Utility of trial-to-trial latency variability of somatosensory evoked potentials for diagnosis of spinal cord demyelination

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Abstract

Traditional measurement of somatosensory evoked potentials (SEPs) depends on averaging of many recordings, which introduces loss of dynamic variability. Single trial extraction provides a new measurement of SEP latency variability for evaluation of the neurodynamic status of the somatosensory pathway. This aim of this study was to apply a single trial SEP to diagnose the severity of demyelination in a chronical spinal cord injury model. The severity of demyelination was evaluated by histological examination with Luxol fast blue staining. Results showed that the latency variability based on a single trial SEP was negatively correlated with the severity of demyelination measured by histology, and the correlation coefficient r =-0.90 and r =-0.95 respectively). These data suggest that single trial SEP can provide a dynamic indicator of spinal cord demyelination.

Keywords: Spinal cord demyelination; somatosensory evoked potentials (SEPs); second order blind identification; single trial extraction; trial-to-trial latency variability

Introduction

Cervical spondylotic myelopathy (CSM) is a common neurodegenerative disorder, and is a leading cause of chronic compression of the cervical spinal cord or nerve roots in subjects older than 55 years of age, with resulting neurological dysfunction.[1] Clinically, diagnosis of CSM is mainly based on clinical manifestations and the score Japanese Orthopaedic Association Scores (JOA) to evaluate spinal cord function and prognosis. However, the onset of CSM is insidious, with varying clinical symptoms and signs, and the traditional detection methods have a low sensitivity and are prone to subjective bias. Thus, new objective evaluation methods are required for early diagnosis and precise prognosis of CSM.[2]

Somatosensory evoked potentials (SEPs) are strongly correlated with disability and postoperative recovery in patients with CSM.[3-5] SEPs reflect brain activity in response to external electrical stimulation of peripheral nerves, and can provide more accurate quantitative analysis of spinal cord function. SEPs are of low cost, easy to use, non-invasive, and have higher success rates, and are widely used to detect functional integrity along the somatosensory pathway. [2, 6] However, it is difficult to obtain the features of SEP signals in clinical measurement because of the weak signal amplitude and high noise background.[7-8]

Ensemble averaging (EA) is a popular method used for clinical measurement of SEPs.[2, 9] In practical application, the amplitude and latency of an observable SEP waveform are the main two measures used to assess potential neurological deficits involving changes in nerve conductivity along the spinal cord.[10] However, the EA method requires a large number of trials for averaging to obtain an interpretable SEP waveform, with a long detection time. As such, deficits may have already occurred before acquiring sufficient trials, especially for intraoperative monitoring.[11] The EA method also obscures the variations in response amplitude, latency, or

phase characteristics with time, but the variations contain non-stationary and dynamical neurological characteristics inside SEP signals from trial-to-trial.[6, 12] As such, the traditional EA method cannot accurately detect SEP features from a large number of across-trials. Thus, a desired approach would involve the extraction of the real SEP features from the real cases.

Several promising blind source separation algorithms, in which the uncorrelated sources can be separated, have been recently reported to provide fast detection and an enhanced signal-to-noise ratio of SEP signals.[13-14] Of these, the second order blind identification (SOBI) is the most appropriate for separating EPs, with advantages of simplicity, reliability, robustness, and applicable to Gaussian signals, especially to short serial signals.[13] To improve the performance efficiency of SOBI, a constrained SOBI algorithm, termed one-unit SOBI with reference algorithm (SOBI-R), was recently proposed.[15] We previously demonstrated effectiveness of SOBI-R for fast SEP extraction with a few channels, [16] and that single trial SEP obtained using SOBI-R can identify early stages after spinal cord injury (SCI). [16] Further, we evaluated the electrophysiological prognostic value of trial-to-trial variability of SEP in patients with cervical myelopathy,[16] and found that the latency variability of trial-to-trial SEP reflects the recovery ratio of CSM patients after surgery, suggesting that changes in latency are more sensitive to spinal cord deficits.[12, 16-18]

The SEP latency is a measure of signal transmission time along the somatosensory pathway (from peripheral nerves to the brain), while the latency of SEP was suggested to reflect the severity of myelopathy in the spinal cord.[16] Thus, abnormal trial-to-trial SEP variability in latency (TTSEP-VL) is closely correlated with the severity of demyelination in CSM.[16] However, the mechanism of how the TTSEP-VL changes according to cervical myelopathy remains unclear.

Based on these findings, and that the major deficits in CSM are axonal lesions with demyelination and axon loss, the overall aim of this study was to evaluate the changes in TTSEP-VL during progressive demyelination following SCI induced by chronical compression. Further, we hypothesize that TTSEP-VL is an indication of spinal cord demyelination in CSM.

Materials and Methods

Animal model of chronic compressive SCI

A total of 36 healthy adult Sprague Dawley rats of both sexes (weight, 250-330 g) were divided equally into a sham control group (without any injury to the cervical spinal cord; Group 1; n=12) and two compressive SCI groups that received chronic compression of the C5 cervical segment (Groups 2 and 3, n=12 per group). All experimental procedures were approved by the Committee on the Use of Live Animals in Teaching and Research of our local institution.

For surgical procedures, all rats received general anesthesia with intraperitoneal injection of ketamine-xylazine mixture (60/10 mg/kg), and with additional doses to maintain adequate intraoperative anesthesia. Chronic compression for SCI with CSM was performed by implantation of water-absorbing materials (3 × 1 × 1 mm size).[2, 9] After posterior incision of the spine, a small space between the adjacent laminae near the facet at the C5 level was opened and enlarged with natural flexion of the spine, and the underlying dura was carefully separated from the laminae. The water-absorbing material was carefully inserted into the right posterolateral side of rat spinal canal at the C5 level. This material expands overtime, with a maximal expansion of 7× volume within 24 h after implantation, to produce chronic cord compression that is maintained for >6 months.[2] After the operation, rats were returned to separate cages. For tissue collection, rats in the sham control group were euthanized at 4 weeks recovery, while SCI

animals were euthanized at 2 weeks (Group 2) or 4 weeks (Group 3), by an overdose of 20% pentobarbital.

Histopathological detection

After perfusion, the cord was immersed in 10% formalin for 12 h, and then embedded in paraffin. The cord was continuously cut into 5 μm-thick sections using a microtome. Sections were stained by luxol fast blue and analyzed by light microscopy (Nikon H600L microscope, Nikon, Tokyo, Japan). Luxol fast blue was used to stain the myelin in white matter of the lateral cord, with a blue color reflecting the myelin content. The area and intensity of myelin staining was measured in the white matter with 10 randomly placed 0.05×0.05 mm^2 regions of interest (Fig. 1) using ImageJ software (Version 1.47v; National Institutes of Health, Bethesda, USA).[19] The mean value of the ten regions of interest was used as the result of each animal. In the injured cord, the severity of demyelination was evaluated by myelin area and staining intensity.

Somatosensory evoked potentials collection

To evaluate the functional integrity of the spinal cord, the upper limb SEP signals were longitudinally collected in all animals before and after compressive SCI. A constant current stimulation (square wave of 3.4 Hz stimulation rate, 0.2 ms duration) was delivered to the stimulation electrodes on the median nerve at the forepaw. Cortical SEPs from four channels were recorded by screw electrodes placed on the skull over the sensorimotor cortex at F3, F4, Fz, and Cz versus Fpz, according to the 10-20 international system (Fig. 2). The raw recorded surface SEPs were amplified 2000× at a sampling rate of 10 kHz, and bandpass filtered from 20 to 2000 Hz (Zhuhai Yiruikeji Co., Ltd., Zhuhai, China). One hundred trials of raw SEP were recorded from each rat.

Single trial extraction of SEP

To precisely detect the single-trial SEP waveforms from a few channels, we used the SOBI-R spatial filtering algorithm,[20] which can isolate signals related to a reference signal from a multi-input signal as follows:

$$S(t) = \sum_{m=1}^{M} s_{m(t)},$$

where $s_m(t)$ is the m-th observed signal and M is the number of observed signals. Different from the classical SOBI, the SOBI-R algorithm was formulated as follows:

$$\min\ G(y) = -\sum_{\tau} E(y(t)y(t-\gamma))^2$$

$$\text{subject to } e(y,r) - \delta \leq 0 \text{ and } E(yy^T) - 1 = 0,$$

where y is the estimated output signal $y(t)$, r is the reference input signal $r(t)$, δ is a threshold of a constraint condition of the closeness, and $e(y,r)$ measures the closeness between y and r.

The Lagrange multipliers method was then used for optimal solution, and the Newton-like learning algorithm was performed to determine the optimal unmixing vector V as:

$$\Delta V = -\mu\left(\frac{\partial^2 L(V,\alpha,\beta)}{\partial V^2}\right)^{-1} \frac{\partial L(V,\alpha,\beta)}{\partial V}$$

$$\Delta\alpha = max\{-\alpha, \varphi(e(y,r) - \delta))\}$$

$$\Delta\beta = \varphi(E(yy^T) - 1),$$

where μ is the learning rate, $L(V,\alpha,\beta)$ is the augmented Lagrangian function, α and β are the Largrange multipliers, and φ is a scalar penalty in the augmented Lagrangian function. The unmixing vector V is updated by $V_{i+1} = V_i + \Delta V$, until the error $|G(y)_{i+1} - G(y)_i|$ is small enough. Thus, the output signal $y(t) = V^T S(t)$ is equal to the desired source signal $y^*(t)$. Here, single trial SEPs were extracted from 100 recordings, and the latency was measured at

single trials. The TTSEP-LV was calculated by the ratio of the standard deviation to the mean value as:

$$\mathrm{TTSEP-LV} = \frac{\text{the standard deviation}}{\text{the mean value}} \times 100\%.$$

Statistical analysis

All statistical analyses were performed with statistical software (SPSS version 16.0 software; SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation. Differences in TTSEP-LV and histological results between the three groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's post-hoc tests, with a significance level α=0.05 at $p < 0.05$. Pearson correlation coefficients between TTSEP-LV and histological results were also calculated using a bilateral test (two-tailed), with $p < 0.05$ and an absolute correlation coefficient $\geqslant$ 0.50 considered a significant linear correlation.

Results

The SEPs were averaged from 100 trials, the SEP waves of SCI rats (purple line) showed a longer latency and decreased amplitude compared with sham animals (blue line) (Fig. 3). The SEP waveforms of 100 single trials extracted by SOBI-R from one rat before and after SCI are shown in Fig. 4. The 100 single trial SEPs from the intact spinal cord showed a similar distribution , and the SEP waveforms were distinctly observed, although showed an irregular distribution compared from the injured spinal cord (even though the waveforms could not be distinguished). By contrast, single trial SEP latency showed a higher variability after SCI compared with the uninjured cord. In addition, the single trial SEP was lower after SCI.

There was a significant differences ($p < 0.05$, ANOVA; Table 1) in TTSEP-LV among the sham surgical control group (15.7±0.86), SCI group at 2 weeks (22.4±0.99) and 4 weeks (26.2±0.65) after chronic compression, and the longer duration of compression after the spinal cord injury, the higher TTSEP-LV. Consistent with these findings, there was a significant decrease ($p < 0.05$, ANOVA) in myelin area of sham controls (598.2±34.3 um^2) compared with SCI rats at 2 weeks (487.4±31.6 um^2) and 4 weeks (430.6±30.1 um^2) after SCI . Further, there was a significant differences ($p < 0.05$, ANOVA) in myelin staining intensity among the sham control group (1.93±0.14), SCI group at 2 weeks (1.37±0.13) and 4 weeks (1.19±0.11) after chronic compression. Moreover, the longer duration of chronical compression after the spinal cord injury, the smaller myelin area and the lower staining intensity.

Finally, there was a strong negative correlation of TTSEP-LV with myelin area ($r = -0.90$, $p < 0.05$) and staining intensity ($r = -0.95$, $p < 0.05$), suggesting that latency variability of the trial-to-trial SEP was correlated with histological outcomes.

Discussion

The pathogenesis of CSM does not fully explain the clinical characteristics.[21] Further, because of a lack of accurate and reliable indicators of neuropathological changes in the spinal cord, the optimal timing and methods for surgical treatment for SCI remain controversial.[22] Neuroelectrophysiological examination can provide a relatively objective quantitative evaluation of nerve function. For example, SEP can predict the course of CSM and the prognosis of decompression surgery.[20, 23-24] Compared with MRI, SEP has the advantages of low cost, ease of use, and a similar sensitivity but better specificity, allowing accurate assessment of the degree of SCI, the nature of the functional loss, the innate nervous system response to injury, and the regeneration of spinal cord neurons.

The main early pathological feature of CSM involves demyelination in the conducting tract of the spinal cord, which leads to axonal loss and neuronal apoptosis.[25] Preoperative SEPs can be used to noninvasively and quantitatively assess the function and pathological changes in the sensory conduction tract in the cervical spinal cord in CSM patients. Indeed, there is a more strict locking time relationship between the EP latency and stimulus, which is not affected by subjective factors, which provides a direct and sensitive indication of demyelinating lesions,[26] and directly reflects the nerve conduction velocity related to the integrity and connectivity of the axon myelin sheath. Neural EPs sometimes have an abnormal latency,[27] and the amplitude of EPs is affected by many factors. Previous clinical studies have found that even if there is a normal amplitude, an abnormal latency can predict poor prognosis,[23-24] and better reflects prognosis than an abnormal amplitude. The SEP latency provides a measure of signal transmission time along the somatosensory pathway, from the peripheral nerves to the brain. Previous studies have reported that an abnormal latency would reflect the severity of myelopathy

in the spinal cord,[16-17, 23] indicating that a delayed SEP latency is closely correlated to the severity of demyelination in cervical myelopathy.

Clinical detection of SEP is based on averaging of 100-300 or more trials, which removes the dynamic characteristics of the neural pathway. However, CSM often involves partial axonal demyelination or degeneration, resulting in a dynamic variation in SEP characteristics, which are not detected by an averaged SEP. A previous study used a single SEP extraction technique to extract a single trial SEP,[20] which can measure the latency variation rate of the spinal cord nerve conduction process. This single trial estimation of SEP allows assessment of the dynamic properties of nerve conductivity along the somatosensory tracts in the spinal cord, and can be used to detect local axonal injury more effectively and with a higher sensitivity than averaged SEPs, which is useful for predicting the potential for neural functional recovery.[12, 15]

In the present study, we examined the underlying neurophysiological changes of TTSEP-LV in a rat model of chronic cervical spinal cord compression,[28] and compared the changes in SEPs with histological examination. Our main findings from analysis of the SEP signal waves were that the trial-to-trial SEPs were changed after SCI, indicating dynamic changes in the nerve pathway. From the results of electrophysiological detection of latency variability in trial-to-trial SEPs, we found a progressive increase in TTSEP-LV with chronic SCI. By histology, we also found a progressive reduction in myelin area and myelin staining intensity with SCI. Further, there was a strong negative correlation of changes in TTSEP-LV with the severity of demyelination after SCI. These data suggest that TTSEP-LV may be a useful electrophysiological tool for clinical assessment of the severity of demyelination after SCI.

SEP mainly reflects the structure and functional status of the conducting fibers in the sensory ascending pathway of the posterior spinal cord (including the ganglion, nucleus cuneiformis, and

dorsal thalamus). SCI can cause damage to the fibers of the sensory ascending pathway, resulting in a decreased number of nerve impulses conducted along the ascending fibers, and decreased synchronous excitation of the cortical sensory nerves. Neurons can also die after SCI, which slows the conduction velocity of nerve impulses, leading to a prolonged latency and decreased amplitude of SEPs.

Conclusions

TTSEP-LV is a new technique for measurement of SEPs based on a single trial. Our data suggest that TTSEP-LV provides accurate *in vivo* assessment of spinal cord demyelination, which may be useful for early and precise diagnosis of myelopathy in the clinic, thus aiding in determining the most appropriate surgical intervention.

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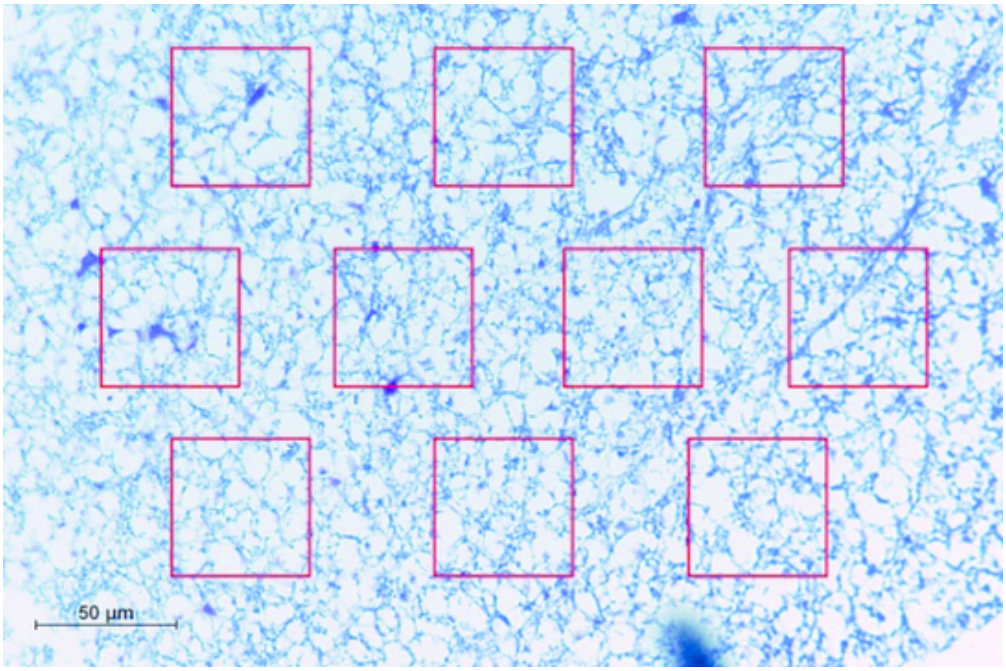
FIG. 1. Ten square regions of interest (ROIs) were randomly drawn to measure the myelin area and staining intensity on sections stained with luxol fast blue (LFB).

FIG. 2. Positions of the recording electrodes overlying the cerebral cortex.

FIG. 3. Averaged somatosensory evoked potentials (SEPs) of 100 trials from the intact and injured spinal cord.

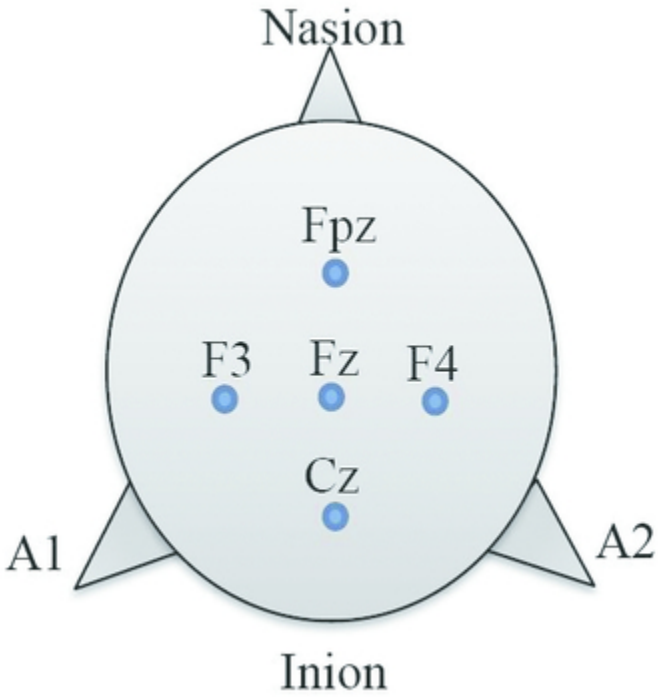
FIG. 4. Trial-to-trial SEPs. (A) Intact spinal cord. (B) Injured spinal cord.

TABLE 1. The parameters variability of the three groups.



Ten square regions of interest (ROIs) were randomly drawn to measure the myelin area and staining intensity on sections stained with luxol fast blue (LFB).

45x30mm (300 x 300 DPI)



Positions of the recording electrodes overlying the cerebral cortex.

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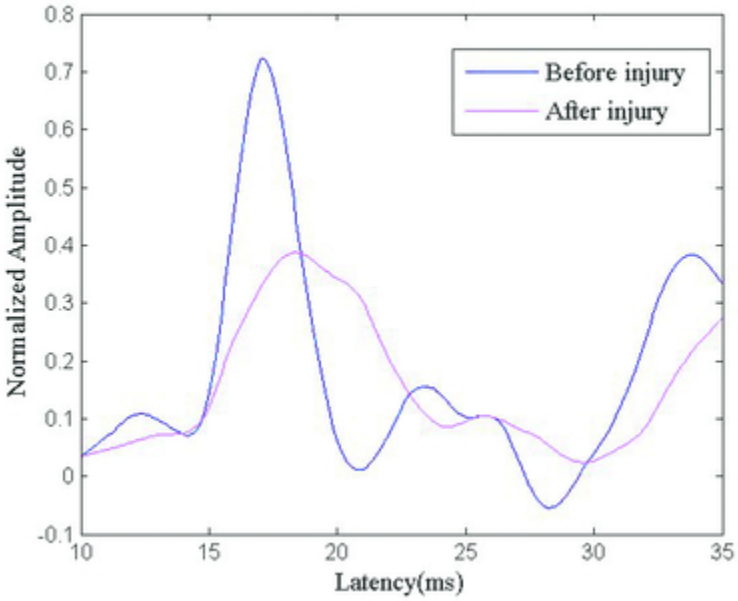

Averaged somatosensory evoked potentials (SEPs) of 100 trials from the intact and injured spinal cord.

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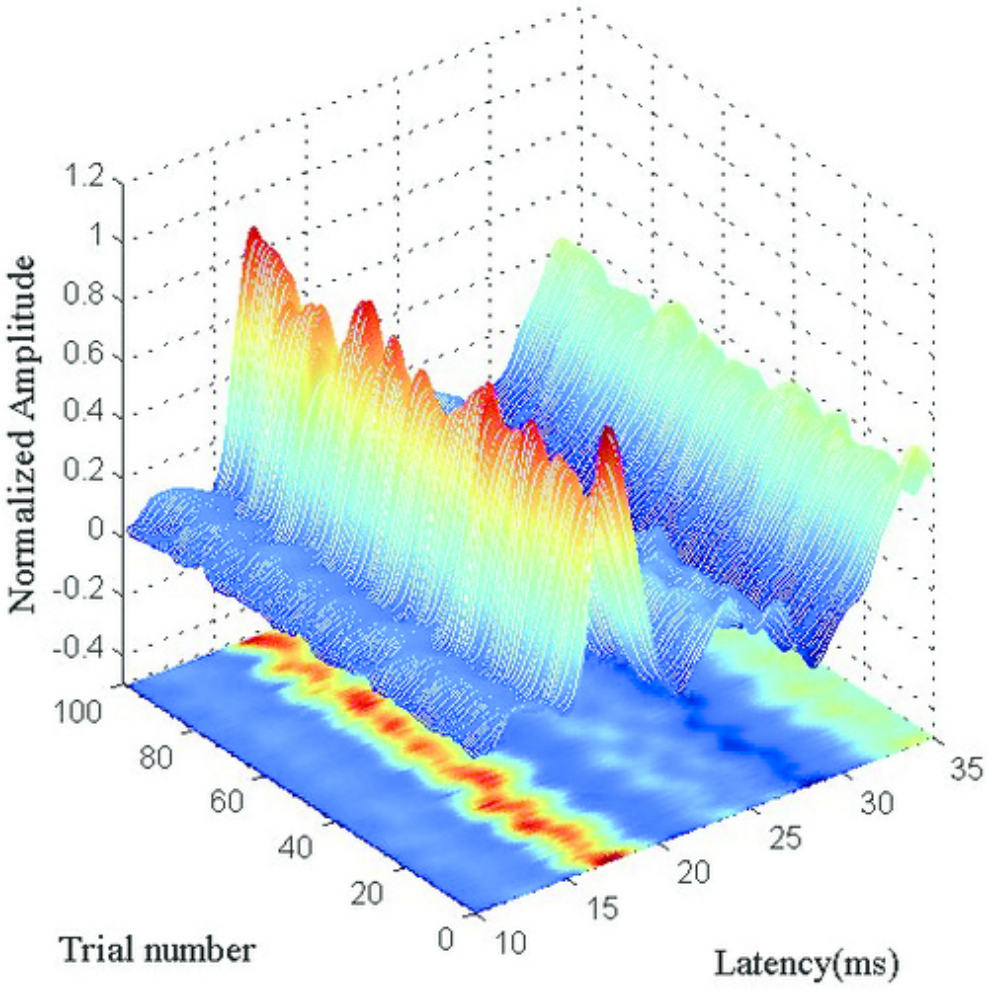
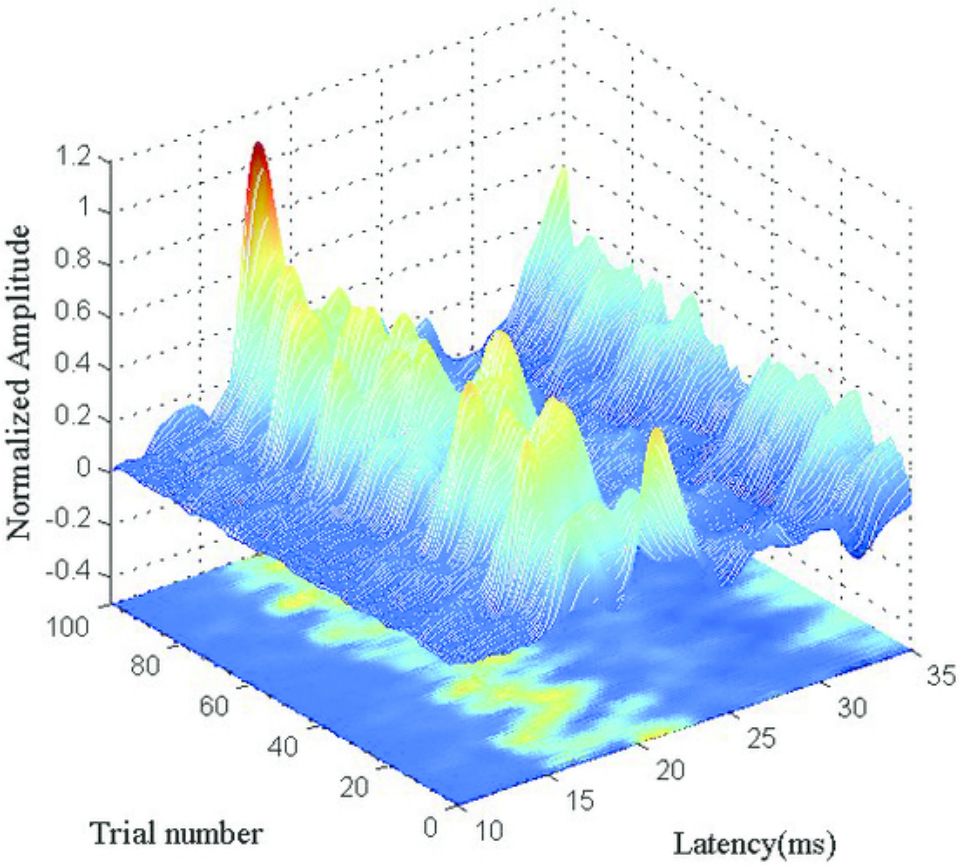


Trial-to-trial SEPs. (A) Intact spinal cord.

42x43mm (300 x 300 DPI)



Trial-to-trial SEPs.(B) Injured spinal cord.

48x43mm (300 x 300 DPI)

Table 1. The parameters variability of the three groups

	Group 1	Group 2	Group 3	ANOVA
SEP latency variability	15.7±0.86	22.4±0.99	26.2±0.65	$p<0.05$
LFB -the myelin area	598.2±34.3 um^2	487.4±31.6 um^2	430.6±30.1 um^2	$p<0.05$
LFB -the staining intensity	1.93±0.14	1.37±0.13	1.19±0.11	$p<0.05$