The usefulness of time-frequency patterns of somatosensory evoked potentials in differentiation of the location of spinal cord lesion

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Abstract

Traditional imaging techniques are not able to identify the levels of neurological impairment caused by spinal cord lesions. As an alternative, electrophysiological methods such as somatosensory evoked potentials (SEPs) are capable of filling this gap. Previous studies show that the latency and shape of median SEP components can be affected by the level of spinal cord lesion. Measuring SEP signals in time-frequency domain has drawn much attention in recent years and is suggested as a tool of preventing from iatrogenic nerve injury during spinal surgery. The relationship between time-frequency characteristics of SEP signal and the affected levels of spinal cord lesion, however, is still unclear. Based on the hypothesis that time-frequency features of SEP are associated with the location of neurological deficit in spinal cord, this paper analyzes the time-frequency distributions (TFDs) of SEPs at different injury levels. Twenty-four rats were divided into one normal group and three injury groups, in which weight drop contusions were delivered to the spinal cord of the rats at C4, C5 and C6 level respectively. The components in TFDs of each group were found, and the comparison result shows that sub-components (frequency<60Hz and amplitude<1.5) belonging to different groups are located in different areas in the time-frequency domain, which infers that each injury level corresponds to a distinct time-frequency distribution pattern. Statistical results show that the TFD of each injury group has one or more regions of interest (ROIs) that are significantly different from the normal group. Importantly, the distributions of $p<0.05$ ROIs for injury groups are distinct from each other, which proves that changes in spinal cord injury level have an effect on the SEP distribution pattern in the time-frequency domain. Based on these findings, it could be concluded that time-frequency patterns of SEPs have potential usefulness in differentiation of the location of spinal cord lesion.

Keywords: Somatosensory evoked potentials, Time-frequency analysis, Spinal cord injury

Background

A lesion in the spinal cord usually leads to autonomic symptoms, limb weakness, and sensory loss in the limbs [1], and it could badly affect the nerve conduction in the central nervous system. Spinal cord lesion is caused by the disease of cord or compression from external injury. Conventional techniques of localizing the affected level of spinal cord lesion are mainly based on imaging, such as magnetic resonance imaging (MRI) and spiral computed tomography (CT). These techniques only focus on the anatomy of the spine, but cannot provide detailed information on neurological deficits in the spinal cord [2,3].

Electrophysiological assessment has been suggested as a better alternative to the conventional clinical diagnosis techniques. Somatosensory evoked potentials (SEPs) has been widely used in intra-operative monitoring of spinal cord surgery since it directly accesses the physiological integrity of the spinal cord [5,6]. SEPs have also been applied to quantitative estimation of the spared function of damaged neuropathway and the degree of injury after spinal cord injuries (SCI) [7]. A complete SEP signal contains a series of components that convey useful information about the physiological mechanisms of the nervous system and reflect sequential activation of the neural structures along the somatosensory pathway [8-11]. Changes of the characteristics (such as latency and amplitude) of the SEP components, therefore, are potential indicators of the locations of spinal cord lesion.

It has been reported that changes of the latency of median SEP components are associated with the levels of spinal cord lesion [12]. Another study indicates that there is a relationship between the level of SCI and the presence/absence of N9, N13 and N20 components in SEP [9]. However, only temporal properties of SEP recordings are used in these studies. Since the number of available temporal properties in an SEP signal is limited, their differentiation degree for lesion levels is inadequate.

Measuring SEP signals both in joint time-frequency domain has gained increasing interests in recent years and was suggested as an effective indicator of SCI [13]. It was shown in [14] that time-frequency analysis (TFA) is able to identify stable time-freuqency components of normal SEP signals. These time-freuqency SEP components will be remarbley altered when neurological deficits occur in the spinal cord, suggesting potential application of TFA to intraoperative spinal cord monitoring [15,16]. Researchers also found the evidence that some detialed time-frequency components in SEP may reflect different origins in the spinal cord [14]. Based on this finding, a hypothesis could be proposed that the analysis of these detailed T-F components may help to identify the location of spinal cord lesion. If the hypothesis is proved reasonable, different patterns in TFA could be found when spinal cord lesions occur in different locations.

In this study, acute SCI was conducted on rats to produce the lesion in the spinal cord. The SEP recordings before and after injury were analyzed using TFA. Based on the assuption that the symptomatic level of SCI could be disclosed by the abnormalitis in time-frequency patterns of SEPs, we aim to find out the evidence that SEP signals recorded from the rats with spinal cord injured at different levels have distinct time-frequency distributions.

Methods

- **Materials**

The animal experiments were performed on twenty four adult rats weighting between 250g and 380g. These rats were divided into 4 groups, including 3 experimental groups and 1 control

group and each contianing 6 rats. Rats in the 3 experimental groups were injuried at C4, C5 and C6 level respectively in the spinal cord.

- **Experimental Procedure:**

During the experiments, each rat was placed in a stereotaxic frame to keep its head stable and anesthetized with isoflurane (2% for induction and 1.5% for maintenance). After a midline scalp incision, a burr-hole 2mm posterior to the right coronal suture and 2mm lateral to the sagittal suture was made on the skull. A steel screw 1.5mm in diameter in the burr-hole served as recording electrode. Another burr-hole 2mm anterior to the right coronal suture and 1mm lateral to the sagittal suture was made, in which another screw served as reference electrode.

After the occipital and nuchal areas were shaved and prepared, a skin incision was made to expose the laminae from C3 to C7 with removal of ligamentum flavum between them through a posterior approach under microscopy. A small space around the facet was opened with laminotomy and enlarged with natural flexion of the spine. The dura underneath was carefully seperated from the laminae to prevent leakage of cerebrospinal fluid.

After that, a contusion was delivered to the rat at a certain level of the spinal cord. Here, the contusion was performed using the NYU-MASCIS impactor [17] by dropping a 10 gram rod from the height of 25mm on the exposed dorsal surface of the spinal cord.

- **Data Collection:**

SEP signals were collected from the rats before and after weight drop contusion. During SEP recording, isoflurane (1.5%) was still used to maintain the anaesthesia. To elicit cortical SEP, a constant stimulator was used to generate 4.1Hz square wave 0.1ms in duration to stimulate the median nerve along the left forelimb of the rat. Stimulation was done with two needle electrodes inserted into the rat' right forelimb adjacent to the median nerve. And another needle electrode inserted into the hindlimb muscles was used as ground. The stimulation intensity was selected to cause mild twitch of the forelimb. During the test, the effectiveness of median nerve stimulation was evaluated continuously by visual inspection on the twitch to ensure that adequate stimulation was applied.

Cortical SEPs were recorded at a sampling rate of 10KHz using the recording electrode over the left sensori-motor cortex. The recorded cortical SEPs were amplified 2000 times and bandpass filtered from 20 to 2000Hz. The sweep time of SEP recording was 50 ms. A total of 200 SEP responses were averaged to achieve a good signal-to-noise ratio (SNR). All the recordings followed this procedure to confirm the reproducibility. The data collection procedures were performed using SEP signal recording equipment (YRKJ-A2004, Zhuhai yiruikeji Co., Ltd., China).

- **Time-frequency analysis:**

Previous studies showed that short-time Fourier transform (STFT) algorithm is suitable for time-frequency analysis of SEP signals [15,16,18]. In this study, STFT algorithm with 35ms window size (Hanning window) was applied to the pre- and post-SCI SEP signals to calculate their time-frequency distributions (TFDs). STFT algorithm was performed on all averaged SEP signals. Each TFD is calculated from 0 to 200Hz in a step of 1 Hz [14].

All the TFDs were log-transformed to make the detailed time-frequency components exposed and then expressed as z-scores to make the comparison among different groups possible [19]. All the components in TFDs were found and the distribution patterns of them were compared among different groups.

- **Statistical analysis:**

For each time–frequency point, a t-test (two-tailed, assuming unequal variances) is used to determine whether the TFDs of each injury group are significantly different from those of the normal group, resulting in three p-value maps in time–frequency domain. In these p-value maps, the time-frequency regions showing significant difference ($p<0.05$) were defined as regions of interest (ROIs). Summary values of ROIs (mean of all ROI pixels [24]) in each injury group were then compared with those in the normal group respectively using T-test as a post-hoc test.

Results

- **Time-frequency analysis results:**

Four SEPs in Fig. 1 (first row) are selected as the representative waveforms of Normal, C4, C5 and C6 groups. According to this figure, SEP waveforms after spinal cord injury change substantially in different forms compared with the pre-SCI status. The time-frequency distributions of these four waveforms calculated using STFT are also shown in Fig. 1 (second row). In comparison with the SEP TFD in normal state, there are obviously different patterns in different injury groups by visual inspection. Next, these TFDs are log-transformed and expressed as z-scores, as shown in Fig. 1 (third row) where the positions of all components are marked with white crosses. It could be noticed that components of the post-SCI groups have different time-frequency distributions from those of the normal group, and C4, C5 and C6 group have distinct component distributions from each other.

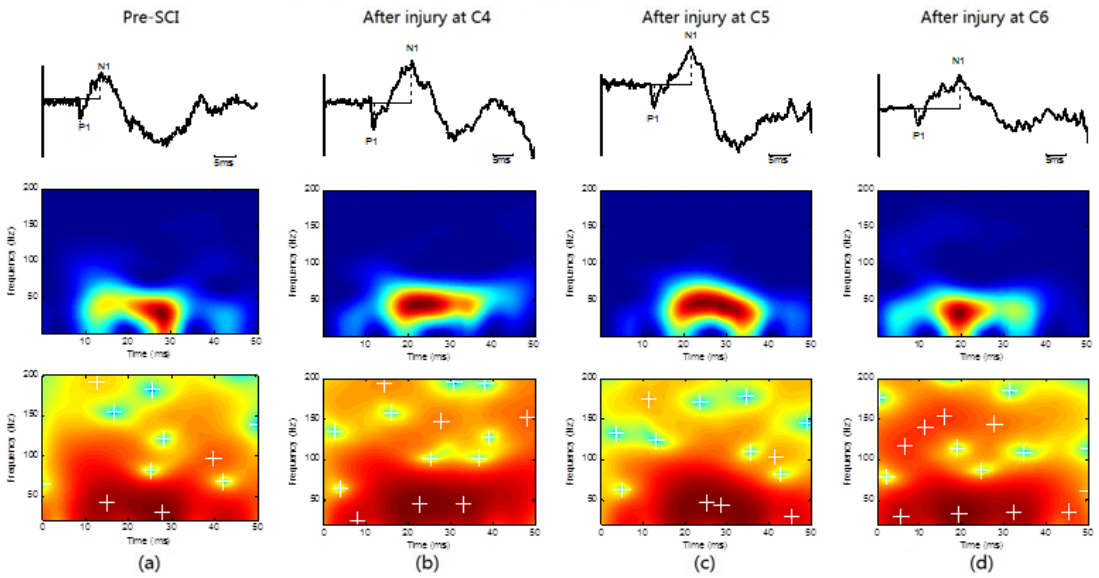

Figure 1. Typical SEP waveforms, their STFT-based TFDs, log-transformed and normalized TFDs (white crosses: the positions of all components). (a) Prior to SCI; (b) After SCI at C4 level; (c) After SCI at C5 level; (d) After SCI at C6 level

- **Component Distribution Comparison Results:**

A component with amplitude higher than 1.5 (z-score value) takes the form of the largest wave (N1) in a typical SEP signal and is named as 'main component' in this study. Components above 60Hz have relatively low amplitudes (absolute value of z-score < 1) and higher variability. They are highly susceptible to the noise and would not be discussed in this paper. Besides them, the components with amplitude lower than 1.5 (absolute z-score value) and frequency below

60Hz are named as 'sub-components' in this study. The distribution patterns of the main and sub-components of SEP signal are compared among groups in time-frequency domain.

Before SCI, main components of SEP are mainly distributed in the area 10-20ms in time domain and 35-50Hz in frequency domain (Fig. 2 (a)), which are consistent with the normal situation of a typical upper limb SEP signal. After injury, main components of SEP start to spread to other regions and have wider distribution ranges in time-frequency domain. It could be observed in Fig. 2 (a) that main components of the three injury groups have mixed distributions, which implies that the level of spinal cord lesion does not have an obvious effect on the postion of the main component of SEP in time-frequency domain.

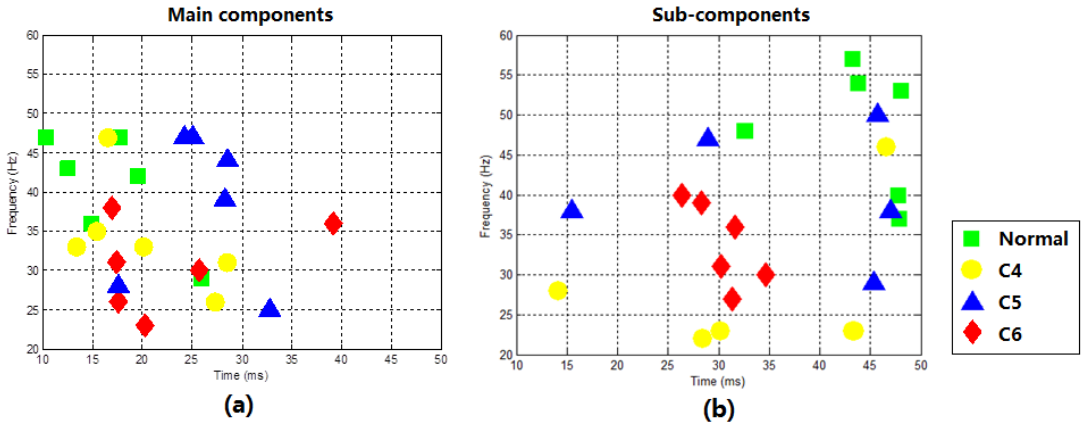

Figure 2. Distributions of the main components and sub-components of each group in time-frequency domain

Time-frequency distributions of the sub-components of normal, C4, C5 and C6 group are demonstrated in Fig. 2 (b). It could be seen that in the normal state sub-components in SEP signal are mainly distributed in the temporal range from 30 to 50ms, and after spinal cord injury they spread to the whole temporal range from 10ms to 50ms. Meanwhile, sub-components of the post-injury groups tend to have lower freqeuncy compared with the normal group. Half of the post-injury sub-components are distributed in the area lower than 35Hz where there is no sub-components of the normal group.

It's evident that sub-components in different post-SCI groups are located in distinct regions. Sub-components of C4 group lie in the areas higher than 45Hz and lower than 30Hz. Sub-components of C6 group concentrate in the region from 25ms to 35ms in time domain and from 25Hz to 45Hz in frequency domain. And the sub-components of C5 group have a more scattered distribution compared with the other groups.

- **Statistical analysis results:**

P-values calculated by t-test are expressed using different colors in Fig. 3. It could be found that there are a number of significantly different areas ($p<0.05$) in each p-value map. Four regions of interest (ROIs) are determined accroding to the distribution patterns of these areas and are plotted using white square frames in fig. 3. It might be noted that the temporal range 0-5ms is easily affacted by stimulation artifact and therefore not included in the ROI A.

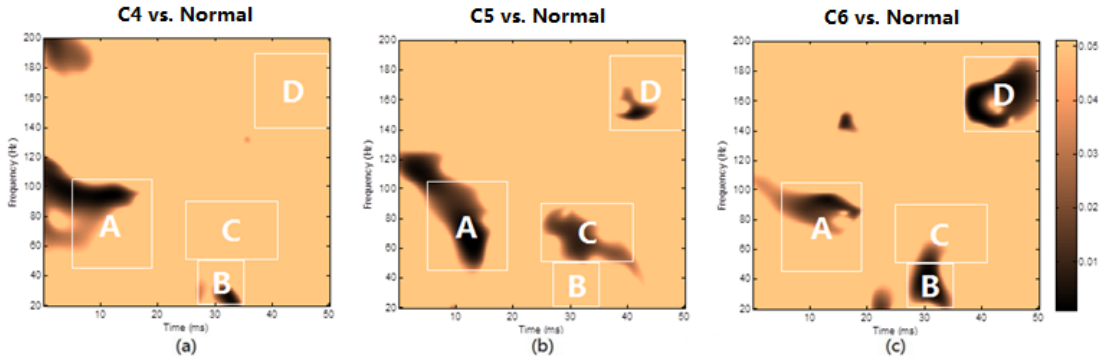

Figure 3. P-value maps and regions of interest (ROIs) drawn based on the results of Student's T-test. (a) C4 vs. Normal; (b) C5 vs. Normal; (c) C6 vs. Normal

Summary values of each injury group calculated within these four ROIs are compared with those of the normal group using t-test as a post-hoc test. The comparison results (p-values) are listed in Table 1.

Table 1: P-values of all the injury groups compared with normal group in ROI A, B, C and D.

	ROI A	ROI B	ROI C	ROI D
Normal	-	-	-	-
C4	**0.028**	0.080	0.475	0.476
C5	**0.002**	0.472	**0.017**	**0.022**
C6	**0.027**	**0.001**	0.092	**0.006**

From Table 1, it could be seen that the three injury groups have significant difference from the normal group within different ROIs. For C5 group, 3 ROIs have p-values less than the commonly used significance level 0.05, which are ROI A, C and D. C6 group also has 3 $p<0.05$ ROIs including ROI A, C and D. As for C4 group, however, there is only one ROI (A) where significant difference could be found.

Discussion

The main target of this study is to determine if the location of spinal cord lesion has an impact on the time-frequency distribution of SEP signal. Since SEP is able to assess the functional integrity of dorsal sensory pathways, the current study tries to examine the time-frequency features of SEP waveforms following a cervical contusion injury to the dorsal section of the rat's spinal cord and to compare the time-frequency patterns of the injury groups.

To evaluate the effect of SCI, it is important to standardize the severity and progress of physical insult in the experiment. For this purpose, the NYU-MASCIS contusion SCI model, which has been widely used clinically for experimental SCI therapeutic and mechanistic research [20,21], was applied in this study. In this model, four contusive SCI grades [22] are standardized by making a 10 gram rod dropped from certain heights including 6.25mm (mild), 12.5 (moderate), 25mm (severe) or 50mm (very severe) onto the dorsal part of spinal cord.

In the animal experiment of this study, a 10-gram rod dropped from the height of 25 mm was applied to delivering the cervical spinal cord contusion, which is a severe injury degree. After the contusion, nerve lesions are produced in the spinal cord at different levels. Note that the

time-frequency distributions of components in SEP signal will likely be different from the results in this paper if the impact parameters are changed. It would be resonable to suppose that contusion at different spinal cord level would also produce different time-frequency pattern when other injury degrees are used. It needs to be recognized that the experiments in the current study are merely associated with acute spinal cord insults which occur within a second rather than compressions evolving over minutes or even longer. If the injury process becomes more chronic, the post-SCI time-frequency distribution of SEP signal would probably be very different.

A widely used time-frequency analysis algorithm, STFT, was applied to calculate the distributions of the recorded SEP signals in time-frequency domain. The STFT results infer that after spinal cord injury the TFD of SEP would change, and the difference in TFDs among the three post-SCI groups are related to the level of the spinal cord lesion.

By comparing the distribution patterns of the components belonging to different groups, it would be more explicit for us to find out the difference among various injury groups in time-frequency domain. According to the result of component distribution estimation, it was found that changes in SCI level have an effect on the TFD of SEP and this effect is mainly embodied in the distribution difference of sub-components (frequency<60Hz and amplitude<1.5) among injury groups. Sub-components of the three injury groups have separated distribution regions in time-frequency domain. For instance, within the region (25-35ms, 25-45Hz) only sub-components of C6 group can be found. And the area below 25Hz only contains the sub-components of C4 group. The presence and absence of sub-components within the above regions, therefore, are potential diagnosis indicators of different lesion levels (C4, C5 and C6). In a practical diagnostic application, for example, if all the sub-components in the collected SEP signal are located within the area from 25ms to 35ms in time domain and from 25Hz to 45Hz in frequency domain, there would be a high probability that this subject has a neurological lesion in its spinal cord at the level of C6. In order to find reliable time-frequency characteristics of each lesion level, experiments involving more animal subjects will be carried out in the future study. And also, the underlying physiological meaning of the above findings needs further test and verification.

After that, Student's t-test is employed to create p-value maps. The results of t-test indicate that each group has one or more ROIs in which its time-frequency distribution is statistically different from that of the normal group, and the $p<0.05$ ROIs of the three injury groups are different from each other. The former means that a contusion to the spinal cord at C4, C5 or C6 level would change the time-frequency distribution of SEP signal. And the later proves that different spinal cord lesion levels correspond to distinct distribution patterns of the SEP signal in the time-frequency domain.

In addition, ROI B and some part (<60Hz) of ROI A and ROI C (Fig. 3) might be associated with the distribution difference of the sub-components among groups (Fig. 2 (b)). For example, most of the sub-components of C6 group are located in the area of ROI B where there is no sub-component of the normal group, which is consistent with the result shown in Table 1 that C6 group is significantly different from the normal group in ROI B. This consistency, however, also needs further confirmation.

In light of the results obtained from the animals, another aim of this study is to give an insight into the application of SEP-based level diagnosis in human patients. Selecting the

appropriate animal model is the backbone of reliably interpreting the results in such a study. Since rats have similar morphological and electrophysiological outcome measures to those of humans, they have been used in many studies involving neurological deficit in spinal cord [20,23]. Therefore, it seems reasonable to extrapolate the conclusions drawn from the rat experiments for the potential utilization in human patients with spinal cord neurological injuries.

Conclusion

According to the above findings, it could be concluded that SEP signals recorded after spinal cord lesions at different levels have distinct time-frequency distributions. Each injury group has a unique distribution pattern which at the same time is distinguished from that of the normal group. This conclusion is consistent with our hypothesis and could be seen as the fundamental of the future work. Based on this prior knowledge, unique SEP time-frequency features of each lesion level will be found in the following study and used as a representive indicator of that level. And these unique time-frequency features corresponding to various lesion levels could probably be applied as a useful level diagnosis tool for spinal cord lesions.

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