



A Study of the Safety of Low Temperature Plasma Radiofrequency Ablation on Rat Sciatic Nerve

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Purpose: Low temperature plasma radiofrequency ablation (LTPRA) has been widely applied for widespread clinical use in a variety of disciplines. However, the safe distance to act in the vicinity of neural tissue has not been determined.

Methods: Adult male Sprague-Dawley rats were subjected to LTPRA surgery performed at 0mm, 1mm and 2mm from the sciatic nerve or by cutting only the skin muscle to expose the sciatic nerve. Paw withdrawal thresholds (PWT) were determined at different time points, neurophysiology was examined, and the sciatic nerve was harvested for light and electron microscopic evaluation and Elisa for expression of proinflammatory cytokines and pain-related markers.

Results: At 14 days postoperatively, both the 0mm and 1mm groups showed a decrease in PWT, a reduction in the peak amplitude of compound muscle action potentials (CMAPs), a decline in the number of Schwann cells, the area of myelin sheaths, and thinner myelin thickness, additionally, both groups exhibited upregulation of TNF- α , IL-1 β , NGF, and C-fos in the sciatic nerve; furthermore, the 0mm group also exhibited slowed nerve conduction velocity, prolonged latency, and myelin vacuolization. The 2mm group exhibited transient reductions in PWT and elevated IL-1 β and C-fos at 14 days, and all of these indicators fully recovered on postoperative day 28.

Conclusion: LTPRA causes only temporary and reversible changes 2 mm away from the sciatic nerve in rats, which can be recovered within 28 days. This study identifies a 2mm safety threshold for LTPRA to mitigate neurological sequelae, informing surgical guidelines.

Keywords: low temperature plasma radiofrequency ablation, nerve injury, safe distance, clinical applications

Introduction

Low temperature plasma radiofrequency ablation (LTPRA) represents a relatively new and innovative technological advancement with considerable promise for future applications.¹ LTPRA is usually performed at temperatures between 40° to 70°.² The energy generated by the bipolar radiofrequency is applied to the cellular electrolyte through the tip of the knife, which is approximately 1mm in diameter, converting the electrolyte into plasma. These plasmas will break the peptide bonds of the tissue, vaporizing and melting the tissue into O², H₂, CO₂, etc. This results in rapid cutting, crumpling and hemostasis of the tissue.

Currently, head, neck, nose, throat, spine disorders and pain are treated with low temperature plasma radiofrequency ablation.^{3–11} In cases of diseases affecting the head and face, LTPRA is often utilized as a therapeutic modality for the treatment of various pathologies, including pharyngeal tumours,^{12–14} adenoid hypertrophy,^{15,16} nasopharyngeal hemangiomas,^{17–19} tonsillar hypertrophy²⁰ and sleep apnea syndrome.^{21–23} Additionally, research has demonstrated the efficiency of LTPRA in the therapy of cervical spondylosis,^{24,25} lumbar disc herniation,⁹ postherpetic neuralgia²⁶ and phantom limb pain.²⁷ This method offers several advantages, including reduced trauma, bleeding, and postoperative complications, as well as accelerated wound recovery.¹⁷

As previously mentioned, LTPRA has been employed in a number of domains, its application is characterized by direct action on the soft tissues surrounding the lesion. However, the study of LTPRA for neuropathic pain caused by peripheral nerve entrapment has not been reported. LTPRA, on the other hand, is a relatively new radiofrequency ablation technique that has a lower temperature during its action compared to conventional thermal radiofrequency.¹¹ However, there is also prior controversy in the application of plasma ablation regarding thermal damage. In a clinical study of plasma-mediated radiofrequency ablation assisted bone cement injections, it was described that plasma-mediated radiofrequency generates less heat in the surrounding tissues, is safer for spinal applications, and that thermal damage at the site of plasma radiofrequency is minimized when applied to cancellous bone.²⁸ In the treatment of bone tumors, plasma-mediated radiofrequency ablation has also shown superiority in terms of precision of the site of action, less damage to surrounding tissues, and less heat, but it is also emphasized that the tip of the probe should be kept at a distance of at least 2 mm from the adjacent neural tissues in the application.²⁹ In the treatment of chronic total occlusion, the PlasmaWire™ System was used, but applying this system to the superficial femoral artery in sheep, a thin tissue coagulation layer suggestive of thermal damage was observed.³⁰ Therefore, due to the uncertainty of thermal damage caused by LTPRA, safety distances must be strictly controlled during the procedure to avoid nerve damage.

The issue of species-specific neural responses based on energy devices has not been addressed. We must admit that there are differences between human beings and other animals, but animal models are able to visually reveal the neurophysiological changes in the nerves after the action of energy devices on them. It is not entirely clear that current clinical protocols based on plasma energy devices do not produce permanent structural changes in neural tissue, so we believe that this issue should first be evaluated in experimental animals. Rats in rodents are often used in studies of nerve damage because their neurophysiology and function are similar to those of humans. In addition, the researchers selected to employ the sciatic nerve of rats as the subject of their study on the effects of pulsed radiofrequency and plasma knives on nerve tissue.^{31,32} This study applied LTPRA to different distances of the sciatic nerve in rats. By detecting changes in nerve function and structure, the safe distance for applying LTPRA technology in rat nerve tissue was determined, with the aim of providing some inspiration for clinical trials.

Materials and Methods

Animals

The rats selected for this study were male Sprague Dawley rats (Jihui, Shanghai, China), with an age range of 8–10 weeks and a weight range of 250–280g. The rats were kept in the Experimental Animal Center of the Shanghai Yueyang Hospital of Integrated Traditional Chinese and Western Medicine (Animal License Number: SYXK (Shanghai) 2023-0050). The experimental rats had free food and water access, and the temperature of the animal room was maintained at 20 °C with 12 hours of alternating day and night light. After one week of adaptation, the rats were randomized into various groups. All experiments were conducted to minimize the animals' number and to reduce their suffering as much as possible. The experimental procedures and protocols followed in this research were authorized by the Experimental Animal Ethics Committee of Yueyang Hospital of Integrative Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine.(approval number: YYLAC-2022-173-17), and animal-related experimental operations were conducted in accordance with the International Association for the Study of Pain's ethical guidelines for the use of laboratory animals.³³

Surgery Procedure

SD rats were anesthetized with isoflurane using an animal gas anesthesia machine (R500, RWD, Shenzhen, China). The rats were positioned laterally in a decubitus posture, and their hindquarters were depilated and disinfected. To fully expose the sciatic nerve trunk, an incision was made at the right hindlimb femoral crest followed by blunt dissection of fascia and muscle. With a low temperature plasma radiofrequency surgical system (LZ-BP-450A, Luangzi, Hunan, China), the radiofrequency energy was operated at 450 kHz, the ablation power was configured as a 1-step (50 W) with a continuous ablation time of 5 seconds for each ablation. The plasma cutter head (LZ-DJ-B-03, Luangzi, Hunan, China) is

attached to the surgical system and the procedure is initiated either directly on the nerve near the bifurcation area or on the muscle area near the sciatic nerve.

The experiment was divided into two sections, section 1: the animals were randomized to four groups, sham, 0mm, 1mm and 2mm. The sham group was sutured after only cutting the skin and muscle to expose the sciatic nerve, the 0mm group was stimulated directly near the sciatic bifurcation with a plasma knife, and the 1mm and 2mm groups were stimulated with the muscle tissues 1mm and 2mm away from the vicinity of the bifurcation site of the sciatic nerve (Figure 1). Changes in mechanical pain threshold were assessed in the rats during 3 days before and 14 days after surgery, and the animals were sacrificed on day 14 after nerve electrophysiology of the sciatic nerve on the operated side was established, and tissue samples from the sciatic nerve for enzyme-linked immunosorbent assay, pathological staining, and transmission electron microscopy. Section 2: Changes in mechanical pain threshold were observed during preoperative d3 and postoperative d28. Animals were executed on day 28, and again neurophysiological examinations were performed prior to execution, followed by collection of nerve tissue from the operated side for ELISA, histological staining, and transmission electron microscopy. In this study, 14 and 28 days postoperatively were chosen to assess nerve injury in the acute or subacute phase.

Behavioral Tests

Mechanical allodynia was assessed by placing the rats on metal grids and covering them with transparent Plexiglas compartments, using von Frey filaments. Vertical stimulation of the skin of the soles of the rat's hind paws with a series of von Frey filaments (2–26 g), starting with 2 g of filaments and increasing gradually, maintaining a 5s dwell each time. If the rat showed a paw retraction response, the rat was tested again with the same number of grams of filaments at 5-min intervals, and the paw withdrawal response was observed on three or more out of five consecutive occasions, then the current grams of filaments were considered to be the withdrawal threshold for that rat.

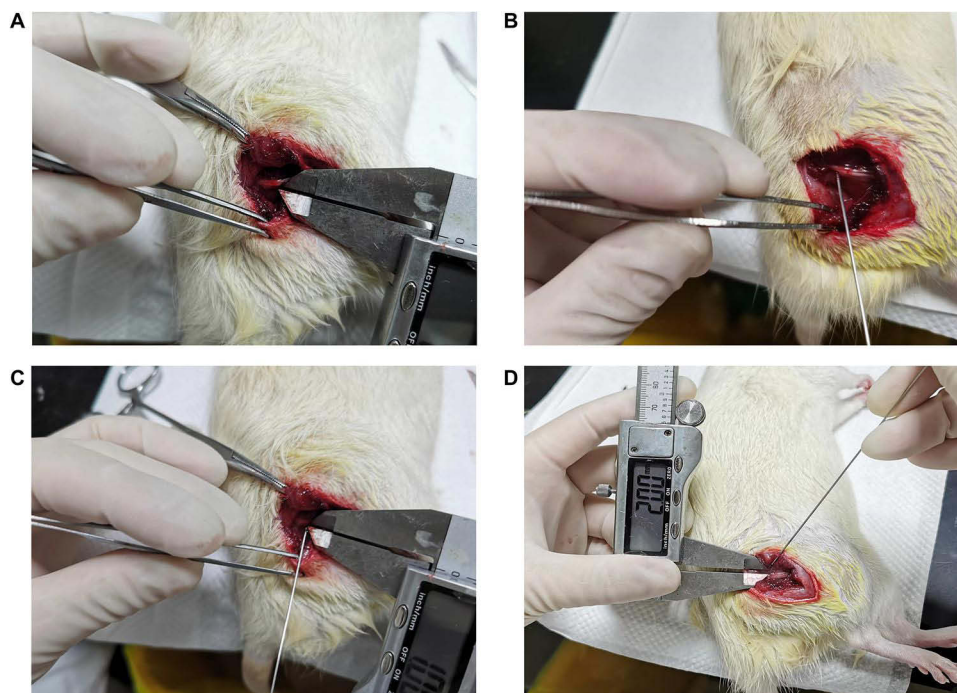


Figure 1 Diagram of the surgical procedure for low temperature plasma radiofrequency ablation of the rat sciatic nerve. (A) Sham group, only the muscle was incised to expose the sciatic nerve. (B) 0 mm group, the plasma knife tip was applied directly to the sciatic nerve near the bifurcation. (C and D) 1mm group and 2mm group, the plasma knife tip was applied to the muscle 1mm and 2mm away from the sciatic nerve.

Electrophysiology Evaluation

Before euthanasia, rats were re-exposed to the sciatic nerve on the operated side under isoflurane anesthesia. Two stimulating electrodes (KD-BHE, KedouBC, Suzhou, China) were positioned at 1cm intervals on the sciatic nerve, both proximal and distal to the previously applied site. The recording electrodes were then inserted into the calf flounder muscle. The nerve was stimulated with an electrophysiological instrument (Dantec keypoint, Denmark), compound muscle action potentials (CMAPs) and latencies were noted, nerve conduction velocities were measured, from which nerve function was assessed.

Enzyme Linked Immunosorbent Assay (ELISA)

The expression levels of TNF- α , IL1- β , c-fos and NGF in rat sciatic nerve were determined by Elisa. Rat sciatic nerve segments were harvested, completely lysed with RIPA lysate and homogenized by grinding on ice. The Elisa kit TNF- α (YJ731064), IL1- β (YJ710928), c-fos (YJ712464), NGF (YJ712345) was purchased from Mlbio, Shanghai. The experiments were carried out strictly accordance with the manufacturer's instructions.

Histological Evaluation and Morphological Analysis

Histological and morphological analyses of the sciatic nerve at the surgical site were conducted. For tissue staining, the obtained sciatic nerve tissues were fixed with 4% paraformaldehyde at 4° C refrigerator for 24 hours, then buried in paraffin wax and 5 μ m transverse slides were prepared at the distal nerve end. The slides were respectively stained with hematoxylin and eosin (HE), toluidine blue (TB), and luxol fast blue (LFB). Observations were made with a light microscope. (Nikon Eclipse E100). For transmission electron microscopy, the nerve samples were fixed with 2.5% pentylene glycol for 2 hours at 4°C and then re-fixed with 1% osmic acid, followed by embedding and cutting into ultrathin sections, and staining the ultrathin sections with lead citrate and uranyl acetate. The ultrastructure of the nerve tissue was examined by transmission electron microscopy (TEM, HITACHI, HT7700, Japan).

The photos of the stained cross sections were taken with a light micrograph with a camera. Sections were statistically analyzed by Image-Pro Plus (Media Cybernetics, USA) software at a field of view of 400x magnification to count the number of Schwann cells in TB-stained slices and to measure the area of myelin positivity in LFB-stained slices. For transmission electron microscopy sections, photographs were taken and the same software was used to statistically analyze the thickness of myelin at a magnification of 1000 \times .

Statistical Analysis

All statistics data were expressed as the mean \pm standard deviation (SD), and statistical analysis was performed by Graphpad Prism 9.5 software. Two groups were compared by *t*-test, and multiple comparisons were made by variance (ANOVA) with Tukey's post hoc tests, with a P value of less than 0.05 being considered significant.

Results

Mechanical Threshold Assessment

Mechanical allodynia was assessed in the sham, 0mm, 1mm, and 2mm groups by determining ipsilateral and contralateral paw withdrawal thresholds (PWT) on preoperative d3 and postoperative d3, d5, d7, d10, and d14 (Figure 2A and B). For the surgical side, during d3 to d14 postoperatively, the paw withdrawal thresholds were significantly decreased in the 0mm ($p<0.05$), 1mm ($p<0.05$) and 2mm ($p<0.05$) groups compared to the sham group, and the PWT was consistently less in the 0mm group than in the 2mm group ($p<0.05$), however there was no apparent significant between the 0mm and 1mm groups. From d3 to d5 days after surgery, the PWT was less in the 1mm group than in the 2mm group ($p<0.01$), but there was no statistical difference between the two groups from d7 to d14 days after operation. The contralateral paw withdrawal thresholds were consistently not statistically different among the four groups. Similarly, ipsilateral and contralateral PWTs were determined on preoperative d3 and postoperative d3, d7, d14, d21, and d28 for the sham and 2mm groups (Figure 2C and D). The results indicated that the paw withdrawal threshold was lower in the 2mm group compared to the sham group from d3 to d21 postoperatively ($p<0.05$), but by postoperative d28, the data between the two

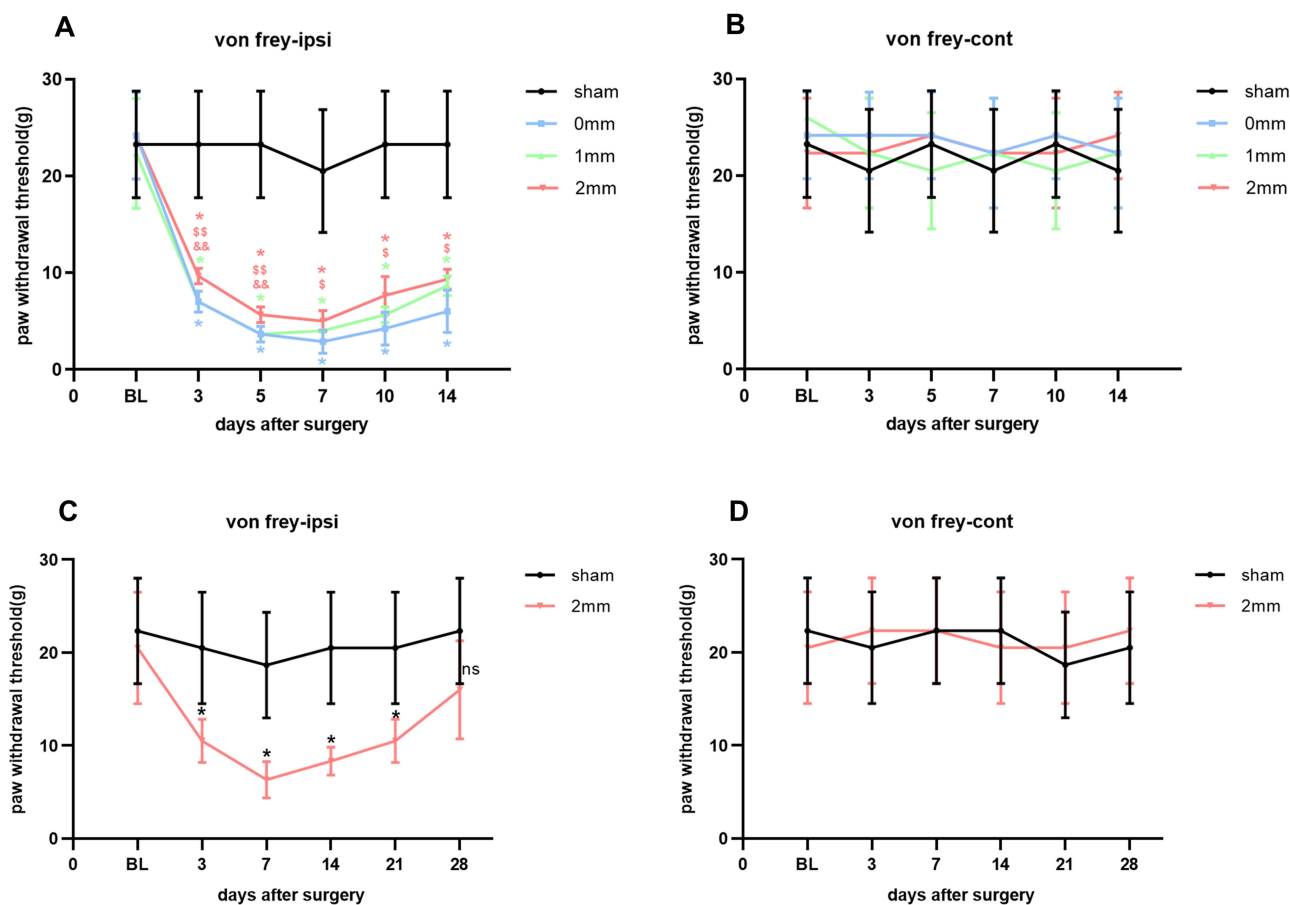


Figure 2 (A and B) Changes in ipsilateral and contralateral paw withdrawal thresholds in the sham, 0 mm, 1 mm, and 2 mm groups ($n=4$ in the sham and $n=6$ in other group). **(C and D)** Changes in ipsilateral and contralateral paw withdrawal thresholds in the sham and 2 mm groups ($n=6$ in two groups). * $p < 0.05$ compared to sham group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ versus to 1mm group.

Abbreviation: ns, not significant.

groups was statistically indistinguishable. In the same way, Contralateral data between the sham and 2mm groups showed no meaningful differences.

Neurophysiologic Examination

The peak amplitude of CMAPs, latency and conduction velocity were recorded by the Biological Signal Acquisition and Analysis System 14 days and 28 days after surgery (Figure 3). Figure 3D shows representative CMAP signal recordings for the sham, 0mm, 1mm, and 2mm groups on postoperative d14 and for the sham and 2mm groups in postoperative d28. Figure 3A shows that at postoperative d14, the nerve conduction velocity in the 0mm group was slower than that in the sham group ($p < 0.001$), but there was no statistical significance between the sham, 1mm and 2mm groups. The nerve conduction velocity was markedly higher in the 1mm ($p < 0.01$) and 2mm ($p < 0.001$) groups than in the 0 mm. The mean peak CMAP amplitudes on postoperative d14 were 22.08, 10.72, 15.5, and 18.54 mV for the sham, 0mm, 1mm, and 2mm groups, respectively. The amplitude in the 0mm ($p < 0.0001$) and 1mm ($p < 0.01$) groups was considerably lower than in the sham group, while there was no obviously different between the sham and 2mm groups. The peak amplitudes of the 1mm ($p < 0.05$) and 2mm ($p < 0.001$) groups were significantly higher than those of the 0mm group (Figure 3B). The 0mm group had a considerably longer latency than the sham ($p < 0.01$) and 2mm groups ($p < 0.05$), but it was not different from that of the 1mm group. The latency of the sham, 1mm and 2mm groups were all relatively close to each other (Figure 3C). On postoperative d28, The nerve conduction velocity in the 2mm group were very close to the sham group (Figure 3E). The peak amplitude of the CMAPs in the sham and 2mm groups was 22.63 and 23.23 mV, respectively, No clear difference in

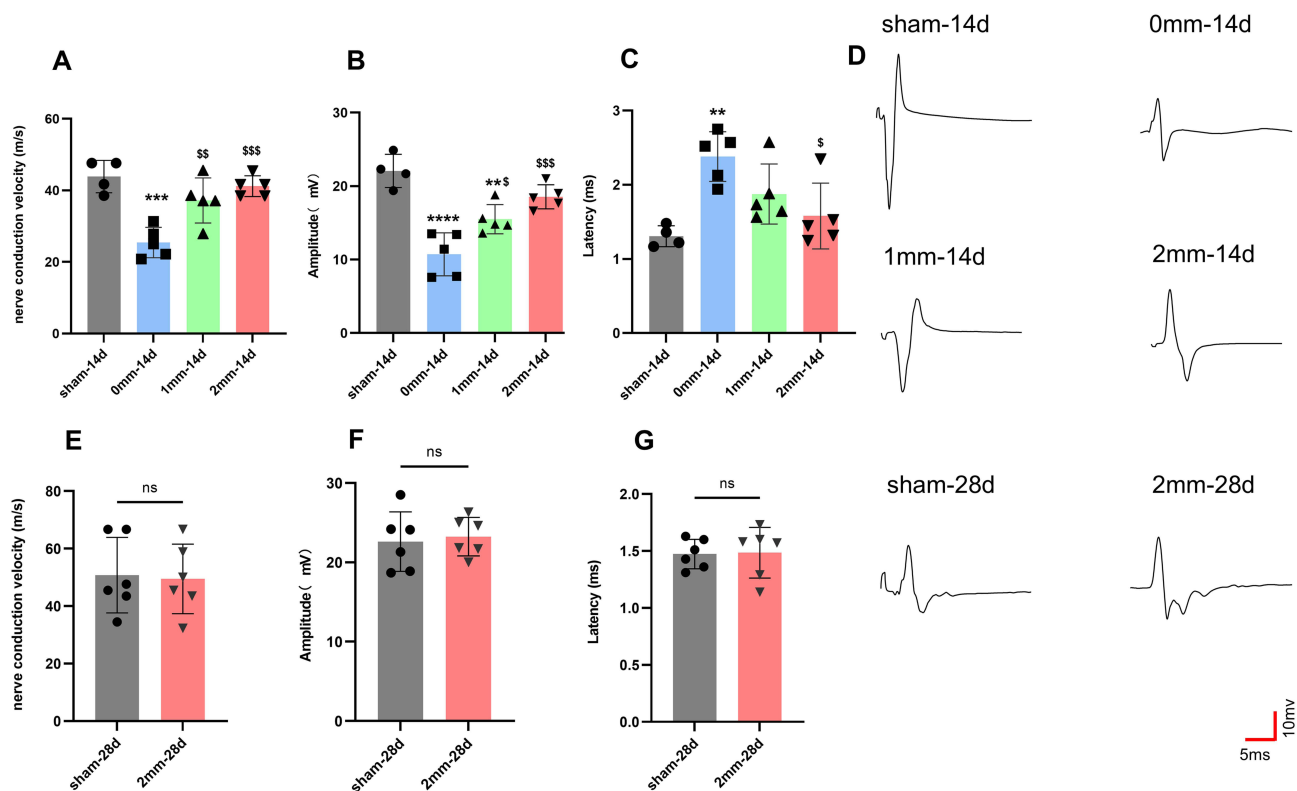


Figure 3 Statistical results of (A) conduction velocity, (B) CMAP peak amplitude, and (C) latency at day 14 postoperatively in the sham, 0mm, 1mm, and 2mm groups ($n=4$ in the sham and $n=5$ in other group). (D) Representative CMAPs recorded on the operated side of each group on postoperative day 14 and day 28. Statistical results of (E) conduction velocity, (F) CMAP peak amplitude, and (G) latency at day 28 postoperatively in the sham and 2mm group ($n=6$ in two groups). $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ versus sham group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$ \$ \$}p < 0.01$ versus 0mm group. **Abbreviation:** ns, not significant.

data between the two groups (Figure 3F). There was no significant difference between the sham group and the 2 mm group on the 28th day after surgery (Figure 3G).

Pro-Inflammatory Cytokines and Pain Markers

Inflammatory factors and pain-related indices in the sciatic nerve were determined by Elisa on postoperative days 14 and 28, respectively. On postoperative d14, TNF- α expression levels were clearly higher in the 0 mm ($p < 0.001$) and 1mm ($p < 0.05$) groups than in the sham group, whereas the difference between the 2 mm and sham groups was not statistically different. TNF- α levels in rat sciatic nerves were significantly lower in 1mm ($p < 0.05$) and 2mm ($p < 0.001$) groups than in 0mm group (Figure 4A). The results of the IL-1 β study demonstrated a clear rise in IL-1 β levels in the sciatic nerve on postoperative d14 in the 0mm ($p < 0.0001$), 1mm ($p < 0.0001$) and 2mm ($p < 0.01$) groups compared with the sham group. Nevertheless, a reduced IL-1 β level was found in the 2mm ($p < 0.01$) group versus the 0mm group (Figure 4B). As can be seen from Figure 4C, the C-fos of the 0mm ($p < 0.001$), 1mm ($p < 0.001$), and 2mm ($p < 0.05$) groups were higher than that in the sham group, and less in the 2mm group than in the 0mm group ($p < 0.05$). For the results of NGF expression, it also showed that the 0mm ($p < 0.001$) and 1mm ($p < 0.05$) groups were higher than the sham group, and the 0mm group was clearly higher than the 2mm group ($p < 0.001$), but the results were similar for the sham and 2 mm groups (Figure 4D). On the 28th day after surgery, the four indicators of TNF- α , IL-1 β , C-fos and NGF were tested again. The changes in sciatic nerve indexes were determined in the sham and 2mm groups of rats. It was found that there was no obvious difference in the indicators between the two groups (Figure 4E-H).

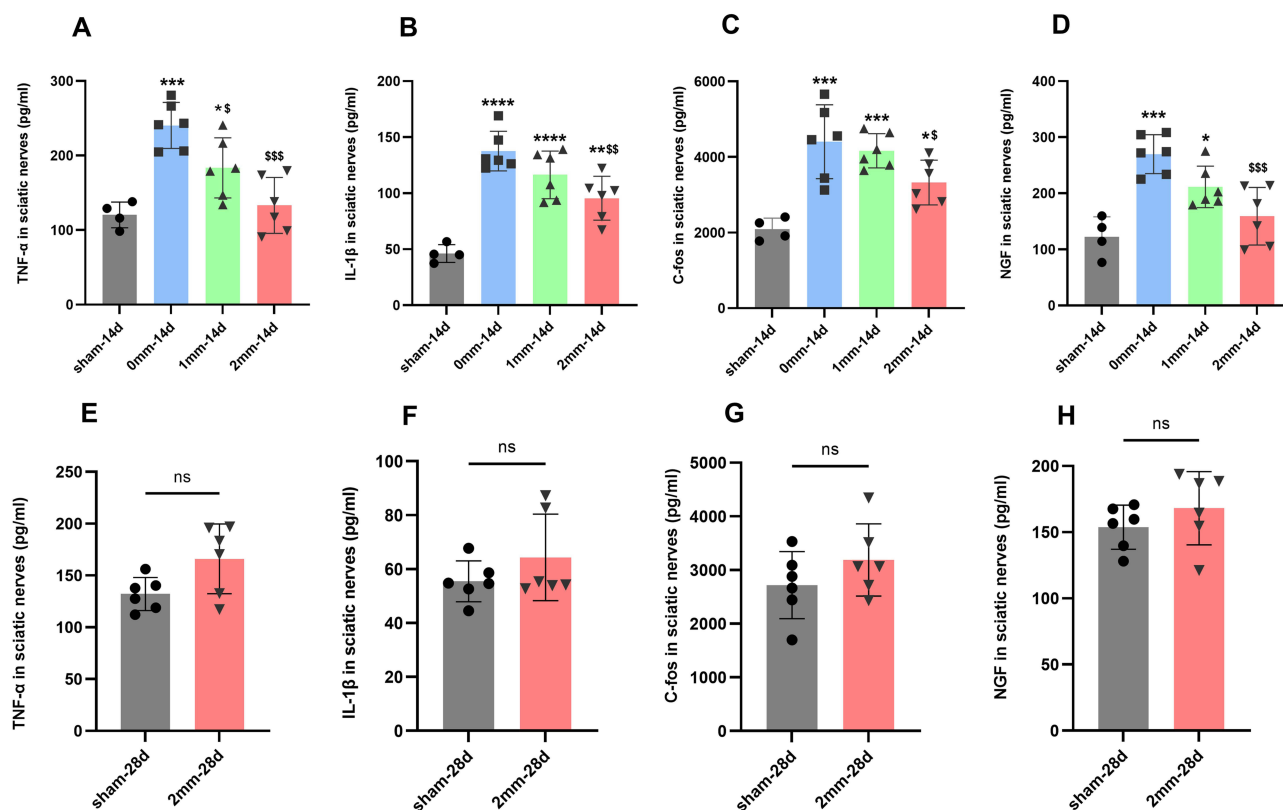


Figure 4 Expression of (A) TNF- α , (B) IL-1 β , (C) C-fos and (D) NGF in sciatic nerves of sham, 0mm, 1mm and 2mm groups on postoperative day 14 by ELISA method (n=4 in the sham and n=6 in other group). Expression of (E) TNF- α , (F) IL-1 β , (G) C-fos and (H) NGF in the sciatic nerve of sham and 2mm groups on postoperative d28 by ELISA method (n=6 in two groups). * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001 versus sham group, $\$$ p < 0.05, $\$$ $\$$ p < 0.01, $\$$ $\$$ $\$$ p < 0.001 versus 0mm group.

Abbreviation: ns, not significant.

Histology

To observe the morphological structure of the tissues, HE, TB and LFB staining was performed on the sciatic nerve sections of rats from several groups at different time points (Figure 5A). From the cross sections of pathological staining, it could be seen that on the d14 after operation, the nerve fibers in the 0mm and 1mm groups were sparse and loosely arranged, and even uneven staining, oedema and vacuoles were found in the 0mm group; the nerve fibers in the sham group and 2mm group were more tightly arranged and evenly stained, there were no vacuoles, as well as the structure of the nerve fibers was intact. The sections on day 28 after surgery showed that the nerve tissue of the sham group and the 2mm group was clear and intact, with no obvious changes.

Next, the density of Schwann cells and the myelin-positive area were determined by means of TB and LFB staining. In terms of the number of Schwann cells, on postoperative d14, the sham group had more than the 0mm (p <0.0001) and 1mm (p <0.01) groups, but the sham group was close to the 2mm group. Compared with the 0mm group, the 1mm (p <0.01) and 2mm (p <0.0001) Schwann cell groups had higher numbers of Schwann cells, and the 2mm group again had more than the 1mm group (p <0.05). Schwann cell counts in the 2mm group remained statistically indistinguishable from the sham group until postoperative d28 (Figure 5B and D). In terms of myelin area, at postoperative d14, the 0mm (p <0.0001) and 1mm (p <0.0001) groups were smaller than the sham group, no obvious difference between 2mm and sham groups, the 2mm (p <0.0001) and 1mm (p <0.0001) groups were larger than the 0mm group, and the 2mm group was larger than the 1mm group (p <0.0001). At postoperative d28, myelin sheath area remained statistically insignificant in the sham and 2mm groups (Figure 5C and E).

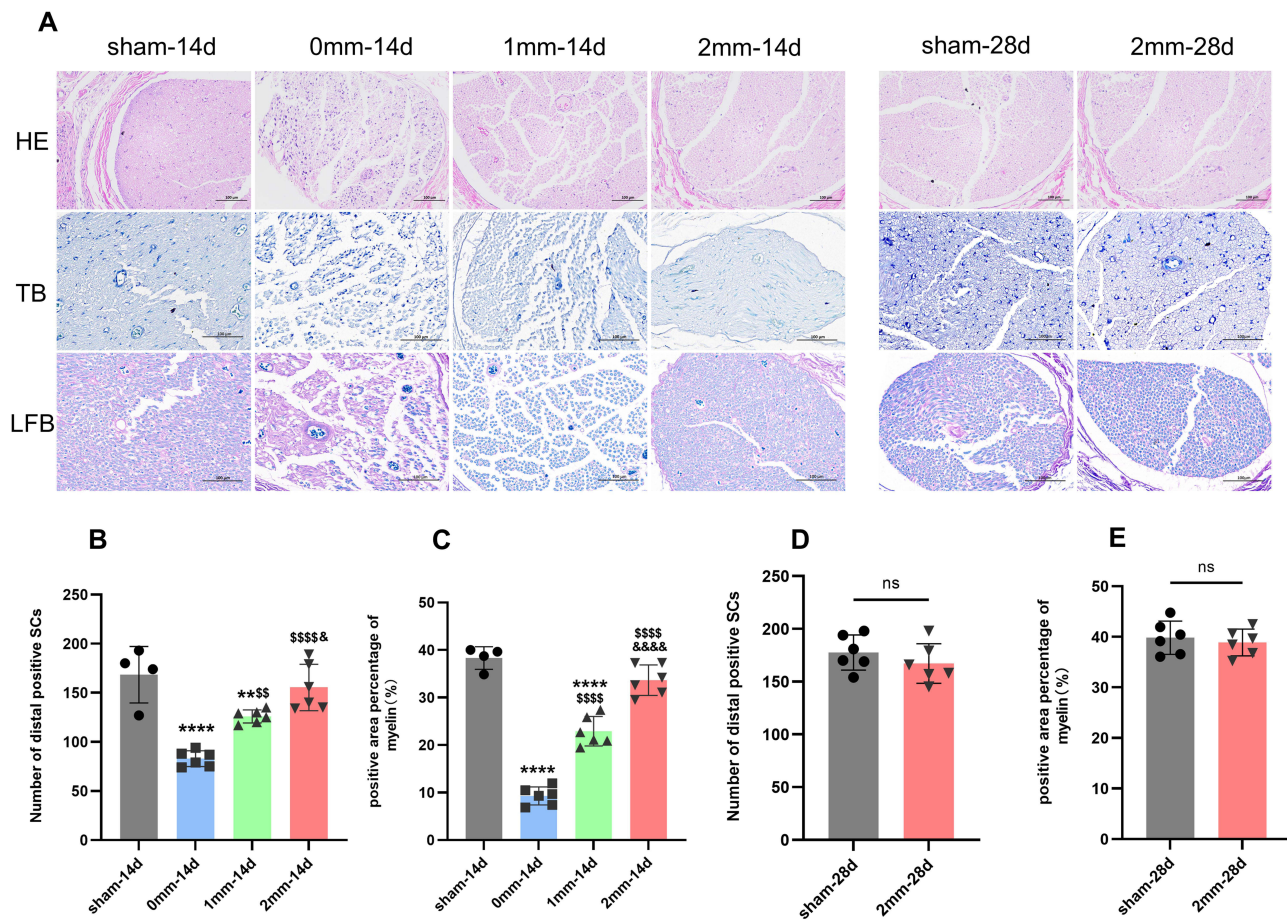


Figure 5 (A) Representative images of HE, TB and LFB staining of the distal sciatic nerve on d14 and d28 post-surgery. 400× magnification, Scale bar=100 μm. On day 14 postoperative, (B) the Schwann cells density in TB staining and (C) the positive area percentage of myelin in LFB staining in the sham, 0mm, 1mm, and 2mm groups (n=4 in the sham and n=6 in other group). On day 28 postoperative, (D) the Schwann cells density in TB staining and (E) the positive area percentage of myelin in LFB staining in the sham and 2mm groups (n=6 in two groups). ** $p < 0.01$, **** $p < 0.0001$ versus sham group, \$ $p < 0.01$, \$\$\$ $p < 0.0001$ versus 0mm group, * $p < 0.05$, &&& $p < 0.0001$, versus 0mm group.

Abbreviation: ns, not significant.

Transmission Electron Microscopy

Ultrastructure of the sciatic nerve cross section in each group observing using transmission electron microscopy (TEM) (Figure 6A). In the sham group, the sciatic nerve fibers were numerous, regular in outline, structurally intact, the myelin sheaths were evenly distributed, neatly arranged and concentric, and the axons were intact. In the 0mm group, the number of nerve fibers was sparse, the structure was damaged, the myelin sheath was stratified and there were many vacuoles. In the 1mm and 2mm groups, the number of nerve fibers was higher, the structure was intact, and the myelin sheaths were generally free of uneven stratification and vacuoles on postoperative d14. Again on postoperative d28, the sham and 2 mm groups were sampled and showed comparable numbers of nerve fibers, uniform distribution of myelin sheaths, no significant delamination and structural integrity and clarity in both groups.

Next, the thickness of the myelin sheath in each group was measured and analyzed. As can be seen from Figure 6B, on the 14th day after surgery, the sham group had a thicker myelin sheath than the 0mm ($p < 0.0001$) and 1mm ($p < 0.01$) groups, but the 2mm group had a myelin sheath thickness similar to that of the sham group. The myelin sheaths in the 1mm ($p < 0.01$) and 2mm ($p < 0.0001$) groups were thicker than those in the 0mm group. Additionally, the myelin sheaths of the 2mm group were thicker than those of the 1mm group ($p < 0.01$). On postoperative day 28, the 2mm group still had a myelin thickness close to that of the sham group (Figure 6C).

In conclusion, LTPRA operates on nerves at different distances and damages nerves to different degrees. Among them, 0mm, which directly acted on the nerve, caused the greatest damage to the nerve, and the rats showed abnormal

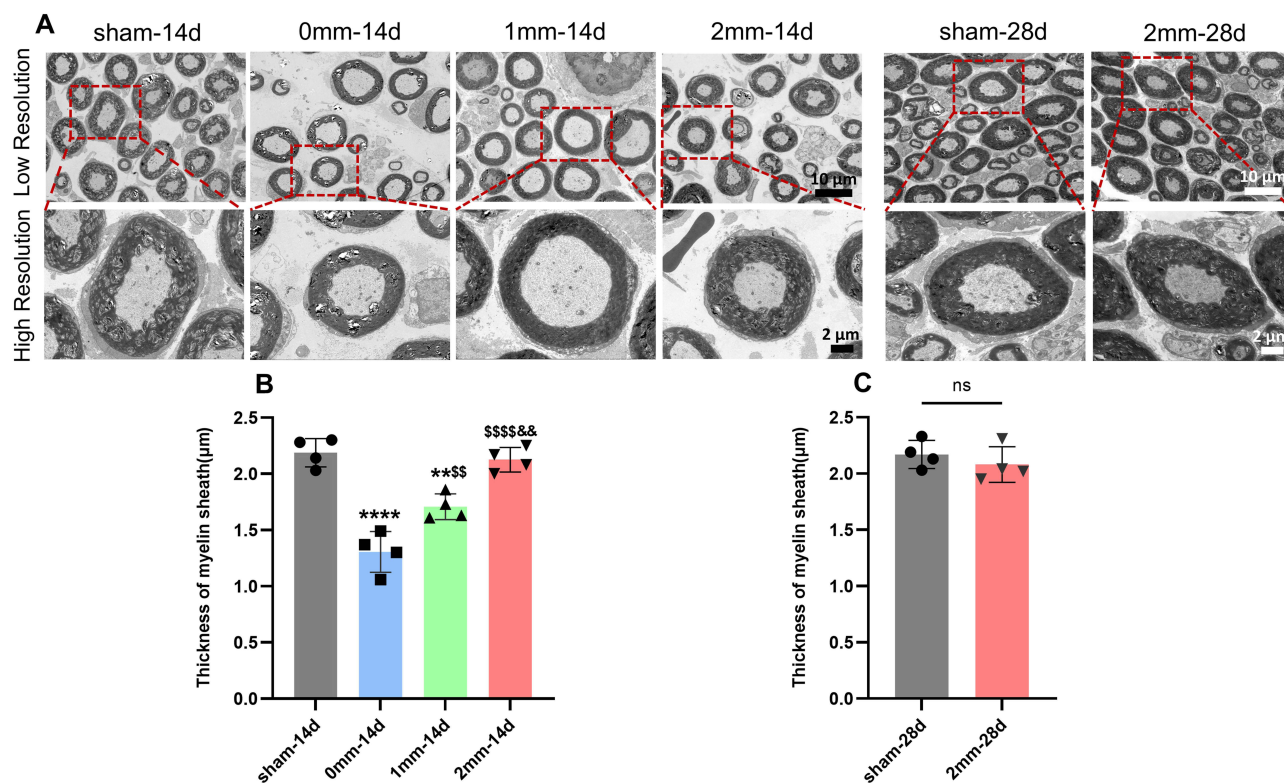


Figure 6 (A) Representative TEM image of a rat sciatic nerve cross section on day 14 and day 28 after surgery. 1000 \times , 3000 \times amplification, scale bar=10 μ m, 2 μ m. (B) On the 14th day after surgery, changes in myelin thickness in the transverse section of the sciatic nerve in the sham group, 0mm group, 1mm group, and 2mm group (n=4 in the group). (C) On the 28th day after surgery, changes in myelin thickness in the transverse section of the sciatic nerve in the sham group, and 2mm group (n=4 in the group). ** p <0.01, *** p <0.0001 versus sham group, \$\$ p < 0.01, \$\$\$ p < 0.0001 versus 0mm group, && p <0.01, versus 0mm group.

Abbreviation: ns, not significant.

nerve function and tissue structure changes after surgery, with the most obvious inflammatory reaction; the stimulation at a distance of 1mm also induced changes in the nerve tissue structure and local inflammatory reaction; and the damage to the nerve was the least at a distance of 2mm, which was close to that of the sham group. However, two weeks after surgery, the paw withdrawal threshold of the rats in the 2mm group had not returned to pre-surgery levels and the local inflammatory response was still present. To further evaluate the long term effects of LTPRA on the nerves at the 2mm distance, we extended the postoperative test period to further confirm the safety of the 2mm distance. At 28 days postoperatively, all tests were performed again on the 2mm group. It was found that there was no meaningful difference between the paw withdrawal thresholds of rats in the 2mm group and those in the sham group. And basically recovered to the preoperative level, besides, the inflammatory response also essentially disappeared. 2mm can be considered as a safe distance for plasma knife operation.

Discussion

In previous studies on low temperature plasma radiofrequency ablation, most of them directly discuss the efficacy of LTPRA of lesions or compare it with other treatments; however, with the expansion of the application range of LTPRA, the safe distance of its application close to the neural tissues has not yet been reported, especially when it is applied to the release of soft tissues at the site of neural entrapment and entrapment.

In this research, to investigate the safe perineural extent of low temperature plasma radiofrequency ablation application, we set different distances, namely 0mm, 1mm, and 2mm, near the sciatic nerve bifurcation in normal rats to perform the ablation procedure. We found that application of the low temperature plasma knife at both distances of 0mm and 1mm induced structural changes in the nerve tissue and a severe local inflammatory reaction, as well as impaired nerve function, and that the distance of 2mm did not induce structural changes in the nerve tissue or functional

abnormalities, and only a local mild inflammatory reaction was observed. Based on this part of the study, we excluded 0mm and 1mm as safe distances and prolonged the postoperative recovery time in the 2mm group to judge its long-term effect. At 28 days postoperatively, the 2mm group was evaluated again and found that the indicators had returned to the preoperative level. Radiofrequency ablation is now widely used in clinical surgery, particularly in head and face surgery, including tonsils, adenoids, turbinates, vocal cord lesions, and head and neck tumors,^{34–38} as well as in the treatment of trigeminal neuralgia, herpes zoster neuralgia and herniated discs.^{39–41} Low temperature plasma radiofrequency ablation has been shown to be superior when comparing bleeding,⁴² postoperative pain scores,⁴³ and postoperative complications.⁴⁰ It is a new technology that is mainly applied to soft tissues⁴⁴ based on radiofrequency ablation to generate plasma composed of charged particles, and the plasma destroys the peptide bonds of the tissue to decompose the target tissue under low-temperature conditions.⁴⁵ Owing to the various advantages of LTPRA, the application of peripheral nerve entrapment loosening promotes higher theoretical feasibility and clinical significance. Therefore, our present study focused on exploring the safe distance of LTPRA on peripheral nerves to provide broader ideas and methods for clinical practice.

In both parts of the experiment, the effects of LTPRA on nerve function were evaluated by means of pain behavioral tests and neurophysiological tests. Three days after surgery, paw withdrawal thresholds decreased in all groups of rats, except that they were higher in the 2mm group than in the 0mm and 1mm groups. At 2 weeks postoperatively, the paw withdrawal thresholds did not return to normal levels in all surgical groups, but at 4 weeks postoperatively, it returned to normal levels in the 2mm group. Electrophysiological experiments suggested that neuroelectric conduction was more affected in the 0mm and 1mm groups, especially in the 0mm group which had lower nerve signal intensity than the other groups and the slowest nerve signal transmission. The results for the 2 mm and sham group were not significantly different.

NGF, which is markedly increased in injured and inflamed tissues, plays an important role in pain and is considered a pain mediator, and the degree of pain correlates with NGF levels.^{46,47} Inflammatory cytokines IL1- β and TNF- α produced by local inflammation promote the production of NGF by several nearby cells.⁴⁸ C-fos is an immediate early gene and transcriptional activation of the gene occurs within minutes of stimulation. A variety of noxious stimulations can induce C-fos expression in the brain and spinal cord.^{49,50} Previous studies have reported that nerve injury leads to sequential up-regulation of C-fos and NGF mRNA levels in local neural tissues, and it is C-fos that mediates the up-regulation of NGF mRNA levels.⁴⁷ Our study showed that LTPRA directly on the nerve caused high expression of inflammatory factors, NGF, and C-fos, as expected, but the ablation procedure at a distance of 2 mm from the nerve also increased the expression of IL-1 β and C-fos in the short term, and it only decreased one month after the procedure. We speculate that this may still be related to the thermal effects of plasma-based radiofrequency energy, although LTPRA operates at a lower temperature than traditional radiofrequency energy, it still exhibits high central target temperatures during operation. The extent of thermal damage caused by this thermal effect remains unclear. Some studies suggest that plasma energy devices can cause thermal damage, and it is important to maintain a safe distance from critical tissues and nerves.^{29,30} This suggests that LTPRA should be applied to soft tissues near nerves at a strictly controlled distance, and that the neuroinflammatory response is still present in the short term after the procedure.

In the peripheral nervous system, Schwann cells are myelinating glial cells that form lipid-rich myelin sheaths around axons and promote nerve regeneration and repair following nerve injury.^{51,52} With regard to nerve tissue morphology, degree of nerve injury was rated by calculating the number of Schwann cells measured by TB staining. The 0mm and 1mm groups had severe nerve injury with a obvious decrease in the Schwann cell count, whereas the 2 mm group had the highest number of Schwann cells among the three groups. The area of myelin stained purple-blue was measured by LFB staining, which was also consistent with the above results. The myelin sheaths and axons of the nerve fibers can be seen more visually under transmission electron microscopy. The myelin sheath in normal nerve tissue was structurally complete, surrounding the axon in concentric circles, with a dense and regular structure and a distribution of nerve fibers of all sizes and diameters.⁵³ The direct action of LTPRA on the nerve destroyed the myelin sheath structure of the nerves and created cavities. There were also changes in myelin thickness, with significant thinning in the 0 mm and 1 mm groups and almost no change in the 2 mm group.

Our current study focuses on the safety of LTPRA on normal rat nerve tissue. It may provide inspiration for clinical applications, but the species-specific differences between humans and rats should not be overlooked. The diameter of the rat sciatic nerve is approximately 2 mm, while the diameter of human peripheral nerves is approximately 8 mm.⁵⁴ The differences between the two nerves necessitate further exploration of the clinical safety of LTPRA.

Conclusion

Low temperature plasma radiofrequency ablation applied directly to the nerve can damage the nerve, and the distance should be controlled during application, 2mm from the nerve may be a safe distance. This study also has certain limitations. First, the study subjects were rats, and further validation is needed for clinical application. Second, the study primarily assessed nerve damage during the acute and subacute phases, with chronic outcomes not explored. Finally, the study did not address repetitive use scenarios such as revision surgery.

Ethics Approval

The experimental procedures and protocols followed in this research were authorized by the Experimental Animal Ethics Committee of Yueyang Hospital of Integrative Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine (approval number: YYLAC-2022-173-17), and animal-related experimental operations were conducted in accordance with the International Association for the Study of Pain's ethical guidelines for the use of laboratory animals.³³

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work. None of the authors of this article have any affiliation with medical device companies or institutions.

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