

## Animal Trial

## **e** Analgesic Effects and Neuropathology Changes of Electroacupuncture on Curing a Rat Model of Brachial Plexus Neuralgia Induced by Cobra Venom

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**Background:** Electroacupuncture (EA) is widely applied to treat neuropathic pain. Brachial plexus neuralgia (BPN) is a common form of chronic persistent pain. Few studies have evaluated the analgesic effects and mechanism of EA using the novel animal model of BPN.

**Objective:** To observe the curative effects of repeated EA on curing BPN induced by administration of cobra venom to the lower trunk of the right brachial plexus.

**Study Design:** Controlled animal study.

**Setting:** Department of Anesthesiology, Pain Medicine & Critical Care Medicine, Aviation General Hospital of China Medical University.

**Methods:** Sixty-six adult male Sprague-Dawley rats were equally and randomly divided into the following groups: normal control (NC), brachial plexus neuralgia (BPN), BPN with sham EA stimulation, BPN with EA stimulation starting on postoperative day 1 (EA1), and BPN with EA stimulation starting on postoperative day 12 (EA12). The BPN model was established by administration of cobra venom to the lower trunk of the right brachial plexus. On postoperative day 1 or day 12, EA (constant square wave, 2 Hz and 100 Hz alternating frequencies, intensities ranging from 1 – 1.5 – 2 mA) was applied to the right “Shousanli” (LI10) and “Quchi” (LI11) acupoints for 30 minutes, once every other day for 12 times in both groups. Mechanical withdrawal thresholds (MWT) were tested with von Frey filaments. Video recordings were conducted to analyze the spontaneous exploratory behaviors. Moreover, the organizational and structural alterations of the right brachial plexus and cervical cord (C8-T1) were examined via light and electron microscopy.

**Results:** Following the production of the BPN model, the MWT of both ipsilateral and contralateral paws demonstrated a profound decrease ( $P < 0.05$ ). But after EA interventions, the MWT showed a significant increase ( $P < 0.05$ ). In comparison to the EA12 group, the analgesic effects of the EA1 group were more significant, and similar results were observed in exploratory behaviors. However, grooming behaviors did not demonstrate significant differences. Meanwhile, on day 12 after surgery it was observed under light microscopy that the inflammatory response in the right brachial plexus and cervical cord (C8-T1) were significantly attenuated after EA stimulation. Furthermore, the demyelination of the brachial plexus and cervical cord (C8-T1) were also reversed.

**Limitations:** Limitations include the fact that there was demyelination of the cervical cord (C8-T1) in the control group because of inappropriate manipulation.

**Conclusion:** Repeated EA contributes significant analgesic effects in the treatment of BPN.

**Key words:** Electroacupuncture, brachial plexus neuralgia, cobra venom, cervical cord, ultra-structural alterations

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**N**europathic pain associated with damage to the brachial plexus is reported to be difficult to treat, as it involves both distinct pathophysiological and etiological factors (1,2). Therapeutic strategies for treating brachial plexus neuralgia (BPN) is usually composed of neural blockade and analgesic drugs. However, long-term use of these modalities has shown a plethora of unexpected side effects resulting in an increase in both fiscal and emotional costs. Therefore, it is important to find a therapy that demonstrates both fewer side effects and a more cost-effective outcome for this illness.

Electroacupuncture (EA) is an enhanced form of acupuncture that utilizes precisely pulsed electrical stimulation and is standardized with regard to frequency, voltage intensity, and wave-form (3,4): as such, EA is considered a beneficial, safe, and cost-effective physiotherapy. Further, new experimental studies have demonstrated that normal acupuncture effectively alleviates acute, chronic neuropathic and inflammatory pain in rats (5,6). Thus, EA is likely to be a more cost-effective alternative to current analgesic modalities.

Previous studies of the analgesic effects and mechanism of EA were mainly concentrated in chronic low-back pain including sciatica and inflammatory pain (7,8). Very few studies evaluated the analgesic effects of EA on brachial plexus neuropathic pain. An adequate understanding of the analgesic effects and mechanism of EA requires reliable animal models. In our study, we employed a novel persistent rat model induced by injecting cobra venom into the right lower trunk of the brachial plexus (9). Then, using this model, we systematically demonstrated the prolonged analgesic effects of EA.

The aim of this study was to explore the analgesic effects induced by EA on a BPN rat model induced by cobra venom. We would use both behavioral assessments as well as neuropathologic and ultra-structural changes in the affected nerves. In addition, we discuss the possible analgesic mechanism of EA on this novel rat model of persistent pain.

## **METHODS**

### **Animals**

A total of 66 adult male Sprague-Dawley rats weighing 250 – 300 g were obtained from The Laboratory Animal Center of the Academy of Military Medical Sciences. All experimental animals were housed under controlled ambient environmental conditions (tempera-

ture 22 – 24°C, relative humidity 40 – 60%, 7 am to 7 pm alternative light-dark cycle, food and water ad libitum). The Animal Care and Use Committee (Beijing, China) approved all animal protocols. The ethical guidelines of the International Association for the Study of Pain were followed in all experimental procedures (10). Animals were randomized into 5 groups: normal control (NC) (n = 12), BPN model (n = 14), BPN with sham EA stimulation (n = 14), BPN with EA stimulation commencing on postoperative day 1 (EA1) (n = 14), and BPN with EA stimulation commencing on postoperative day 12 (EA12) (n = 12).

### **Brachial Plexus Neuralgia Model Establishment**

To produce a standardized BPN model, cobra venom was injected into the lower trunk of the right brachial plexus (9). Briefly, rats are anesthetized using a 4% solution of chloral hydrate (0.8 mL/100g body weight, Sigma) injected intraperitoneally. Following routine sterilization of the skin, the right lower trunk of the brachial plexus is exposed at the clavicle by blunt dissection through the pectoralis major muscle group. The cobra venom 4 µL (mixture of 0.4 mg lyophilized cobra venom, Venom Research Institute of Guangxi Medical University) was injected into the nerve sheath using a 10µL microinjector (Shanghai Gaoge Industry). During this procedure, we were extremely cautious to avoid leakage of the cobra venom into surrounding tissues. For the NC group, the experimental rats underwent the same procedure except injecting saline instead of cobra venom. The muscle and skin were then closed with 4.0 silk sutures. The pain behaviors for the left forelimb and both hind paws of each rat were measured again, 24 hours after the procedure.

### **Electroacupuncture Treatment Procedure**

EA treatment was performed according to a novel, unrestrained, unседated, and conscious method reported by Lao et al in 2004 (11). After cleaning the rat's skin with alcohol swabs, the investigator swiftly inserted disposable acupuncture needles (gauge #32, 0.5 in. in length) into the right (operative) forelimb at the equivalent anatomical landmarks of human "Shousanli" (LI10) and "Quchi" (LI11) acupoints (Fig. 1A). In humans, LI10 is located on the dorsum of the forearm, 2 inches below the elbow. LI11 is located at the depression medial to the extensor carpi radialis, at the lateral end of the cubital crease. The needles were fixed with adhesive tape (Fig. 1B). The entire procedure

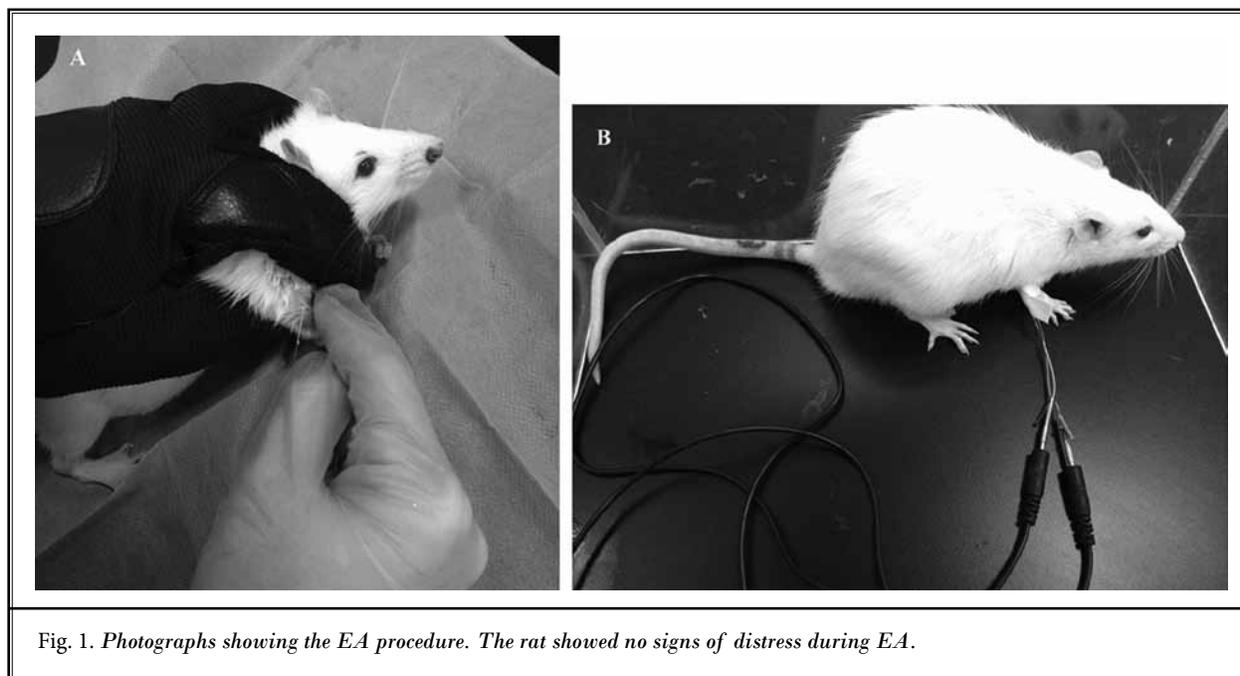


Fig. 1. Photographs showing the EA procedure. The rat showed no signs of distress during EA.

lasted less than 20 seconds and caused little distress to the animal. The animals were released into a small transparent plastic cage to receive EA stimulation using a Han's Acupoint Nerve Stimulator (HANS, LH series, Peking University). The frequency of EA stimulation was held 2/100 Hz variable, current intensity was maintained at 1 mA for 10 minutes, then increased to 1.5 mA for 10 minutes, and finally increased to 2 mA for 10 minutes: the total procedure time was 30 minutes. The electrical stimulation was administered to the muscles and not directly to the skin or the peripheral nerve, and caused a mild muscle twitch. If the needles dropped during EA, they were inserted instantly again.

In order to avoid tolerance, the EA intervention was given once every other day for 12 consecutive courses of treatment for rats in the BPN with EA1 (beginning on the first day after operation) and BPN with EA12 (beginning on the twelfth day after operation). For the sham EA control group, acupuncture needles were inserted into the same acupoints, LI10 and LI11, but without electrical current stimulation. This same procedure was administered once every other day for 12 days following the first day after operation.

### Behavioral Assessment

Two sets of behavioral tests were conducted 3 days before the operation and 3, 6, 9, 12, 15, 20, 25, 30, 40, and 60 days after the operation to investigate the

analgesic effects of EA on the rats. The 2 tests were conducted during daylight hours (7 am to 7 pm). In experiment one, mechanical withdrawal threshold (MWT) of the rats was detected and calculated by the up-down method (12). The rats were placed in a clear plastic chamber (20×20×20 cm) on the surface of an elevated wire mesh (1.0×1.0 cm cell), which allowed full access to the paws. Before each test, the rat acclimatized to the environment for approximately 15 minutes until the spontaneous behaviors (for example exploratory behavior or grooming activities) ceased. A series of calibrated von Frey hairs (Stoelting, Chicago, IL) with stiffness between 0.41 and 15.10 g were applied perpendicular to the mid-plantar surface of the left forelimb and both hind paws with adequate force to cause mild bending against the paws, and held for approximately 6 – 8 seconds. A positive response occurred when the paw was sharply withdrawn or flinched.

In experiment 2, spontaneous behavior was examined using a video camera which was placed 0.4 m in front of a transparent plastic cage (30×30×20 cm). At least one-fourth of the recorded view required that the rat's body to be visible to the camera. The time of each rat's free behaviors was observed for 7 minutes and the analysis of changes in exploratory and grooming behaviors was performed after video recording was completed (13).

## Morphology Studies

On day 12 and 60 after surgery, 2 rats of the NC group, BPN group, BPN with sham EA group, BPN with EA1, and BPN with EA12 group were respectively sacrificed and HE staining and electron microscopic examination were performed. These morphology methods were designed to observe pathologic and ultra-structural alterations in neurons and nerve fibers. The rats were deeply anesthetized with the same anesthetics mentioned previously and then euthanized. The tissue of the lower trunk of the right brachial plexus and cervical cord (C8-T1) were rapidly removed to an ice plate, and then suspended in 10% formalin. Tissue samples were cut at 10 mm thick sections that were stained, fixed, and examined with light microscopy.

In addition, for electron microscope examination, the animals were anesthetized with the same anesthetics mentioned previously and then perfused with warm saline, followed by a mixed solution of 4% paraformaldehyde and 2% glutaraldehyde (Sigma, St. Louis, MO, USA). The 2 kinds of tissue mentioned previously were immersed in 3% glutaraldehyde for 24 hours and then rinsed with 0.1M PB 3 times. The samples were fixed with 1% osmium tetroxide (Sigma, St. Louis, MO, USA)

for 2 hours, dehydrated, embedded in araldite, and cut into 1  $\mu\text{m}$  plastic sections that were stained in uranil acetate and observed under an electron microscope.

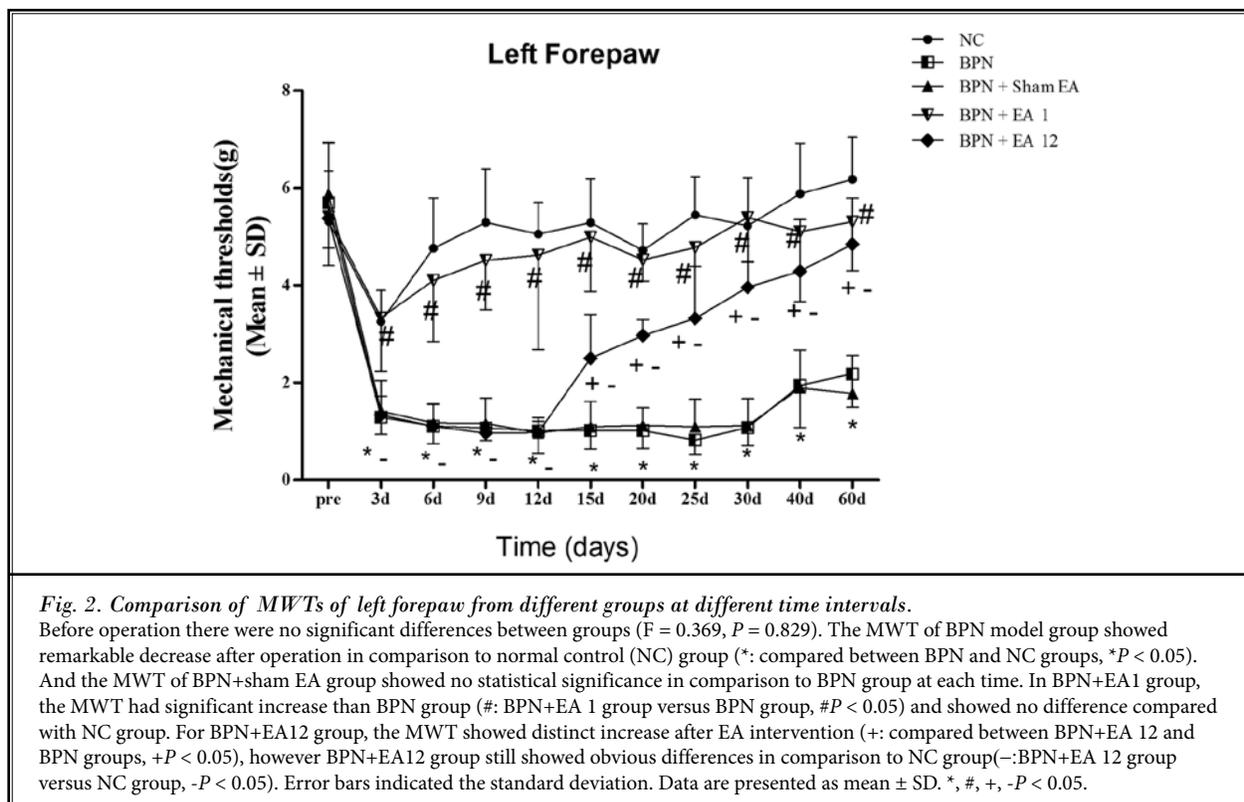
## Statistical Analysis

Data from the behavioral tests were presented as mean  $\pm$  SD and analyzed with repeated measures data of ANOVA. The least significant difference (LSD) test was used to compare the differences between every 2 groups. And  $P < 0.05$  was regarded as the level of statistical significance.

## RESULTS

### Effects of EA on Pain Mechanical Threshold

As shown in Fig. 2 to 4, before the operation there were no significant differences between groups (left forepaw:  $F = 0.369$ ,  $P = 0.829$ ; left hind paw:  $F = 0.290$ ,  $P = 0.880$ ; right hind paw:  $F = 0.393$ ,  $P = 0.812$ ). The MWT on the left forepaw and both hind paws of the BPN model group showed remarkable decreases after operation in comparison to the NC group ( $*P < 0.05$ ). The MWT of BPN with sham EA group showed no statistical difference in comparison to the BPN group



at all time intervals. In BPN with EA1 group, the MWT demonstrated a significant improvement when compared to the BPN group ( $\#P < 0.05$ ), and in comparison to the NC group the MWT of left forepaw showed no difference, however the thresholds of both hind paws had distinct decreases ( $\&P < 0.05$ ). The effect of EA appeared greater and more immediate in the contralateral forepaw than both hind paws. Furthermore, the MWT of left hind paw and right hind paw showed no statistical significance at each interval. For BPN with EA12, the MWT showed distinct increase after EA intervention ( $+P < 0.05$ ), whereas the BPN with EA12 group still showed obvious differences in comparison to NC group ( $-P < 0.05$ ). The data suggested that EA played an ameliorative effect on BPN.

### Effects of EA on Spontaneous Behaviors

As shown in Figs. 5 and 6, prior to the operation there were no significant differences between groups no matter what the frequency or time of exploratory and grooming behavior. (Exploratory behavior frequency:  $F = 0.325$ ,  $P = 0.858$ ; time:  $F = 0.233$ ,  $P = 0.917$ ; grooming frequency:  $F = 0.343$ ,  $P = 0.846$ ; time:  $F = 0.605$ ,  $P = 0.663$ ). The frequency and time of exploratory and grooming behavior in the BPN model group showed a remarkable decrease after operation in comparison to the NC group ( $*P < 0.05$ ). And there was no statistical significance in comparison to

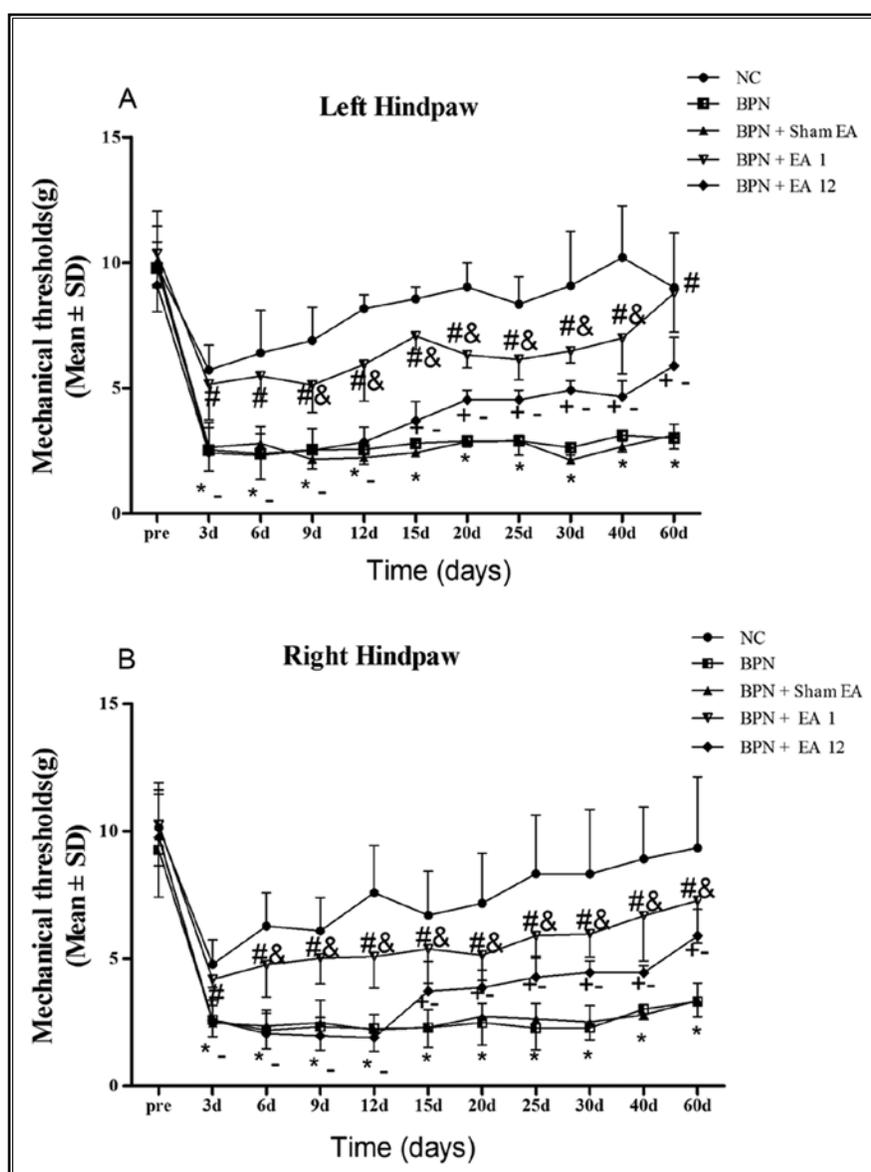
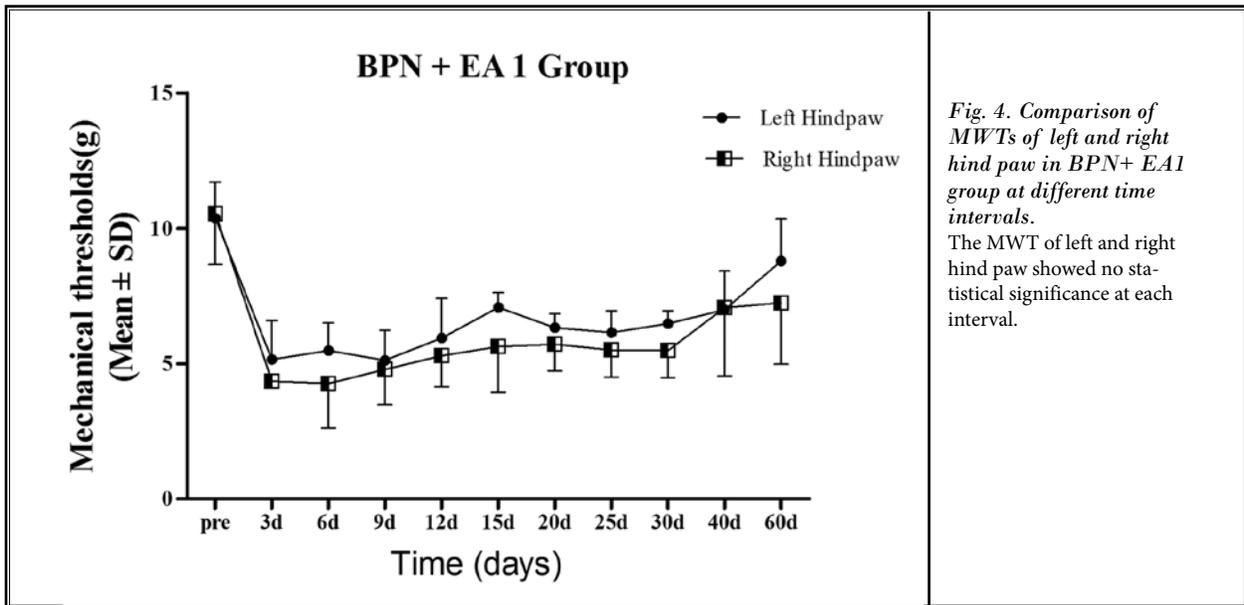
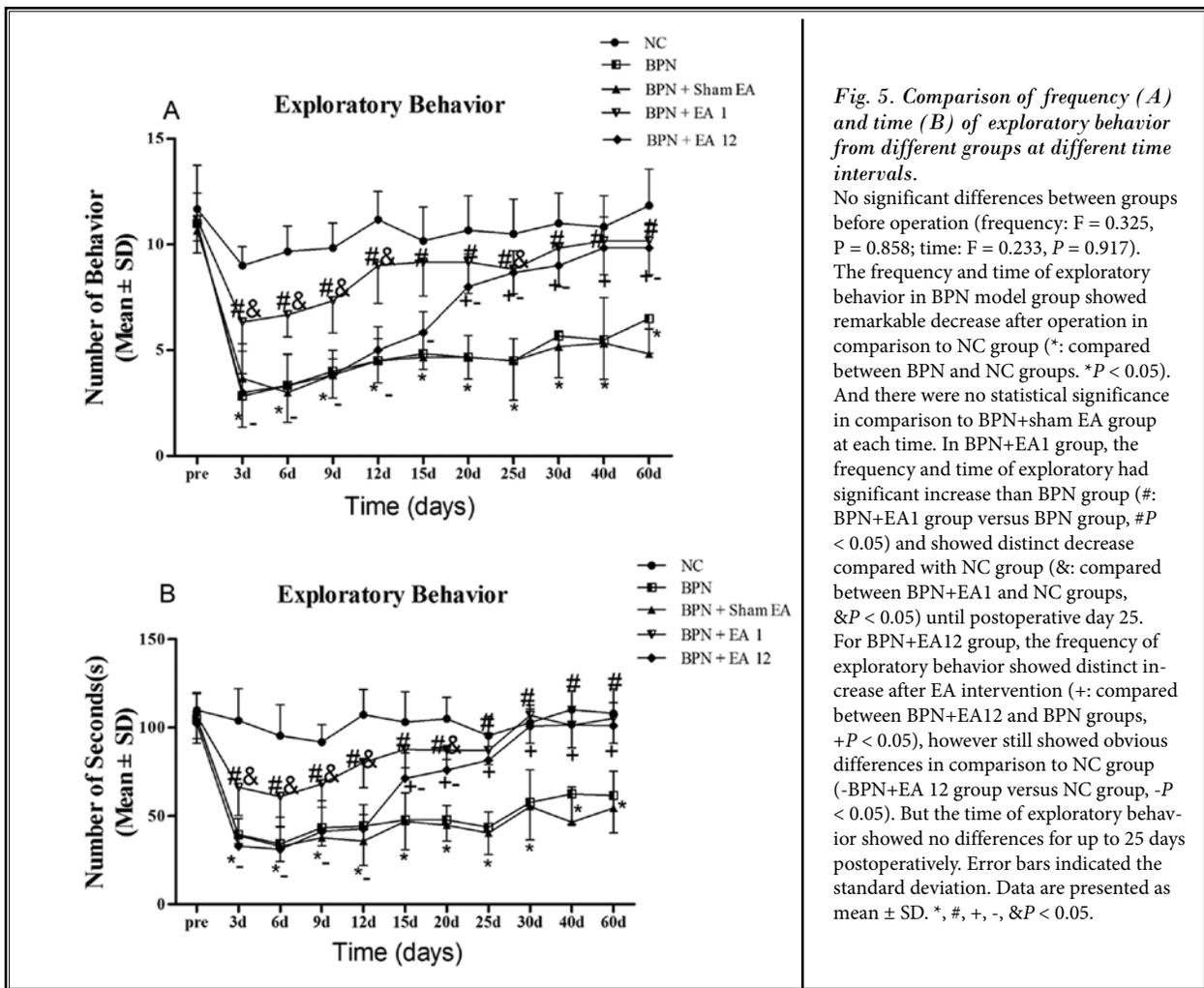


Fig. 3. Comparison of MWTs of left and right hind paws from different groups at different time intervals.

Measurements were taken at the left hind paw (A) and right hind paw (B). Before operation there were no significant differences between every 2 groups (left hind paw:  $F = 0.290$ ,  $P = 0.880$ ; right hind paw:  $F = 0.393$ ,  $P = 0.812$ ). The MWT of BPN model group showed remarkable decrease after operation in comparison to normal control (NC) group ( $*: compared between BPN and NC groups, *P < 0.05$ ). And the MWT of BPN + sham EA group showed no statistical significance in comparison to BPN group at each time. In BPN+EA1 group, the MWT had significant increase than BPN group ( $\#: BPN+EA1 group versus BPN group, \#P < 0.05$ ) and showed decrease compared with NC group ( $\&: compared between BPN+EA1 and NC groups, \&P < 0.05$ ). For BPN+EA12 group, the MWT showed distinct increase after EA intervention ( $+: compared between BPN+EA12 and BPN groups, +P < 0.05$ ), however still showed obvious differences in comparison to NC group ( $-: BPN+EA12 group versus NC group, -P < 0.05$ ). Error bars indicated the standard deviation. Data are presented as mean  $\pm$  SD. \*, #, +, -, &P < 0.05.



**Fig. 4. Comparison of MWTs of left and right hind paw in BPN+ EA1 group at different time intervals.**  
The MWT of left and right hind paw showed no statistical significance at each interval.



**Fig. 5. Comparison of frequency (A) and time (B) of exploratory behavior from different groups at different time intervals.**

No significant differences between groups before operation (frequency:  $F = 0.325$ ,  $P = 0.858$ ; time:  $F = 0.233$ ,  $P = 0.917$ ). The frequency and time of exploratory behavior in BPN model group showed remarkable decrease after operation in comparison to NC group (\*: compared between BPN and NC groups,  $*P < 0.05$ ). And there were no statistical significance in comparison to BPN+sham EA group at each time. In BPN+EA1 group, the frequency and time of exploratory had significant increase than BPN group (#: BPN+EA1 group versus BPN group,  $\#P < 0.05$ ) and showed distinct decrease compared with NC group (&: compared between BPN+EA1 and NC groups,  $\&P < 0.05$ ) until postoperative day 25. For BPN+EA12 group, the frequency of exploratory behavior showed distinct increase after EA intervention (+: compared between BPN+EA12 and BPN groups,  $+P < 0.05$ ), however still showed obvious differences in comparison to NC group (-BPN+EA 12 group versus NC group,  $-P < 0.05$ ). But the time of exploratory behavior showed no differences for up to 25 days postoperatively. Error bars indicated the standard deviation. Data are presented as mean  $\pm$  SD. \*, #, +, -, &  $P < 0.05$ .

BPN with sham EA group at each time interval. In the BPN with EA1 group, the frequency and time of exploratory had a significant increase compared to the BPN group ( $\#P < 0.05$ ) and showed a distinct decrease compared to the NC group ( $\&P < 0.05$ ) until 25 days postoperatively. For BPN with EA12 group, the frequency of exploratory behavior showed a distinct increase after EA intervention ( $+P < 0.05$ ); however, it still showed obvious differences in comparison to the NC group ( $-P < 0.05$ ). But the time of exploratory behavior showed no differences for up to 25 days postoperatively.

For grooming behavior, in the BPN with EA1 group, the frequency of exploratory behavior showed a significant decrease compared to the BPN group ( $\#P < 0.05$ ) and showed no differences compared with the NC group up to 20 days postoperatively. However, the grooming time showed no difference compared with the BPN group until 60 days postoperatively but did show statistical significance in comparison to the NC group until postoperative day 30 ( $\&P < 0.05$ ). For BPN with EA12 group, the frequency of grooming behavior showed a distinct increase after EA intervention ( $+P < 0.05$ ), however the time of grooming still showed obvious differences in comparison to the NC group ( $-P < 0.05$ ) until postoperative day 30. The data obtained with exploratory behaviors indicated that EA had better analgesic effects on BPN, whereas the results of grooming did not.

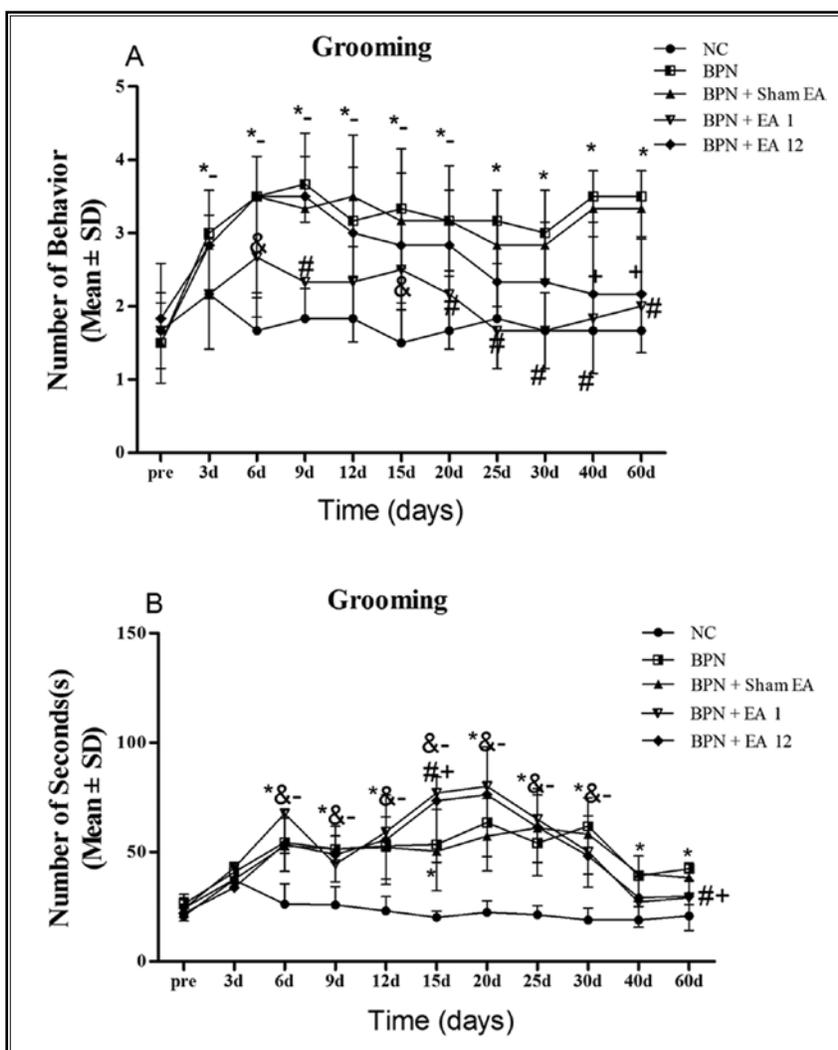


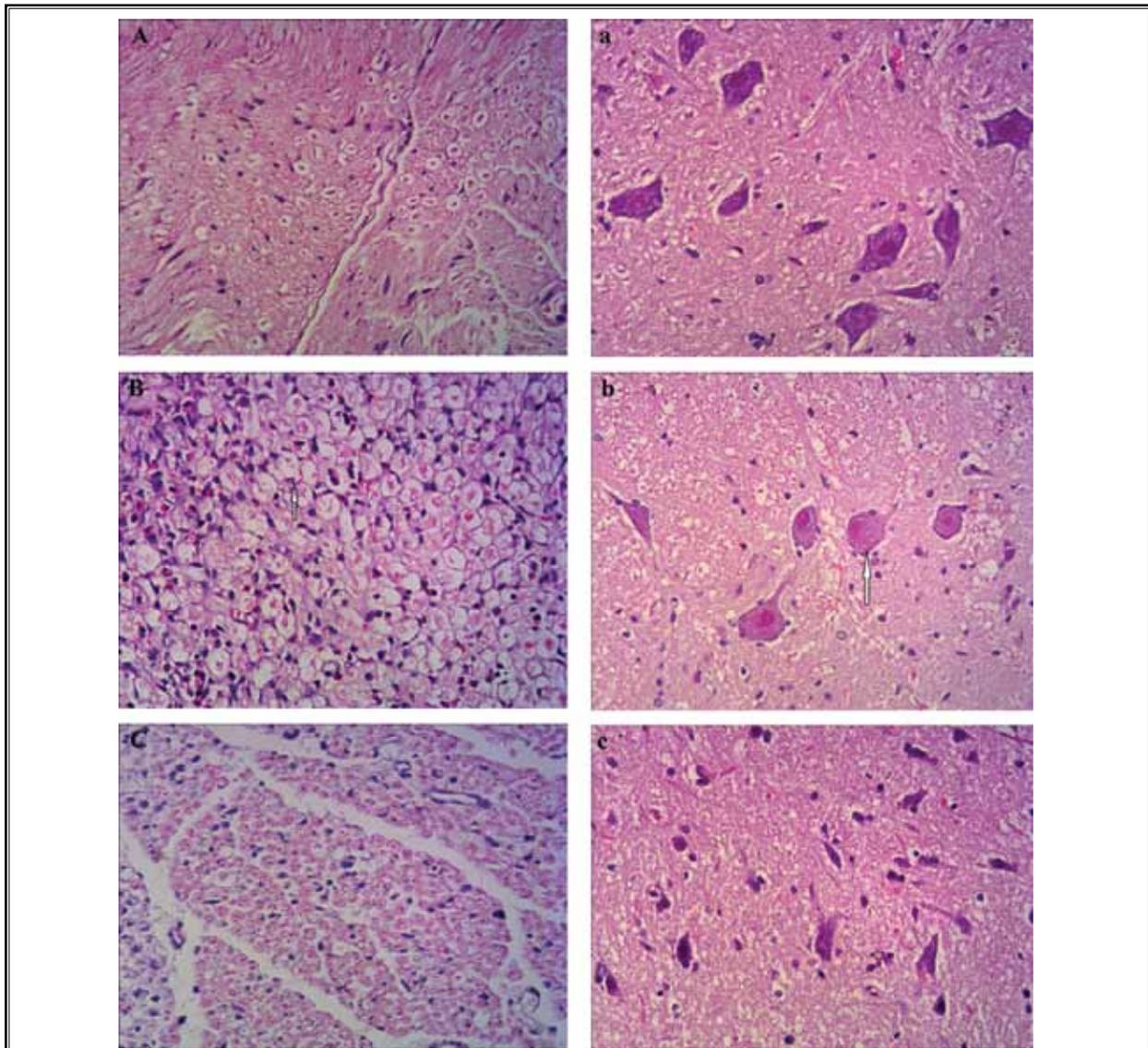
Fig. 6. Comparison of frequency (A) and time (B) of grooming behavior from different groups at different time intervals.

No significant differences between groups before operation. (Frequency:  $F = 0.343, P = 0.846$ ; Time:  $F = 0.605, P = 0.663$ ) No matter frequency nor time the brachial plexus neuralgia (BPN) model group showed remarkable increase after operation in comparison to normal control (NC) group (\*: compared between BPN and NC groups.  $*P < 0.05$ ). And there were no statistical significance in comparison to BPN+sham EA group at each time. In BPN+EA 1 group, the frequency of grooming had significant decrease than BPN group ( $\#P < 0.05$ ) and showed no differences compared with NC group up to 20 days postoperatively. However, the grooming time showed no differences compared with BPN group until postoperative day 60 but did show statistical significance in comparison to NC group until postoperative day 30 ( $\&P < 0.05$ ). For BPN+EA 12 group, the frequency of grooming behavior showed distinct increase after EA intervention ( $+P < 0.05$ ), however the time of grooming still showed obvious differences in comparison to NC group ( $-P < 0.05$ ) until postoperative day 30. Error bars indicated the standard deviation. Data are presented as mean $\pm$ SD. \*,#,+,-,&P < 0.05.

### Neuropathologic Changes

Compared with the NC group, tremendous inflamma-

tory cells were observed in peripheral nerves of the BPN model group on postoperative day 12 (Fig. 7B). Swelling in the neuron body, Nissl body col-



*Fig. 7. Comparison of the neuropathology changes in brachial plexus (A B C) and C8-T1(a b c) in rats of different groups under light microscopy on postoperative day 12. (HE 400)*

A(a): NC group, B(b): BPN group, C(c): BPN+ EA 1 group. The normal nerve bundle and neuron were observed in NC and BPN+ EA 1 groups. However, tremendous inflammatory cells were found in peripheral nerve of BPN group. In addition to, the phenomons of the neuron body swelled, Nissl body collapsed and the nuclei were offset were observed in C8-T1 area.

lapse, and the offset nuclei were found in the C8-T1 area (Fig. 7b). However, the brachial plexus and C8-T1 of the BPN with EA1 group did not exhibit obvious neuropathologic change (Fig. 7C, c). On postoperative day 60, the organization of the peripheral nerve and C8-T1 had no significant neuropathologic changes in the BPN with EA1 and 12 groups (Fig. 8B, b; C, c). For the BPN group, a few inflammatory cells were observed in the

brachial plexus (Fig. 8A). Whereas, swollen neurons were also found in the C8-T1 area (Fig. 8a). The results indicated that an inflammatory response participated in the formation of neuropathic pain, and EA had anti-inflammatory effects.

#### **Ultra-structural Alterations**

On postoperative day 12, electron microscopic

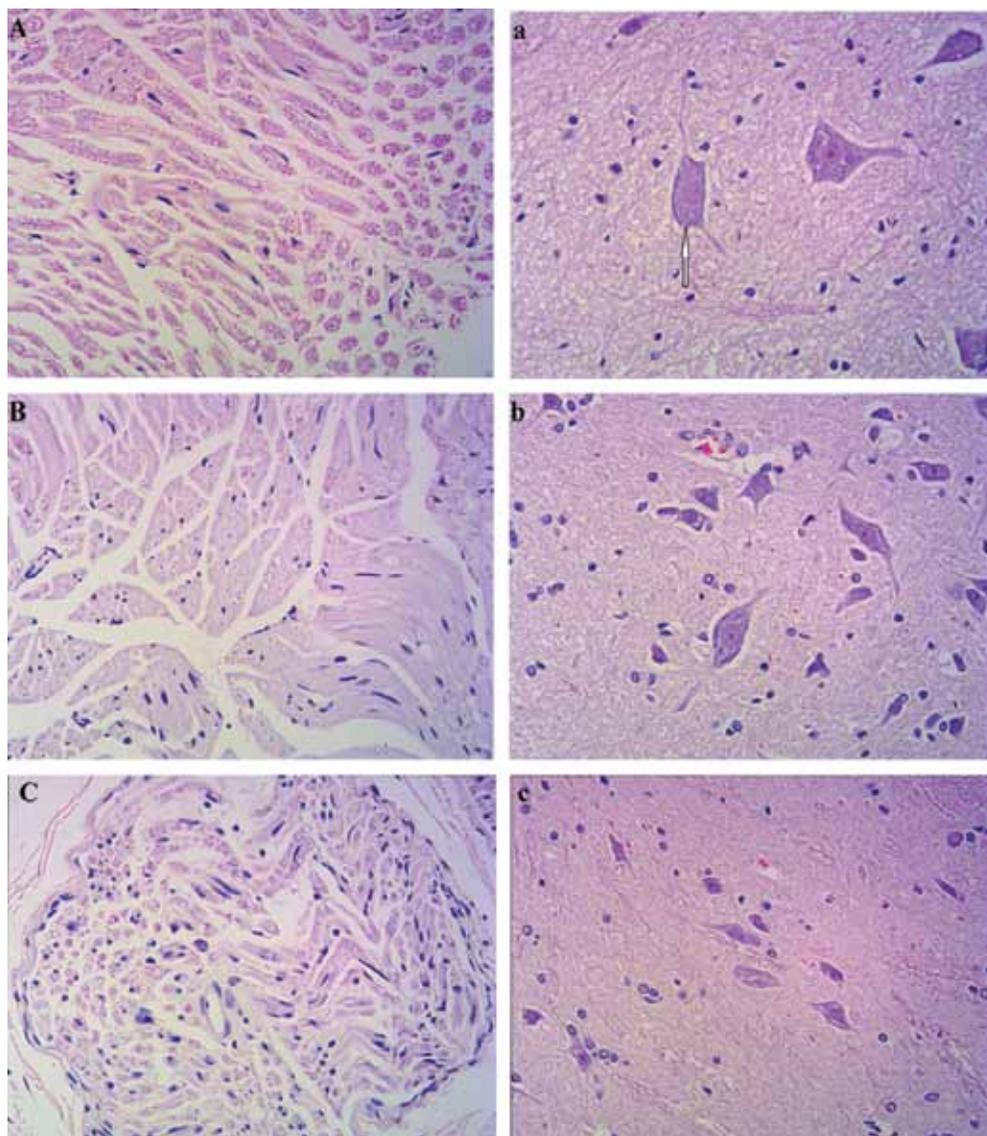
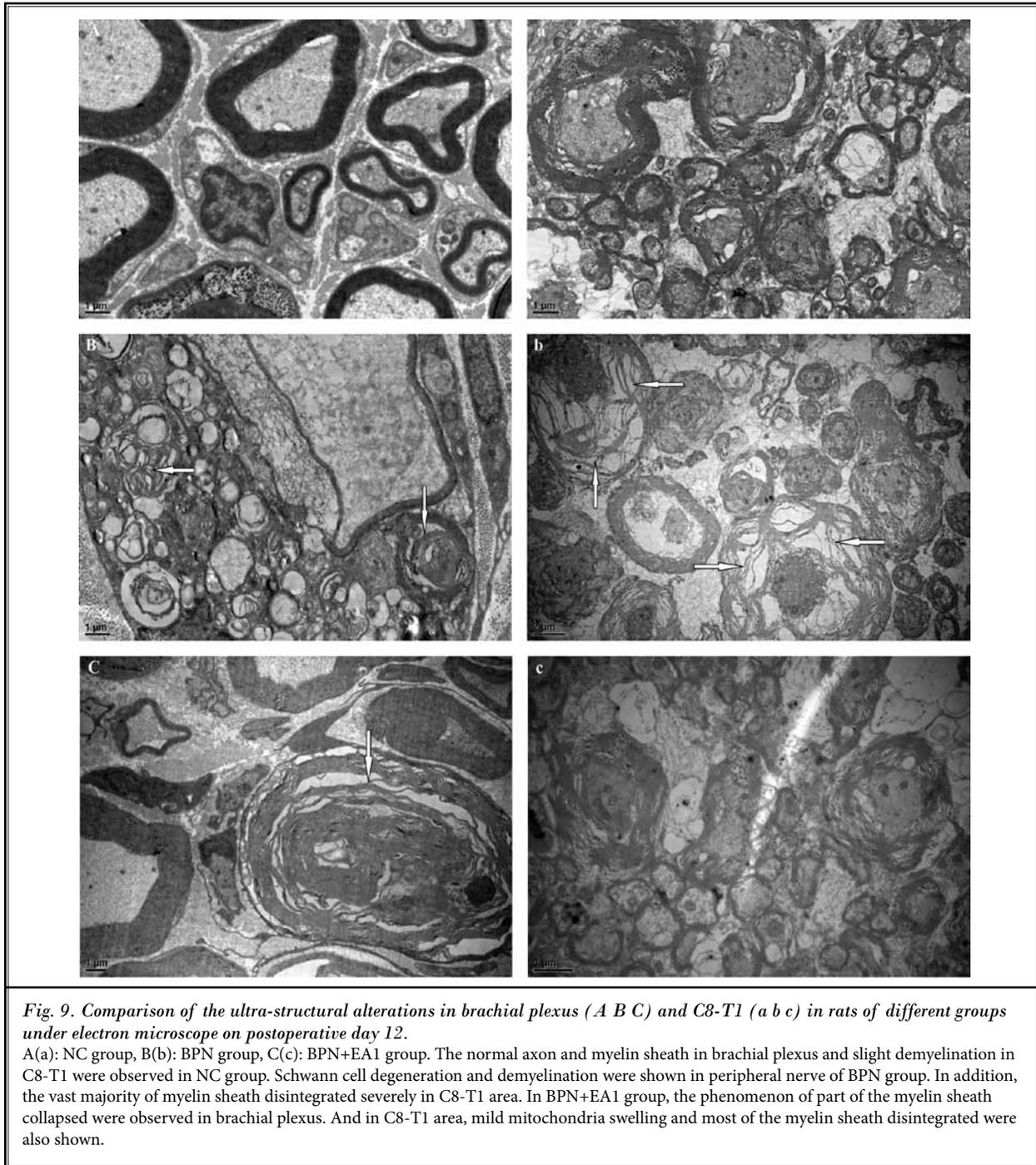


Fig. 8. Comparison of the neuropathology changes in brachial plexus (A B C) and C8-T1 (a b c) in rats of different groups under light microscopy on postoperative day 60 (HE 400).

A(a): BPN group, B(b): BPN+EA1 group, C(c): BPN+EA12 group. For BPN group, few inflammatory cells were observed in brachial plexus. Whereas, swollen neurons were also found in C8-T1 area. The organizations of peripheral nerve and C8-T1 had no significant neuropathology changes of BPN+EA 1 and 12 groups.

tests demonstrated normal axon and myelin sheath in the brachial plexus with just a slight demyelination in C8-T1 in the NC group. Schwann cell degeneration and demyelination were shown in the peripheral nerves in the BPN group (Fig. 9B). In addition, the vast majority of myelin sheaths disintegrated severely in the C8-T1 area (Fig. 9b). In the BPN with EA1 group, the phenomenon

of partial myelin sheath collapse was observed in the brachial plexus (Fig. 9C), and C8-T1 area along with mild mitochondrial swelling and disintegration of most of the myelin sheath (Fig. 9c). On postoperative day 60, the axon and myelin sheath in the brachial plexus and slight demyelination in C8-T1 were observed in the BPN with EA1 group (Fig. 10B, b). Schwann cell degenera-



tion and demyelination were shown in the peripheral nerves of the BPN and BPN with EA12 groups (Fig. 10A, C). In addition, there was severe disintegration in the vast majority of myelin sheaths in the C8-T1 area of

the BPN group (Fig. 10a). In the C8-T1 area of the BPN with EA12 groups, myelin sheath disintegration was observed (Fig. 10c). Given the results above, EA appears to significantly reverse nerve demyelination.

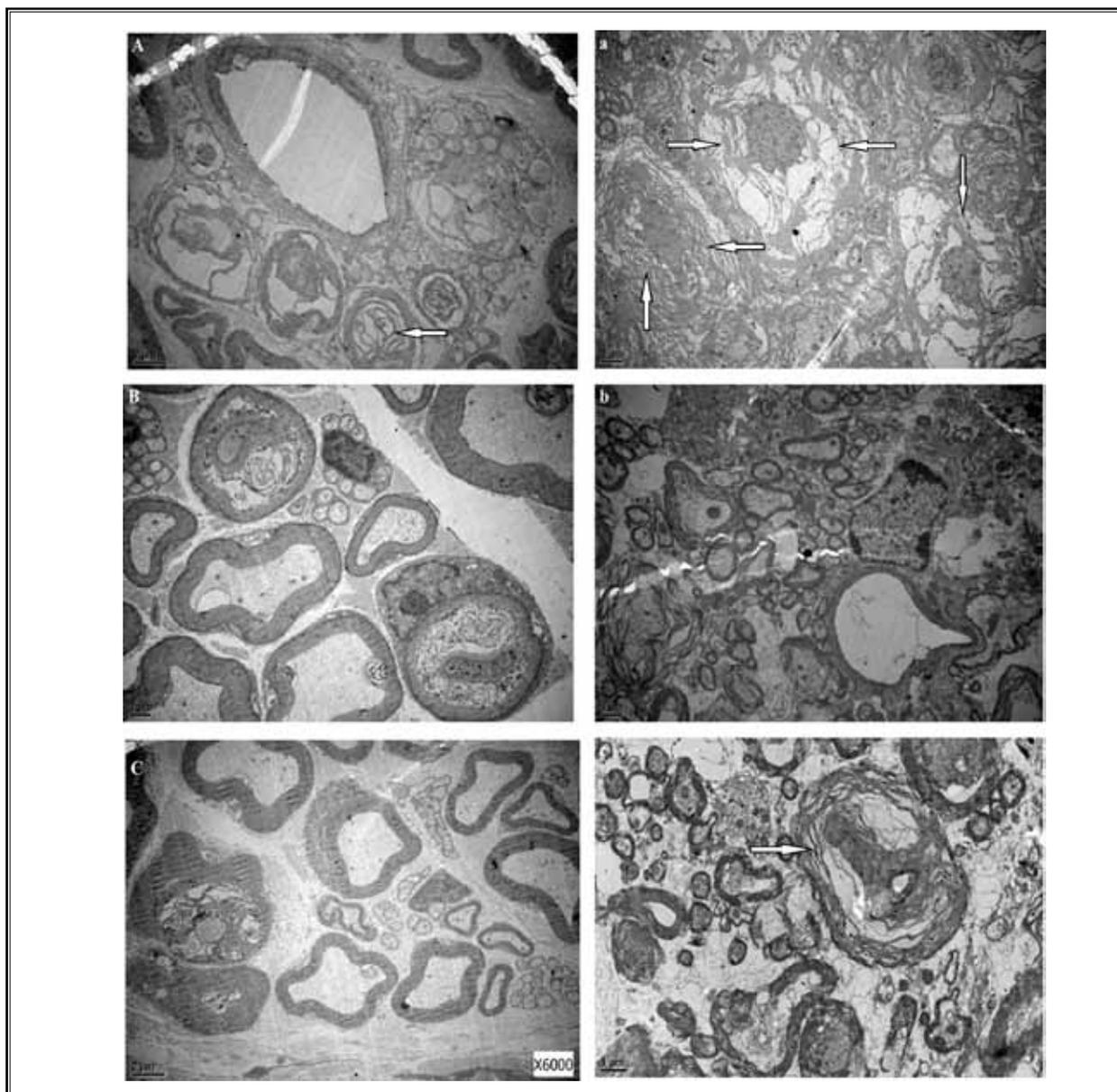


Fig. 10. Comparison of the ultra-structural alterations in brachial plexus (A B C) and C8-T1 (a b c) in rats of different groups under electron microscope on postoperative day 60.

A(a): BPN group, B(b): BPN+EA1 group, C(c): BPN+EA12 group. Schwann cell degeneration and demyelination were shown in peripheral nerve of BPN group. In addition, the vast majority of myelin sheath disintegrated severely in C8-T1 area of BPN group. The basic normal axon and myelin sheath in brachial plexus and slight demyelination in C8-T1 were observed in BPN+EA1 group. In C8-T1 area of BPN+EA12 groups, the phenomenon of most of the myelin sheath disintegrated were observed.

## Discussion

### Successful Establishment of the Rat Model of Brachial Plexus Neuralgia

EA is widely used in clinical practice and research to reduce neuropathic pain (14). However, the analge-

sic effects and mechanisms still need to be elucidated. Previous studies primarily applied the chronic constrictive injury (CCI) model, produced by ligating the sciatic nerve (15). In the present study, we established a chemical damage model following administration of cobra

venom to the brachial plexus in the rats, a modified Liu CC's method (9). On the third day after operation, all cobra venom-injected rats showed a severe deformity of the right forepaw. And the MWT of left forelimb and both hind paws presented significant differences in comparison to preoperative measures. Additionally, the consistency of both hind limbs mechanical pain threshold decrease indicated the segmental features of spinal cord injury. Further, the results of the neuropathologic and ultrastructural alterations also confirmed this point. In view of these facts, we believe that we successfully demonstrated a rat model of BPN.

### Acupoints and EA Frequency Selection

It is well known that acupoints are the triggers of acupuncture analgesia, which is based on the uneven distribution and density of nerve endings in the body. Concerning the total number of acupoints, it has increased to 361 in the modern Traditional Chinese Medicine textbook (16). However, in acupuncture research, the commonly used acupoints are limited to a smaller number. The 3 most frequently used acupoints are "Hegu" (LI4 n = 345), "Zusanli" (ST36 n = 299), and "Neiguan" (PC6 n = 259) based on a search on the Science Citation Index Expanded (SCI-Expanded) from 1899 to 7/24/2010. In traditional acupuncture medicine, pain is believed to be caused by a blockade of meridians that are referred to as channels "Jing" and their branches "Luo." "Shousanli" (LI10) and "Quchi" (LI11) acupoints were reported to alleviate many kinds of upper limb pain. Moreover, a study demonstrated that the acupoints of rats were anatomically identical to those of humans (17). In the current study, we selected the LI10 and LI11 to research the analgesic effects of EA.

The frequency of EA is a key parameter because of its influence on EA's analgesic mechanism. Neurochemical studies including human and animals revealed that different frequencies cause the brain and spinal cord to release different types of opioid peptides (18). EA stimulation of 2 Hz accelerated the release of enkephalins and endorphins in the brain and spinal cord, whereas 100 Hz favored the release of dynorphins (19,20). To obtain ideal therapeutic effects, in the current study, we selected 2 Hz and 100 Hz alternating frequencies that in theory released both types of opioid peptides.

### The Analgesic Effects of EA Stimulation

In the present study, the results of MWT and exploratory behaviors confirmed the analgesic effects of EA in treating BPN. A robust and long-lasting reduction

in hyperalgesia was observed after EA stimulation intervention through MWT and spontaneous behaviors tests. However, in comparison with both hind paws, the MWT of left forelimb recovered more quickly. The causes of this may be the following: firstly, the distance of the left upper limb was closer to the acupuncture needles than both hind paws. After all, the analgesia of acupuncture results from the direct impact on the meridian-collateral structure and function (21). Secondly, the selected acupoints and the stimulation parameters maybe other considerable limitations. Furthermore, the neurons that participate in pain conduction of the forelimb and hind paws should be different. Moreover, analgesic mechanisms of EA are mediated by the descending pain inhibitory system that are mainly composed of spinal opioid, adrenergic, serotonergic, cholinergic, and GABAergic receptors (22,23). The involved neurotransmitters of the forelimb and hind paws are likely to be more diverse.

Meanwhile, we also found that the analgesic effects of the EA1 group showed significant improvement compared with the EA12 group. In other words, the EA stimulation effectively attenuates mechanical hyperalgesia in the acute period. This suggests that we should take more active treatments during the acute clinical period. Therefore, the analgesic effects of EA stimulation observed in the present study could provide a basis for the clinical application in the treatment of BPN.

Under normal situations, if the reduced MWT were reversed, the grooming behaviors should also subsequently reduce. Whereas, our current study demonstrated that the grooming behaviors increased after EA stimulation intervention, and the grooming behaviors relieved slowly when EA stimulation was undone. This indicated that EA stimulation could persist a period of time after removing.

### Neuropathologic Changes and Ultrastructural Alterations of Peripheral and Central Nervous System after the Intervention of EA

Results of the present study showed that, following the cobra venom injection, the inflammatory reaction of the brachial plexus and cervical cord increased remarkably, and the demyelination changes were observed in the peripheral and central nervous systems. Nevertheless, contrary to all expectations, we found that the inflammatory reaction was suppressed and the demyelination phenomenon decreased significantly after EA intervention. Regarding these findings, we

further confirmed that the anti-inflammatory effects and inhibition of nerve demyelination probably were the mechanism of EA on curing the novel BPN model induced by cobra venom in rats.

### The Clinical Interventions of EA Stimulation

The results of this experimental animal study presents that EA could produce definite analgesic effects in the treatment of BPN. In addition, early intervention with EA stimulation could prevent or reverse ultra-structural alterations of peripheral and central nervous system. Even though the analgesic adjuvant drugs, such as pregabalin, could play a similar therapeutic effect (9), long-term use of these drugs can produce serious side effects. However, EA stimulation is a beneficial, safe, and cost-effective modulating therapy. Given these factors, EA might become another type of efficacious clinical treatment or prevention of BPN.

### Fiber C Presumably May Play a Crucial Role in Acupuncture Analgesia

As far as we know, acupuncture performed the analgesic effects by exciting all sorts of different types of afferent fibers. However, which kinds of afferent fibers mediate EA analgesia are still controversial. Some study demonstrated that EA induced the potent analgesic effects via exciting A $\delta$ -type afferent (group III) and C-type afferent (group IV) (24). In the current study, we built the BPN model using the administration of cobra venom to the right lower trunk of the brachial plexus. And the cobra venom was able to make the myelinated nerve fibers demyelinate. Whereas, the EA stimulation still had available analgesic effects. Given the present

results, C-type afferent probably plays an important role in EA analgesia.

### Limitations

The fact that there was slight demyelination of the cervical cord (C8-T1) in the control group under electron microscope on postoperative day 12 was secondary to inappropriate manipulation.

### CONCLUSIONS

Taken together, these data indicated that EA attenuated neuropathy pain induced by administration of cobra venom to the lower trunk of the right brachial plexus and the analgesic effects of EA were related to relieving the inflammatory response in peripheral and central nervous systems. Furthermore, EA could be used to treat neuropathy pain as a complementary and alternative medicine. The present study may indicate a discussion on the possible roles of C-type fiber in the therapeutic effects of EA and provide insight on the mechanism.

### Disclosures

No disclosure or conflicts of interest for any author. Hui Liu helped conduct the study, collect and analyze the data, and prepare the manuscript. Xiao-Yan Qian helped design and conduct the study. Jian-Xiong An helped design the study, analyze the data, and approve the final version. Yi-De Jiang helped conduct the study and collect the data. Doris K. Cope and John P. Williams helped prepare the manuscript and approve the final manuscript.

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