



Effect of long-term electroacupuncture stimulation on recovery of sensorimotor function after peripheral nerve anastomosis

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ABSTRACT

Background Recently, application of electroacupuncture (EA) to stimulate nerve regeneration has become a mainstream treatment in clinical rehabilitation and related basic research, but the efficacy of long-term stimulation has not been confirmed.

Objective To evaluate the influence of long term EA on peripheral nerve injury (PNI) from multiple angles.

Method Twenty-four rats were divided into three groups: control, PNI and PNI+EA. In the latter two groups, PNI was modelled by transection followed by re-anastomosis of the sciatic nerve. In the PNI+EA group only, EA was delivered using a discontinuous wave with frequency 5 Hz, pulse width 2 ms, and intensity approximately 2 mA, until the affected limb was observed to twitch slightly. The treatment was given for 15 min each time, six times a week (continuously for 6 days followed by a 1-day break) for a total of 8 weeks. The effects of EA on anastomotic sciatic nerve regeneration were evaluated using the sciatic function index (SFI), mechanical withdrawal thresholds, thermo-nociceptive thresholds, conduction velocity of the sciatic nerve and bilateral gastrocnemius wet weight.

Results From weeks 2 to 4 after modelling, the SFI recovery rate in the PNI+EA group was faster than that in the PNI group. In week 4, the SFI of the PNI+EA group was significantly higher than that of the PNI group ($p < 0.05$). However, a significant effect of EA was no longer evident from weeks 5 to 8. There was no effect of acupuncture on anti-amyotrophy and conduction velocity of the sciatic nerve at 8 weeks after modelling. EA did not shorten the paw withdrawal threshold time, but appeared to alleviate thermo-nociceptive sensitivity.

Conclusion Long term repeated stimulation of the same site with EA does not appear to be conducive to the functional recovery of an injured sciatic nerve in rats.

INTRODUCTION

Peripheral nerve injury (PNI) is one of the most common traumatic disorders and has a remarkable impact on the daily life of patients.^{1,2} Severe nerve injury has a

devastating effect on patients' quality of life. However, despite using microsurgical techniques and different repair methods, a fully functional outcome, especially with respect to motor function, is rarely achieved.³

Acupuncture is a method of Chinese medical treatment, which has been used in practice for more than 2000 years. Electroacupuncture (EA), a modified acupuncture technique, has been used for several decades in the treatment of PNI.⁴⁻⁶ Research has demonstrated that EA has a positive effect on the repair of injured nerves, leading to improvements in behaviour, electrophysiology and morphology.⁷⁻⁹ Several research studies have shown that low-frequency EA is a better approach to the promotion of nerve regeneration after traumatic injury.¹⁰ The clinical outcomes from rehabilitation medicine must be taken into consideration and the treatment has to be safe and effective. Although EA is considered to be a safe and effective way to assist patients' rehabilitation after PNI, its efficacy over longer time periods has not been confirmed. Therefore, the aim of this study was to evaluate the influences of long-term EA on PNI from multiple angles.

METHODS

Animals and grouping

This experiment was conducted in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. Twenty-four healthy, pathogen-free, male Sprague-Dawley rats with a body mass of 190 ± 20 g, were provided by the Experimental Animal Centre, Tianjin University of Traditional Chinese Medicine. The laboratory environment was as follows:



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temperature 18–22°C; indoor light exposure approximately 8 hours; relative humidity about 45%; free access to water and food; and six rats per cage. According to a random number table, rats were randomly divided into control, PNI and PNI+EA groups. Each group consisted of eight animals. In each group, four rats underwent determination of sciatic function index (SFI) and gastrocnemius wet weight, and the other four rats were used to evaluate mechanical withdrawal thresholds, thermo-nociceptive thresholds and conduction velocity of the sciatic nerve.

Modelling

All animals were deeply anaesthetised by intraperitoneal injection of 10% chloral hydrate (0.4 mL/100 g). Their left gluteal regions were shaved and cleaned with medicinal alcohol. A 2 cm vertical incision was made at the rear of the middle femoral shaft on the left side to expose the biceps femoris muscle. The sciatic nerve was then dissociated by blunt dissection and transected with micro-scissors 0.5 cm below the sciatic notch. The injured sciatic nerve was anastomosed by suture of the epineurium and perineurium with 9–0 microsurgical thread, and the shin was then sutured with 3–0 thread. The above operation was performed by a single operator.

Procedures by group

In the PNI+EA group, the sciatic nerve injury model was established by transection injury of the sciatic nerve stem followed by re-anastomosis, as described above. The following day, after successful modelling, acupuncture needles (Hwato brand, 0.25 mm outer diameter, 13 mm length; Suzhou Acupuncture Supplies Factory) were inserted at GB30 (*Huantiao*), located within the depression in front of the femoral greater trochanter at the leading edge of the hip joint, and ST36 (*Zusanli*), located 5 mm lateral to the anterior tubercle of the tibia, both on the affected side, to a depth of about 5 mm such that the muscle twitched instantly and the toes trembled. An EA therapeutic apparatus (Hwato SDZ-V, Suzhou Medical Equipment Factory) was then connected; the anode was connected to GB30 and the cathode to ST36. A discontinuous wave was applied at a frequency of 5 Hz, pulse width 0.2 ms and intensity of about 2 mA, and the affected limb was observed to twitch slightly. The treatment was given for 15 min each time, six times a week (continuously for 6 days followed by a 1-day break) for 8 weeks. Rats in the PNI group were kept under the same conditions after modelling without any treatment. Rats in the control group were kept under the same conditions without modelling or treatment.

Calculation of sciatic function index

The SFI has been widely used to quantify motor recovery from sciatic nerve injury in a number of different injury models in rats. The video recording technique for measuring the SFI used in this study has

previously been reported by Smit *et al* and Varejao *et al*.^{11 12} In this study, a digital camera and Adobe PhotoShop software were used to record the print of the hind paw of the rats, and data were collected.

To analyse the walking track, a Plexiglas runway (12×15×70 cm) was used with a mirror placed under the runway at an angle of 45°. In this way, a split screen provided a lateral and a plantar view of the rat. Recordings were made of the hind feet using a digital SLR camera (EOS 7D, Canon, Corp., Japan) on a stand positioned 150 cm from the runway. Footprints were obtained before modelling and each week thereafter. Walking movements of a minimum of four complete runs were recorded. Three separate images of the experimental (E) and non-experimental (N) foot were loaded onto a computer. From the digitised footprints, the following variables were measured using Adobe PhotoShop CS 6.0 tools (Adobe Systems Inc., California, USA): the distance between the first and fifth toe (toe spread; TS); the distance between the second and fourth toe (intermediate toe spread; ITS); and the distance from the heel to the top of the third toe (print length; PL). To calculate the SFI these dynamic factors were incorporated into the following formula:

$$SFI = -38.3 \left(\frac{EPL-NPL}{NPL} \right) + 109.5 \left(\frac{ETS-NTS}{NTS} \right) + 13.3 \left(\frac{EIT-NIT}{NIT} \right) - 8.8.$$

Mechanical withdrawal thresholds

Mechanical withdrawal thresholds were determined for all rats before modelling and weekly thereafter (for 8 weeks). Rats were placed in an acrylic cube (20×20×17 cm) on an elevated metal mesh grid and allowed to acclimatise for 30 min before testing. We used an ascending series of von Frey filaments (North Coast Medical, Morgan Hill, California, USA) that delivered approximately logarithmic incremental forces (ranging from 0.008 to 4 g). Each monofilament, starting with the lowest force, was applied six times to the mid-plantar region of the hind paw before the next higher force monofilament. The monofilament that produced a response of paw withdrawal, flinching or licking in three of the six applications was defined as the 50% mechanical withdrawal threshold, as previously described.¹³

Thermo-nociceptive thresholds

Thermo-nociceptive thresholds were determined for all rats before modelling and weekly thereafter (for 8 weeks). A focused light from a 12.5 W projection bulb was applied to the middle of the plantar surface of the hind paw (3 mm diameter).^{9 14} Rats were placed in an acrylic cube (20×20×17 cm) on an elevated glass pane and allowed to acclimatise for 30 min

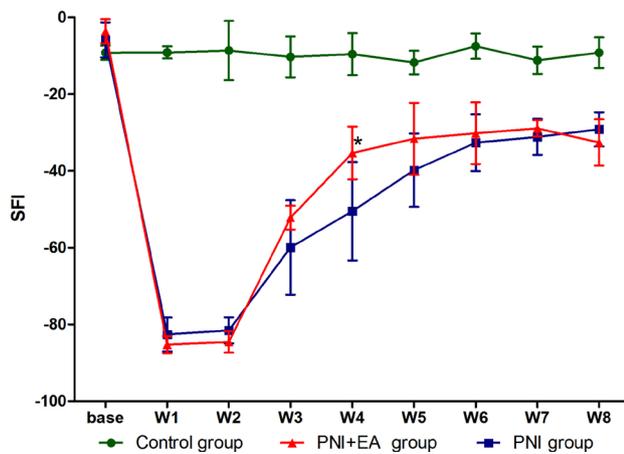


Figure 1 The sciatic function index (SFI) of control, PNI and PNI+EA groups was recorded at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after modelling. * $p < 0.05$ compared with the PNI group at 4 weeks after modelling. EA, electroacupuncture; PNI, peripheral nerve injury.

before testing. The projection bulb was turned off as soon as the mouse removed its paw and a digital timer connected in series measured the paw withdrawal latency to an accuracy of 0.1 s. A cut-off latency of 20 s was used to avoid the possibility of tissue damage.

Conduction velocity of the sciatic nerve

After 8 weeks of modelling, rats in each group were anaesthetised as described above and the left sciatic nerve was surgically exposed. The conduction velocity of the sciatic nerve was measured as described by Martins *et al.*¹⁵ A hook-shaped bipolar stimulating electrode was placed on the injured sciatic nerve, 0.5 cm inferior to the sciatic notch. The recording electrode was inserted into the gastrocnemius muscle at a point 4.5 cm away from the stimulating electrode and a ground electrode was placed between the stimulating and recording electrodes. Another ground electrode was inserted into the muscle adjacent to the nerve. Recordings were made on Power Lab and analysis software (AD Instruments, Australia). A square-wave pulsed stimulus with a low intensity and duration of 0.02 ms was applied to elicit the motor action potential. The intensity was gradually increased until a supramaximal stimulus was reached. The latency was measured as the time from the stimulus onset to the initial rise of a motor response. The conduction velocity was calculated from the latency and distance between the electrodes.

Bilateral gastrocnemius wet weight

After 8 weeks of EA, the rats in each group were anaesthetised as described above, the bilateral gastrocnemius was surgically removed and the wet weight was measured.

Statistical analysis

SPSS 21.0 statistical software was used to analyse the data, and the results are presented as mean \pm SD.

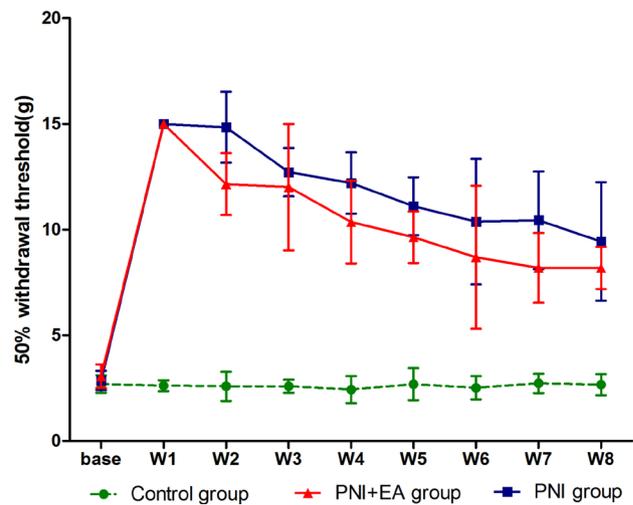


Figure 2 Mechanical withdrawal thresholds of control, PNI and PNI+EA groups were recorded at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after modelling. EA, electroacupuncture; PNI, peripheral nerve injury.

Variance was applied to evaluate integral differences and equal variance was compared between the two groups by test of least significant difference. A p value < 0.05 was considered statistically significant.

RESULTS

Observation of general status

After modelling, the intake of food and water and defecation of rats in all groups were normal. Infection and ulcers were not found on the distal limb. The rats walked by dragging toes or bouncing after modelling. Gait began to recover 2 weeks after modelling.

Sciatic function index

As shown in [figure 1](#), in the first week after modelling, the SFI in the PNI+EA and PNI groups decreased rapidly and then began to recover after 2 weeks. Between weeks 2 and 4 after modelling, the SFI recovery rate in the PNI+EA group was faster than that in the PNI group. From weeks 5 to 8 after modelling, the SFI recovery rate in the PNI+EA model slowed down, compared with the PNI group, and there were no significant differences between the two groups ($p > 0.05$).

Mechanical withdrawal thresholds

As shown in [figure 2](#), in the first week after modelling, the paw withdrawal threshold (PWT) in the PNI+EA and PNI groups increased significantly. Between weeks 2 and 8 after modelling, the PWT in the PNI+EA and PNI groups decreased gradually; there were no significant differences in PWT between the PNI+EA and (untreated) PNI groups ($p > 0.05$).

Thermo-nociceptive thresholds

As shown in [figure 3](#), in the first week after modelling, the paw withdrawal response to heat stimulation of the PNI+EA and PNI groups had a latency of

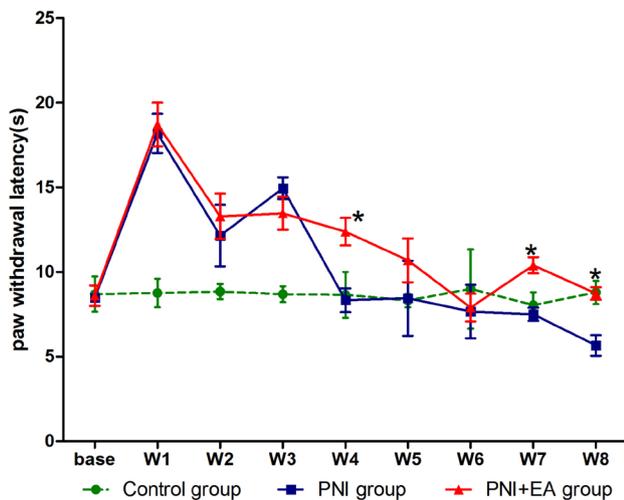


Figure 3 Thermo-nociceptive thresholds of control, PNI and PNI+EA groups were recorded at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after modelling. * $p < 0.05$ compared with PNI group at 4, 7 and 8 weeks after modelling. EA, electroacupuncture; PNI, peripheral nerve injury.

around 20s, showing that there were no nociceptive responses to heat stimulation. Between weeks 2 and 6 after modelling, paw withdrawal latency (PWL) in the PNI+EA group reduced gradually and reached normal levels at 6 weeks. The PWL in the PNI group returned to normal by 4 weeks after modelling. However, PWL in the PNI group was markedly lower than that in the PNI+EA and control groups at 8 weeks after modelling ($p < 0.05$).

Conduction velocity of the sciatic nerve

As shown in figure 4, sciatic nerve conduction velocity in the PNI and PNI+EA groups was significantly lower than that in the control group ($p < 0.05$) when measured at 8 weeks after modelling; there was no significant difference between the PNI+EA group and the PNI group.

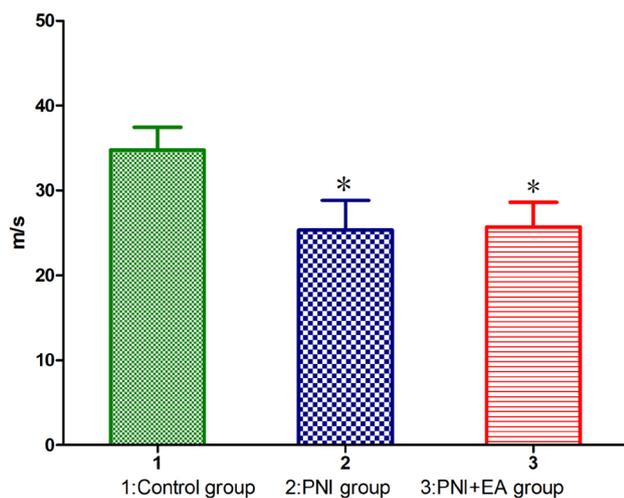


Figure 4 The conduction velocity of the sciatic nerve in the control, PNI and PNI+EA groups at 8 weeks after modelling. * $p < 0.05$ compared with the control group. EA, electroacupuncture; PNI, peripheral nerve injury.

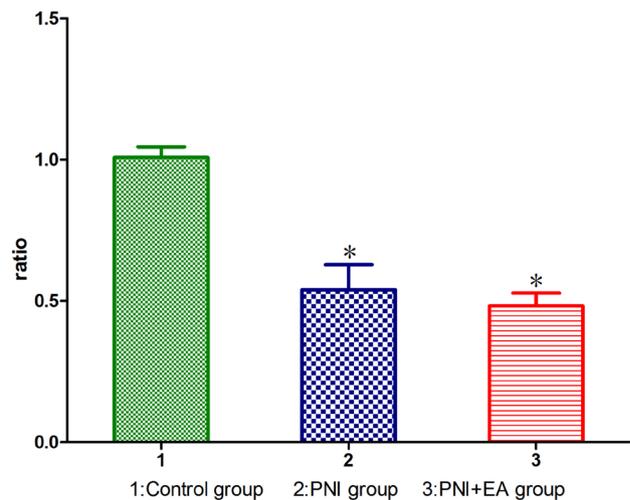


Figure 5 The ratio of bilateral gastrocnemius wet weight in the control, PNI and PNI+EA groups at 8 weeks after modelling. * $p < 0.05$ compared with the control group. EA, electroacupuncture; PNI, peripheral nerve injury.

Bilateral gastrocnemius wet weight

As shown in figure 5, the ratio of bilateral gastrocnemius wet weight in the PNI and PNI+EA groups was significantly lower than that in control group ($p < 0.05$) 8 weeks after modelling; there was no significant difference between the PNI+EA group and the PNI group.

DISCUSSION

The primary goal of nerve repair is to allow re-innervation of the target organs by guiding regenerating sensory and motor axons into the environment of the distal nerve with minimal loss of fibres at the site of repair. Many factors have to be taken into consideration when trying to predict the outcome of peripheral nerve repair, including type, location and extent of nerve injury, timing of surgery and surgical technique.¹⁶ Over the last century, much has been learnt of peripheral nerve pathophysiology and the introduction of microsurgical nerve repair has been a breakthrough. However, despite meticulous surgical techniques and different repair methods, a fully functional outcome, especially of sensorimotor function, is rarely achieved.

Recently, application of EA to stimulate nerve regeneration has become a mainstream treatment in clinical rehabilitation and related basic research. A clinical study by Tang *et al* showed that direct electrical stimulation of the injured ulnar nerve using acupuncture needles combined with rehabilitation accelerates nerve regeneration.¹⁷ Ho *et al*, studying the influences of EA treatments on transected median nerve regeneration from morphological, electrophysiological and functional angles, suggested that EA can promote recovery of transected median nerve morphology and function.¹⁸ Hoang *et al* showed that EA exerts a positive influence on motor recovery and is effective for the

treatment of pain symptoms that develop during target re-innervation 40 days after an operation.⁹ Accordingly, we know that EA can promote nerve regeneration and functional recovery, but its long-term effect is unknown.

The SFI has been widely used to assess motor recovery from sciatic nerve injury in rats. This study confirms that EA can significantly promote the recovery of motor function in the first 4 weeks, but its effect is not obvious after that time point. Furthermore, differences between groups in bilateral gastrocnemius wet weight and conduction velocity of the sciatic nerve were not significantly different at 8 weeks post-modelling. However, at week 8, EA significantly improved PWL and alleviated pathological pain, which is consistent with previous studies.⁹

Research shows that the rate of axonal regeneration is generally estimated to be 1 mm per day, varying broadly from 0.5 to 9 mm per day.¹⁹ Experimental studies have shown that the transected sciatic nerve of rats regenerates to target organ after anastomosis of 3–4 weeks.²⁰ Consequently, many researchers choose 2–4 weeks as the time frame over which to investigate the efficacy of EA. Therefore, we selected a more sustained period of 8 weeks to investigate the longer term efficacy of EA in order to have a more comprehensive understanding of the role of EA in peripheral nerve injury.

There are two potential explanations for the mechanism underlying the promotion of nerve regeneration by EA. One is that neural adhesion molecules, nerve growth factor and other nerve growth and nutritional factors move electrophoretically towards the cathode resulting in a concentration gradient that stimulates nerve regeneration. The other is that axonal injury causes an influx of Ca²⁺ ions into the proximal end of the severed axon.⁶ In this study, the regenerative axons would have been likely to reach ST36 at 5 weeks after modelling. Between weeks 5 and 8 after modelling, the distal regenerative axon may have been located at the far end of ST36, beyond the two needle electrodes. Consequently, at this time, EA may have been unable to promote regeneration of the nerve.

CONCLUSION

This study provides evidence that repeatedly stimulating the same site using EA for more than 4 weeks does not contribute to the recovery of injured nerve function.

Contributors MZ and YZ performed the experiment and prepared the manuscript. YB participated in the discussion and helped with the experiment. YG designed the study protocol. All the authors have read and approved the final version of the manuscript accepted for publication.

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Competing interests None declared.

Ethics approval This study was prospectively approved by the animal ethics committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2015033).

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