Electroacupuncture improves neuronal function by stimulation of ascending peripheral nerve conduction in rats with spinal cord injury

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OBJECTIVE: To establish a method of transient sciatic nerve blockade and to examine the involvement of the ascending peripheral nerve pathway in the therapeutic effect of electroacupuncture at Zusanli (ST 36) in rats with spinal cord injury (SCI).

METHODS: We examined the transient effect of daily lidocaine administration into the posteromedial part of the greater trochanter on sciatic nerve function using electrophysiological examination and histopathology of the sciatic nerve. Rats were divided into three groups: an SCI group (SCI without treatment), an SCI with electroacupuncture treatment (SCI-EA) group, and an SCI with nerve block and electroacupuncture (SCI-NB-EA) group (nerve block was achieved by lidocaine administration to transiently block the ascending peripheral nerve pathway). Behavioral tests and electrophysiological examinations were performed to evaluate recovery of neurological function.

RESULTS: Sciatic nerve conduction was normal immediately before daily lidocaine administration. Histopathological analysis also indicated normal sciatic nerve, confirming that lidocaine nerve blockade was suitable and reversible for transiently eliminating nerve transmission. Neurological function in the SCI-EA group was superior to that in the SCI group, while no differences were found between the SCI and SCI-NB-EA groups.

CONCLUSION: Electroacupuncture treatment can promote recovery of neurological function. Facilitation of nerve conduction may play an important role in this recovery.

INTRODUCTION

Spinal cord injury (SCI), which is common in humans and dogs, can be classified based on the underlying cause (traumatic vs non-traumatic). Traumatic injuries result acute trauma, such as from traffic accidents and falls, while non-traumatic injuries are caused by herniated disks, tumor compression, and spinal cord diseases.1 These injuries often lead to pain, spasms, problems
with bladder and bowel regulation, respiratory failure, and permanent disability and can, therefore, severely affect the quality of life. Advances in understanding the pathophysiology of SCI are constantly being made; however, the prognosis of SCI patients is generally poor and treatment options are limited. The complexity of the mammalian nervous system and the limited ability of the central nervous system (CNS) to regenerate mean that improved treatment strategies still need to be explored.

Acupuncture is effective in the treatment of various diseases and has been recommended by the World Health Organization. It is used to treat SCI and is an important component of Traditional Chinese Medicine. It has achieved dramatic therapeutic effects in clinical practice, particularly in veterinary medicine. A substantial body of evidence indicates that acupuncture/electroacupuncture is efficient in treating SCI. However, the pathway involved in facilitating natural healing after acupuncture/electroacupuncture remains unclear.

Blocking neural signaling is an approach to investigate the relationship between electroacupuncture stimulation and nerve conduction. To the best of our knowledge, no reports have evaluated the involvement of nerve pathways in the therapeutic effect of electroacupuncture on SCI. In this study, we used lidocaine, a reversible nerve blocker, to prevent signal transmission only during electroacupuncture stimulation. This method has the advantage that it reliably retains the normal function and integrity of peripheral nerves compared with peripheral nerve transection.

Electrophysiology techniques, including determination of F-waves and somatosensory evoked potentials (SEPs) are sensitive and objective ways of evaluating neurological function. An F-wave is a late response produced by antidromic activation of motor neurons. F-waves are preferred for detecting disorders of the peripheral nerves conduction and the spinal cord anterior horn cells activated by minimum F-wave latency and an F-wave occurrence rate parameters. SEPs can be elicited by sensory stimuli and used to measure the electrical activity of the cerebral cortex motor area, thus facilitating a functional assessment of the somatosensory system pathway from the peripheral nerves to the cortex. Electrophysiological parameters can be used to assess nerve dysfunction, reveal specific sites of neuropathy, and identify the nature of a lesion; however, it is important to note that SEP amplitude is a more significant parameter both in operation monitoring and in assessment of mild lesions of the spinal cord than SEP latency. Overall, these parameters are important in neurological research and clinical neurological examination. However, detailed electrophysiology studies of electroacupuncture in experimental animals are limited. In this study, we used electrophysiological examination to elucidate whether nerve block by lidocaine was suitable and reliable for transient elimination of nerve transmission. Subsequently, the relationship between ascending peripheral nerve transmission and the effects of electroacupuncture treatment in rats with SCI was evaluated by behavioral analysis and electrophysiological examination.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats of specific pathogen free grade (SLC, Shizuoka, Japan) at 8-9 weeks of age, weighing 260-300 g, were used in this study. Three rats were housed per cage (20 cm x 38 cm x 24 cm) at a temperature of (23 ± 2 ℃) and a relative humidity of 60% ± 10% under a 12 h light/12 h dark cycle (lights on at 6 am) and given free access to water and food (CRF-1; Charles River, Yokohama, Japan). Animal management was in accordance with the Azabu University Animal Experiment Guidelines, April 2000. All experiments were approved by the Committee for Animal Experimentation at Azabu University.

**Nerve block**

Rats were anesthetized deeply with 1% isoflurane (Mylan Inc., Osaka, Japan). Both hindlimbs were subjected to sciatic nerve block by injecting 0.5 mL 1% lidocaine (Nagase Medical Co., Ltd., Hyogo, Japan) into the posteromedial part of the greater trochanter, pointing in an anteromedial direction. In a preliminary study, we confirmed that F-waves disappeared within 5 min of lidocaine administration (Figure 1). This effect lasted for 30-60 min.

![Figure 1. F-waves before and after sciatic nerve block by lidocaine in a normal rat](image1)

A: in normal rats, various F-wave latencies and waveforms were observed approximately 20% above the supramaximal intensity of the M-wave responses; B: after the sciatic nerve was completely blocked by lidocaine, M-wave amplitudes reduced and F-waves disappeared.
**Electrophysiological examinations**

Rats were anesthetized with isoflurane and rectal temperature was monitored and maintained at 36.8 °C using an animal hot plate (TK-43; Vectronics, Osaka, Japan). Electrophysiological examinations were performed in both hindlimbs and the obtained values were averaged. A system for the measurement of evoked potentials (MEB-9402; Nihon Kohden, Tokyo, Japan) was used for the following examinations:

**F-wave examination**

Measurements were performed in the prone position. A recording electrode (a needle electrode) was inserted into the interosseous muscle through the plantar surface of the foot. An indifferent electrode (a needle electrode) was inserted into the lateral aspect of the fifth digit, and a reference electrode (a disk electrode) was placed in the lateral aspect of the heel. A stimulation needle electrode was placed on the tibial nerve above the ankle. Thirty-two serial stimuli were applied at a frequency of 1 Hz with a duration of 200 µs. The stimulus intensity was approximately 20% above the supra-maximal intensity for the M-wave response, typically quantified by the distal motor latency. The number of obtainable F-waves and the minimum latency, defined as the shortest latency from the stimulus artifact to the first deflection (positive or negative) of an F-wave, were measured.19

**SEP examination**

Rats were anesthetized with isoflurane, and the head was shaved and sterilized with 70% alcohol. After a skin incision was made on the head, the periosteum was stripped, and the cranial bone was cleaned. Four transcranial screw electrodes were implanted into the cranial bone in the following order; one electrode was initially implanted into the nasal bone parallel to the orbit as an indifferent electrode, two electrodes were implanted into the parietal bone 3 mm posterior and ± 1 mm lateral to bregma as recording electrodes that receive sensory signals from the sensory pathways from the hindlimbs up to each hemisphere of the somatosensory cortex,20 and the last electrode was set in the central part of the interparietal bone as a reference electrode. The electrodes were placed in light contact with the dura so they would not compress the dura or other brain structures. The screw electrodes were fixed to the cranium bone with cement (Hy-Bond Carbo Cement, Shofu, Kyoto, Japan). Prior to SEP recording, rats were stabilized under anesthesia. The stimulation needle electrodes were placed on the tibial nerve above the ankle in the prone position. Stimulation was performed at an intensity of 1 mA, a duration of 200 µs, and a frequency of 1 Hz. The SEP was acquired from the opposite hemisphere to the hindlimb. The signal-to-noise ratio was improved by an ensemble averaging of 200 sweeps. SEPs were measured twice at an interval of 1 min. The amplitudes were measured and averaged.

**SCI**

Rats were anesthetized with isoflurane. Body temperature was maintained at 37 ℃ with an animal hot plate throughout the surgery. The dorsal skin was shaved and sterilized with 70% alcohol. A dorsal longitudinal incision was made to expose the T7-T10 vertebrae, and a laminectomy was performed at the T8-T9 level. The exposed dorsal surface of the spinal cord was subjected to a weight-drop injury (10 g × 20 mm) using a handmade impactor composed of a copper tube (3 mm inner diameter) and an iron bar (10 g, 2.5 mm diameter) with a tip of about 1.5 mm. The spinal cord tissue was kept intact before the weight-drop injury. The wound was cleaned with normal saline, and the incision was closed by suturing the muscle layers and clipping the skin. The bladder was emptied 1-2 times daily until the micturition reflex was recovered. In a preliminary experiment, we observed that sensory and motor functions in rats were completely unaffected by a sham operation (laminectomy only).

**Electroacupuncture**

Rats were anesthetized with isoflurane during electroacupuncture treatment. Stainless steel needles (0.20 × 25 mm; Suzhou Acupuncture & Moxibustion Appliance Co., Ltd., Suzhou, Jiangsu Province, China) connected to an electrostimulator (Lasper-A; Technolink Co., Ltd., Niigata, Japan) were inserted on both sides of the Zusanli (ST 36, a posterolateral position in the knee, about 5 mm under the malleolus) acupoint until "De Qi" occurred. Stimulation intensity was adjusted to induce a slight twitch of the hindlimbs, then administered at a frequency of 1 Hz for 20 min.

**Behavioral analysis**

Behavioral function was examined using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale.15 Rats were individually placed in an open field (1 m × 1 m) for 4 min and scored by two investigators. The degree of injury severity was scored as ranged from 0 (complete paralysis) to 21 (normal locomotion) according to the method of Barros Filho et al.22 It was categorized into three stages according to isolated joint movement for which three attributes were identified (scores 0-7), sweeping hindlimb movements (scores 8-13), and coordinated forelimb and hindlimb movement (scores 14-21). The score of normal rats was 21.

**Sciotic nerve histopathology**

Rats were anesthetized with isoflurane. A sciotic nerve segment near the posteromedial part of the greater trochanter was excised and immersed in 10% formalin neutral buffer solution (Wako Pure Chemical, Osaka, Japan) overnight at 4 ℃. The samples were then embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin and eosin (HE).27,28 Sections were evaluated via microscopy (× 400; Axio Scope.A1, Carl Zeiss, Jena, Germany).
Experimental procedure
To examine the effects of daily lidocaine administration on sciatic nerve conduction, lidocaine was administered into the posteromedial part of the greater trochanter once a day for 14 d (Lido, n = 5). Examination of F-waves was performed on days 1, 3, 7, 10, and 14 immediately before lidocaine administration. Control rats received the same electrophysiological examination over the same time course (control, n = 4). Segments of the sciatic nerve were obtained from one rat from each group at the end of the experiment for hematoxylin-eosin staining and histopathological analysis. For the SCI experiment, electrodes for recording SEPs were implanted in all rats 4-5 d before SCI. SCI model rats were randomly divided into three groups. The SCI group was subjected to SCI without treatment (SCI, n = 6). The electroacupuncture treatment group was subjected to the same procedure as the SCI group and underwent daily electroacupuncture treatment after the SCI operation (SCI-EA, n = 6). The nerve block group was subjected to the same procedure as the SCI-EA group and sciatic nerve conduction was blocked by lidocaine before electroacupuncture treatment (SCI-NB-EA, n = 6). On post-operative days 1, 3, 7, 10, and 14, BBB tests and SEP examinations were performed to evaluate the restoration of motor and sensory functions, respectively.

Statistical analyses
Data are presented as the mean ± standard error of the mean (SEM) values. All statistical analyses were performed using a bell curve in Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan). Comparisons among the experimental groups were evaluated with the paired Student’s t-test or Bonferroni test. P values < 0.05 were considered statistically significant.

RESULTS
Sciatic nerve function
F-wave examination was performed immediately before the next lidocaine administration. The F-wave occurrence rate was used to examine changes in hindlimb excitatory motor neuron function during daily lidocaine administration. The F-wave occurrence rate to 32 serial stimuli was 100%. No obvious changes were observed, except between the Lido and control groups (P < 0.05) at 3 d after the initiation of daily lidocaine administration (Figure 2A).

To examine total changes in hindlimb motor neuron conduction after daily lidocaine administration, the minimum F-wave latency of rat hindlimbs was measured in response to 32 serial stimuli immediately before the administration of lidocaine. No obvious changes between the Lido and control groups were found within 14 d of initiating daily lidocaine administration (Figure 2B).

Histopathology of the sciatic nerve
Normal architecture was observed in HE-stained sections of sciatic nerve specimens of the control group. This consisted of myelinated nerves of different sizes, a central axon appearing as a dot or wavy lines, and a large Schwann cell nucleus surrounding the empty space of the lipid myelin sheath. In addition, some small blood vessels were scattered between nerve fibers (Figure 3A). The Lido group tissue sections appeared similar to control group sections; typical neuropathological changes such as inflammatory cell infiltration, demyelination, vacuolization, and degenerative changes were not observed (Figure 3B).

![Figure 2 Fluctuation in F-wave parameters after daily lidocaine administration](image2)

![Figure 3 Histopathological images of sciatic nerve sections stained with hematoxylin and eosin (×400)](image3)

A: normal nerve tissue from the control group showed normal myelinated nerves of different sizes and some small blood vessels; B: nerve tissue from the Lido group was similar to that from the control group, without inflammatory cell infiltration, demyelination, vacuolization, or degenerative changes. Black arrow: cross section or longitudinal section of an axon, blue arrow: empty space in a lipid myelin sheath, yellow arrow: Schwann cell nucleus, red arrow: blood vessels.
SEP amplitude after SCI
SEP amplitude was used to define the recovery of the spinal cord along the sensory pathway. SEP values on the day before SCI were (60 ± 7), (72 ± 6), and (48 ± 6) µV in the SCI, SCI-EA, and SCI-NB-EA groups, respectively; there were no statistical differences among the groups. On the first day after SCI, SEP amplitudes were significantly decreased in all three groups compared to before the operation. Over the following 7 days, the amplitude of the SCI-EA group gradually recovered. The amplitude was significantly higher in the SCI-EA group compared with those in the SCI \((P < 0.05)\) and SCI-NB-EA \((P < 0.01)\) groups, but no differences were found between the SCI and SCI-NB-EA groups (Figure 4).

Behavioral changes after SCI
To evaluate motor function, behavior was examined using the BBB test. After SCI, all rats were paralyzed, and they moved by pulling themselves forward with their forelimbs. Hindlimb locomotor activity improved gradually in all groups throughout the entire follow-up period. Fourteen days after the operation, autonomous walking was restored. However, the coordination of forelimbs and hindlimbs was different. The scores on day 14 in the SCI-EA group were significantly higher compared with those in the SCI \((P < 0.05)\) and SCI-NB-EA \((P < 0.01)\) groups (Figure 5).

DISCUSSION
In this study, we used a rat spinal cord contusion model, which has pathophysiological changes similar to those observed in clinical cases of SCI\(^{29,30}\) to evaluate the recovery of neural function with Zusanli (ST 36) electroacupuncture treatment. We used electrophysiological and behavioral examinations to assess recovery. We also reversibly blocked the ascending peripheral nerve pathway using lidocaine and evaluated whether the therapeutic effect had changed. To analyze the ef-
fect of daily lidocaine administration on sciatic nerve conduction, we made histopathological observations and examined F-waves to assess sciatic nerve function. Zusanli (ST 36) is one of the main acupoints along the hindlimb Yangming stomach meridian, according to the theory of traditional Chinese medicine. Previous SCI studies showed that stimulation at Zusanli (ST 36) improves spinal cord blood flow and neuronal function and reduces inflammation. Therefore, it is widely used in the clinical treatment of motor dysfunction disease. The tissue surrounding Zusanli (ST 36) is adjacent to the peroneal nerve, which is a branch of the sciatic nerve. Blocking the sciatic nerve affects Zusanli (ST 36) signal transmission to the CNS; therefore, we considered that Zusanli (ST 36) is the most suitable acupoint for this study.

Lidocaine alters neuronal signal conduction by blocking fast voltage-gated Na⁺ channels in the membrane. This reversibly blocks the signal conduction of local neurons to upper neurons by stopping neural activity. The minimum F-wave latency did not significantly change compared with control rats when measured immediately before daily lidocaine administration. Although the F-wave occurrence rate changed on day 3 after lidocaine injection, no statistical differences were found between the Lido and control groups on all other days. Therefore, we believe that the change in occurrence rate on day 3 is not a notable finding. The histopathological analysis confirmed that the sciatic nerve near the daily lidocaine injection site was normal. These results showed that repeated lidocaine administration did not cause any long-term effect on neurologic function, at least, during the 14-d daily administration. Thus, lidocaine is suitable and reliable for examining temporal nerve conduction block.

After injury, SEP amplitudes and BBB scores were significantly decreased. Following this, SEP amplitudes and BBB scores were significantly improved in the SCI-EA group compared with the SCI group on days 7-14 and day 14, respectively. Notably, no differences were observed between the SCI and SCI-NB-EA groups. These data support the notion that electroacupuncture treatment of SCI. These data will help elucidate the mechanisms behind electroacupuncture treatment and provide avenues to further explore effective patterns of electroacupuncture stimulation that can improve its curative effects in veterinary and human medicine.

REFERENCES


