ahmed, H. Al-Nashash, and N. Thakor, "Using spectral coherence for the detection and monitoring of spinal cord injury," presented at the GCC Ind. Electr. Electron. Conf., Manama, Bahrain, 2007.

[23] W. Young, "Spinal cord contusion models," *Prog. Brain Res.*, vol. 137, pp. 231–254, 2002.

[24] N. S. C. I. S. Center, *Spinal Cord Injury: Facts and Figures at a Glance*. Birmingham, AL: Univ. of Alabama, Dec. 2003.

[25] D. Rowed, J. McLean, and C. Tator, "Somatosensory evoked potentials in acute spinal cord injury: Prognostic value," *Surg. Neurol.*, vol. 9, pp. 203–210, 1978.

[26] C. Li, D. Houlden, and D. Rowed, "Somatosensory evoked potentials and neurological grades as predictors of outcome in acute spinal cord injury," *J. Neurosurg.*, vol. 72, pp. 600–609, 1990.

[27] Z. Hongxuan, Y. Oweis, H. Mozaffari-Naeini, S. Venkatesha, N. Thakor, and A. Natarajan, "Continuous quantitative motor evoked potentials for spinal cord injury detection," in *Proc. IEEE EMBS Conf. Neural Eng.*, 2005, pp. 430–433.

[28] M. Rangaraj, *Biomedical Signal Analysis: A Case-Study Approach*. New York/Piscataway, NJ: Wiley/IEEE Press, 2002.

[29] S. Stearns and R. David, *Signal Processing Algorithms*. Englewood Cliffs, NJ: Prentice-Hall, 1988.

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