RESEARCH ARTICLE

Effect of the combination of high-frequency repetitive magnetic stimulation and neurotropin on injured sciatic nerve regeneration in rats

Jie Chen[1], **Xian-Ju Zhou**[2, 3, *], **Rong-Bin Sun**[1, *]

1 Department of Orthopedics, the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, Changzhou, Jiangsu Province, China

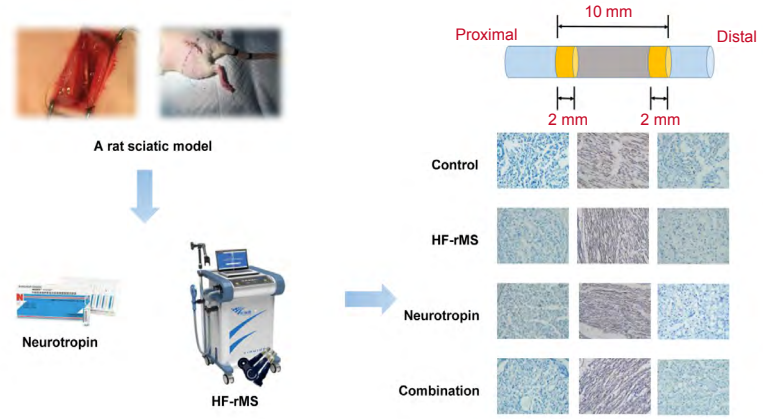
2 Laboratory of Neurological Diseases, Department of Neurology, the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, Changzhou, Jiangsu Province, China

3 Department of Neurology, Integrated Hospital of Traditional Chinese Medicine, Southern Medical University, Guangzhou, Guangdong Province, China

Funding: *This study was funded by the National Natural Science Foundation of China, No. 81471338 and No. 81671284 (to XJZ); the Changzhou Science & Technology Program of China, No. CE20145045 (to XJZ); the Changzhou High-Level Medical Talents Training Project of China, No. 2016CZLJ018 (to XJZ).*

Graphical Abstract

Peripheral high-frequency repetitive magnetic stimulation (HF-rMS) or neurotropin promotes peripheral nerve regeneration



Correspondence to:
Xian-Ju Zhou, PhD, xianju_zhou@yahoo.com;
Rong-Bin Sun, MS, rongbin_sun@sina.com.

orcid:
0000-0003-1744-556X (Xian-Ju Zhou)
0000-0003-2900-7276 (Rong-Bin Sun)

doi: *10.4103/1673-5374.264461*

Received: *November 10, 2018*
Accepted: *March 5, 2019*

Abstract

Repetitive magnetic stimulation is effective for treating posttraumatic neuropathies following spinal or axonal injury. Neurotropin is a potential treatment for nerve injuries like demyelinating diseases. This study sought to observe the effects of high-frequency repetitive magnetic stimulation, neurotropin and their combined use in the treatment of peripheral nerve injury in 32 adult male Sprague-Dawley rats. To create a sciatic nerve injury model, a 10 mm-nerve segment of the left sciatic nerve was cut and rotated through 180° and each end restored continuously with interrupted sutures. The rats were randomly divided into four groups. The control group received only a reversed autograft in the left sciatic nerve with no treatment. In the high-frequency repetitive magnetic stimulation group, peripheral high-frequency repetitive magnetic stimulation treatment (20 Hz, 20 min/d) was delivered for 10 consecutive days after auto-grafting. In the neurotropin group, neurotropin therapy (0.96 NU/kg per day) was administrated for 10 consecutive days after surgery. In the combined group, the combination of peripheral high-frequency repetitive magnetic stimulation (20 Hz, 20 min/d) and neurotropin (0.96 NU/kg per day) was given for 10 consecutive days after the operation. The Basso-Beattie-Bresnahan locomotor rating scale was used to assess the behavioral recovery of the injured nerve. The sciatic functional index was used to evaluate the recovery of motor functions. Toluidine blue staining was performed to determine the number of myelinated fibers in the distal and proximal grafts. Immunohistochemistry staining was used to detect the length of axons marked by neurofilament 200. Our results reveal that the Basso-Beattie-Bresnahan locomotor rating scale scores, sciatic functional index, the number of myelinated fibers in distal and proximal grafts were higher and axon lengths were longer in the high-frequency repetitive magnetic stimulation, neurotropin and combined groups compared with the control group. These measures were not significantly different among the high-frequency repetitive magnetic stimulation, neurotropin and combined groups. Therefore, our results suggest that peripheral high-frequency repetitive magnetic stimulation or neurotropin can promote the repair of injured sciatic nerves, but their combined use seems to offer no significant advantage. This study was approved by the Animal Ethics Committee of the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, China on December 23, 2014 (approval No. 2014keyan002-01).

Key Words: *axon; myelinated nerve fibers; nerve regeneration; neurological rehabilitation; neurotropin; peripheral nerve injury; repetitive magnetic stimulation; sciatic nerve; trauma*

Chinese Library Classification No. *R454; R364*

Introduction

Peripheral nerve injury, caused by trauma or medical disorders, affects over one million people worldwide every year (Carvalho et al., 2018). The majority of patients with peripheral nerve injury require surgery to restore nerve integrity, including nerve graft implantation, end-to-end repairs, and nerve conduit reconstruction (Zhang et al., 2016, 2019). Clinical and experimental investigation has achieved remarkable progress in biological and cellular strategies for reconstruction, but do not always result in a satisfactory outcome in peripheral nerve injury functional recovery (Grinsell and Keating, 2014). The speed and quality of axonal growth determine the ultimate regeneration of injured nerves at the cellular level (Malin et al., 2009). In previous studies, many methods have been shown to accelerate nerve regeneration, including electrical stimulation (Huang et al., 2013; Gordon and English, 2016), energy extracorporeal shock wave (Hausner et al., 2012), X-ray irradiation (Jiang et al., 2017a, b) and low level laser therapy (Mashhoudi et al., 2017). However, these are not commonly used in clinical settings and the optimal treatment remains unclear. Hence, it is imperative to develop better approaches for nerve reparation.

Repetitive transcranial magnetic stimulation (rTMS) provides non-invasive brain stimulation, which can modulate cortical excitability in a frequency-dependent manner: cortical excitability increases with high-frequency stimulation (> 5 Hz) and decreases with low-frequency stimulation (< 1 Hz) (Thut and Pascual-Leone, 2010; Zhang et al., 2018). High-frequency rTMS (HF-rTMS) is accepted in various fields as a therapeutic tool for neuropathic pain (Hosomi et al., 2013), major depression (George et al., 2010) and the negative symptoms of schizophrenia (Prikryl et al., 2013). Despite the wide application of HF-rTMS in the central nervous system (Lefaucheur et al., 2014), there have been few studies of magnetic stimulation of the peripheral nervous system improving the regeneration of damaged nerves.

Previous studies have shown that repetitive magnetic stimulation (rMS) can affect the nerve regeneration microenvironment and activate multilevel neuroplastic changes by stimulating spinal cord or corresponding spinal nerve roots when treating posttraumatic neuropathies following spinal or axonal injury (Zhivolupov et al., 2012; Jiang et al., 2016). These findings raised the possibility that the application of rMS to peripheral nerves could be a new therapy for improving clinical outcomes in total peripheral nerve injury.

Neurotropin (NTP) is the non-protein fraction extracted from rabbit skin inflamed by treatment with the vaccinia virus (Kawai et al., 2018). It has been used clinically for treating neuropathic pain by activating the descending pain inhibitory system (Takahashi et al., 2005), including subacute myelo-optic neuropathy hyperesthesia and post-herpetic neuralgia, and chronic pain, including cervico-omo-brachial syndrome and lower back pain (Kudo et al., 2011; Okazaki et al., 2013; Masuguchi et al., 2014). Recently, Matsuoka et al. (2018) revealed NTP as a potential treatment for peripheral nerve injury in demyelinating diseases by promoting differentiation of Schwann cells both *in vivo* and *vitro*. However, whether NTP is effective against more severe injuries, including transection models or crushed sciatic nerve injury, remains unknown.

Sensory and motor function defects are typical symptoms that negatively impact patients' daily functioning and quality of life after peripheral nerve injury (Quan et al., 2016; Zhou et al., 2018). In our clinical practice of treating peripheral nerve injury complicated with neuralgia, it appeared that the functional recovery of injured peripheral nerves (such as the ulnar, radial and sciatic nerves) was improved by HF-rMS or NTP application, and a better effect was achieved by their combination. In this study, we established an injured peripheral nerve model, and investigated the effect of HF-rMS, NTP and their combination on this peripheral nerve injury model, seeking evidence for these new treatments in improving clinical outcomes in peripheral nerve injury. To our best knowledge, this has not been reported previously.

Materials and Methods

Animals

In total, 32 specific-pathogen-free B6 male Sprague-Dawley rats aged 9–10 weeks and weighing 240–300 g were provided by Cavens Animal Research Laboratories, Changzhou, China (animal license No. SCXK (Su) 2016-0010). To represent a realistic environment, the rats were held at a 12-hour light/dark cycle at 07:00 to 19:00. Additionally, the rats had free access to bottled water and food. This study was approved by the Animal Ethics Committee of the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, China on December 23, 2014 (approval No. 2014keyan002-01). All precautions were taken to minimize the suffering of all animals and the number of animals used.

Surgery procedure

The sciatic nerve injury model was established as previously described (Hausner et al., 2012; Geuna, 2015). Briefly, the rats received anesthesia with intraperitoneal administration of 10% chloral hydrate (Yulonghz, Qingdao, China) at a dose of 400 mg/kg. The area below the level of midriff was depilated and cleansed for surgery. Under aseptic conditions, an incision was made along the crista femora within the rear left limb to expose the sciatic nerve. The origin and proximal end of sciatic nerves were determined before cutting away a 2 mm-long segment of nerve from the ganglia. Subsequently, a 10 mm segment of the distal sciatic nerve was cut away. By rotating 180 degrees, the nerve segment was replaced between the distal and proximal stumps of transected sciatic nerves. Each end was restored continuously with interrupted sutures (Ethilon 9-0, Ethicon-Johnson & Johnson, Shanghai, China) under an operating microscope (Leica M750, Leica Microsystems, Vienna, Austria) (**Figure 1**). Following the surgical procedure, the gluteal muscle and skin incisions were closed with non-absorbable suture (Mersilk 4-0, Ethicon-Johnson & Johnson) in all groups.

After establishment of the sciatic nerve injury model, the rats were randomly divided into four groups (n = 8 each). The control group received only a reversed autograft in the

left sciatic nerve with no treatment. In the HF-rMS group, HF-rMS of the peripheral sciatic nerve was applied for 10 consecutive days (once per day). In the NTP group, NTP was intraperitoneally injected for 10 consecutive days (once per day). Animals in the combined group (HF-rMS + NTP) received the combination of peripheral HF-rMS and intraperitoneal injection of NTP for 10 consecutive days (once per day). All treatments were applied one week after autografting.

Peripheral high-frequency rMS and neurotropin administration

HF-rMS was delivered using a 70 mm eight-figure coil connected to a commercially available magnetic stimulator (Wuhan Yi Rui De Ltd., Wuhan, China). The stimulation site was at the intersection of the notochord and proximal end of the reconstructed sciatic nerve. The rat was fixed in a customized cloth bag when receiving stimulation. The stimulation parameters were 20 Hz frequency, stimulation intensity at 20% 1-second train duration, inter-train interval of 14 seconds, and 20 trains per session. Each session of rMS consisted of 1600 pulses/day delivered within 20 minutes. The control and NTP groups were fixed in the device but no rMS stimulation was given.

NTP was purchased from Nippon Zoki Pharmaceutical Co. (Osaka, Japan). The dosage of NTP was 0.96 NU/kg per day according to the clinical dosage in adults. All medicine was given by intraperitoneal injection. The control and HF-rMS groups were injected with saline.

Behavioral testing

The functional recovery of the injured nerves was assessed using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale (Basso et al., 1995). This rating scale is commonly used to assess the functional recovery of animals with injuries to the spinal cord, and has been shown to be a useful tool for assessing peripheral nerve injury recovery time (Bervar, 2000). BBB scores were assessed at 1 week after surgery (1 day before the 10-day postoperative treatment) and 1 day after the whole treatment (or after 10-day treatment).

A score of 0 represents no spontaneous movement, a score of 14 indicates full limb coordination and fully supporting weight and a score of 21 indicates normal movement. A trained observer who was blind to the experimental design conducted the BBB recordings in a quiet environment. During each session, animals were observed for 4 minutes in a circular metal container without a lid.

Functional assessment of re-innervation

To evaluate motor function, the sciatic functional index (SFI) was calculated, as described previously, pre- and post-treatment (Bain et al., 1989; Varejao et al., 2001). In addition, rats were tested within a dark shelter at the end of a confined walkway. White paper covered the path that the rat was expected to walk, and red ink was applied to its rear paws to record footprints over the paper. The parameters measured were as follows: print length (PL), distance between heel and toe; toe spread (TS), distance between first toe and fifth toe; and intermediary toe spread (ITS), distance between second toe and fourth toe. PL, TS, and ITS measurements were all collected on both the experimental (E) and normal (N) rear legs. Finally, each animal's footprints were used to calculate the SFI using the formula: SFI = 38.3(EPL–NPL)/NPL + 109.5(ETS–NTS)/NTS + 13.3(EITS–NITS)/NITS–8.8. The SFI varied from 0 to –100: a score of 0 represented normal nerve function; a score of –100 represented full function loss.

Morphological analysis

The animals that received auto-grafts were sacrificed with anesthesia by 10% chloral hydrate as mentioned above, 1 day after treatment. The left sciatic nerve samples were excised and fixed in 10% neutral buffered formalin for histopathological examination as previously described (Ikumi et al., 2018; Paradiso et al., 2018).

Preparation for staining: Semithin sections (1 μm) were cut at 2 mm distal and proximal from the center to the graft for toluidine blue staining to determine the numbers of myelinated fibers. To detect axon lengths, the medium sections left were made into 4 μm paraffin strips using a microtome for immunohistochemical staining. The tissue was embedded in paraffin by patching and baking the slices. Finally, routine dewaxing and hydration were conducted for subsequent staining.

Toluidine blue staining: Sections were stained with 1% toluidine blue (Solarbio, G3661, Shanghai, China) for 25 minutes, rinsed in running water, decolorized in 95% acetic acid twice for 2 seconds each, decolorized in 100% ethanol twice for 2 minutes each, cleared in xylene three times for 5 minutes each and sealed with resin.

Immunohistochemical staining: Sections were washed in phosphate-buffered saline (PBS) for 5 minutes, rinsed in 3% methanol-H_2O_2 solution (Sangon, H1976, Shanghai, China) for 10 minutes at room temperature and washed in PBS three times for 5 minutes each. Sections were hot retrieved at high temperature for 4 minutes and at low temperature for 20 minutes, returned to room temperature, washed in PBS three times for 5 minutes each and blocked in 5% bovine serum albumin (Beyotime, P0081, Shanghai, China) for 60 minutes at room temperature. After the removal of excess liquid, sections were incubated in 50 μL anti-NF200 antibody (BM0100; Boster, Wuhan, China) overnight at 4°C, rewarmed at 37°C for 45 minutes, washed in PBS three times for 5 minutes each time, incubated in 50 μL biotin-labeled goat anti-mouse secondary antibodies (SA1020; Boster) at 37°C for 30 minutes and washed in PBS three times for 5 minutes each. Subsequently, these sections were incubated in 50 μL streptavidin-biotin for 30 minutes at 37°C, washed in PBS three times for 5 minutes each, stained in 3,3′-diaminobenzidine solution (ZL-9018; ZSGB-BIO, Beijing, China) for 8 minutes under the microscope (Leica M750, Leica Microsystems, Vienna, Austria), washed in PBS three times for 5 minutes each, re-stained in hematoxylin for 5 minutes, differentiated in hydrochloric acid ethanol solution (Thremo, 7211, Shanghai, China) for 2–5 seconds, washed in water for

15 minutes, then dehydrated, permeabilized and sealed.

Microscopic examination: A camera with a microscope attached recorded pictures of the entire longitudinal section and cross-sectional areas of the nerve (Leica M750, Leica Microsystems, Vienna, Austria). To calculate the average number, three visual fields were randomly selected at the proximal and distal ends using a 40× objective lens to count the number of myelinated fibers marked by toluidine blue staining. The length of total NF200-positive axons was calculated with ImageJ imaging analysis software (version-2.0, National Institutes of Health, Bethesda, MD, USA) in the middle of the specimen, by the length of the three field views (20×).

Statistical analysis

All data are expressed as the mean ± SEM. SPSS 25.0 statistical software (IBM, Armonk, NY, USA) was utilized for statistical analysis. The numerical variables of the four groups were compared using one-way analysis of variance followed by Student-Newman-Keuls *post hoc* test. $P < 0.05$ was considered statistically significant.

Results

Behavioral and functional recoveries

Before treatment, neither the BBB scores nor SFI showed any statistical difference between groups (**Figure 2**). After treatment, both the BBB scores and SFI in the four groups were better than before treatment (all *P*-value = 0.000). Furthermore, the BBB scores and SFI in three treatment groups were improved compared with the control group (all *P*-value = 0.000). However, when compared with the HF-rMS or NTP group, the combined group failed to exhibit any significant improvement in BBB score ($P_{\text{HF-rMS}} = 1.000$, $P_{\text{NTP}} = 0.680$) or SFI ($P_{\text{HF-rMS}} = 0.610$, $P_{\text{NTP}} = 0.530$) (**Figure 2A** and **B**). There was no difference in the BBB score or SFI between the HF-rMS group and the NTP group.

Morphological analysis of grafted nerve

As illustrated in **Figure 3A** and **B**, under a 40× objective lens, the growths of myelinated fibers at the transverse section of the distal and proximal ends in the HF-rMS, NTP and combined groups were improved compared with the control group. Similarly, the elongation of axons on the longitudinal sections in the HF-rMS, NTP and combined groups was improved over that in the control group.

The numbers of myelinated fibers at both the proximal and distal cross-sessions in the HF-rMS, NTP and combined groups were significantly increased compared with the control group (all *P*-value < 0.05, proximal: $P_{\text{HF-rMS}} = 0.009$, P_{NTP} and $P_{\text{Combination}} = 0.000$; distal: $P_{\text{HF-rMS}} = 0.001$, $P_{\text{NTP}} = 0.000$, $P_{\text{Combination}} = 0.002$). However, the numbers of myelinated fibers in the combined group were not significantly different to those in the HF-rMS (proximal: $P = 0.226$; distal: $P = 0.846$) or NTP groups (proximal: $P = 0.967$; distal: $P = 0.336$; **Figure 3B** and **C**).

The total axon lengths in the intermediate sections of the HF-rMS, NTP and combined groups were longer than those in the control group (all *P*-value < 0.05, $P_{\text{HF-rMS}} = 0.000$, $P_{\text{NTP}} = 0.003$, $P_{\text{Combination}} = 0.001$). There was no significant difference in the length of axons between the HF-rMS ($P = 0.140$), NTP ($P = 0.626$; **Figure 3D**) groups and the combined group.

Discussion

In this study, the evaluation of behavioral function pre- and post-treatment revealed that HF-rMS over the injured sciatic nerve, intraperitoneal injection of NTP and the combination of HF-rMS and NTP improved the functional recovery of the injured nerve. Furthermore, our histopathological examination showed that peripheral HF-rMS over the injured sciatic nerve, intraperitoneal injection of NTP and the combined treatment increased the numbers of myelinated fibers and improved the continuity of axons after injury. In addition, HF-rMS was first applied in the treatment of peripheral nerve injury, suggesting a potential new approach for inducing the regeneration of peripheral nerves in a rat sciatic injury model. NTP also had a significant effect on the recovery of injured nerves, but we failed to observe any advantage of combining the two treatments.

It is well known that rTMS changes brain electrical activity by using magnetic coils to stimulate through the skull (Wu et al., 2018). Investigations in the central nervous system suggest that magnetic stimulation improves the release of neurotransmitters and neurotropic factors (nerve growth factor and brain derived neurotrophic factor), enhances synaptic plasticity and increases blood circulation (Banerjee et al., 2017; Soundara et al., 2017), particularly at high-frequency. In this study, we applied high-frequency stimulation based on a previous study of magnetic stimulation (Kumru et al., 2017). rMS stimulates peripheral nerves through the skin and subcutaneous soft tissue and causes contraction of skeletal muscles. This study applied rMS to the peripheral nerve system and although its mechanisms of action are not entirely clear, some studies provide suggestions. For example, Jiang et al. (2016) and Zhivolupov et al. (2012) reported that the application of impulse magnetic stimulation to the spinal cord can improve the microenvironment for nerve growth by reducing inflammation, the degree of edema, demyelination and the numbers of apoptotic cells. Van Soens et al. (2010) also found that rMS shortened muscle-evoked potential latencies and increased amplitudes and effectively contributed to functional recovery of the injured spinal cord in an animal model. Stolting et al. (2016) also assumed that rMS could enhance muscle regeneration through limiting the amount of inflammatory infiltration and the formation of scaring, thus avoiding muscle atrophy post-trauma, and by inducing muscle hypertrophy while increasing muscle turnover and metabolism. There is also evidence that pulse accumulation may promote angiogenesis (Crowe et al., 1997; Ohta et al., 2005) and that rMS could produce positive effects on lymphangiogenesis in rat lower extremities (Lee et al., 2012). According to these studies, we speculated that peripheral HF-rMS can alter the excitability of injured nerves and the plasticity of neuromuscular junctions, and induce the production of re-

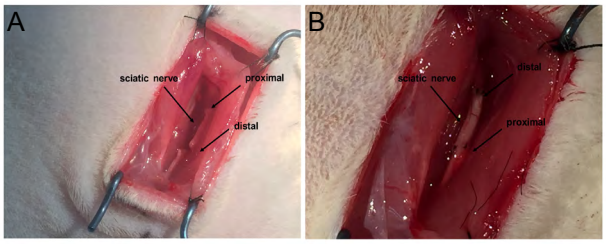


Figure 1 Schematic diagram of sciatic nerve autograft.
The 10 mm-nerve segment was cut (A) and rotated through 180°, and then each end was restored continuously with interrupted sutures (B).

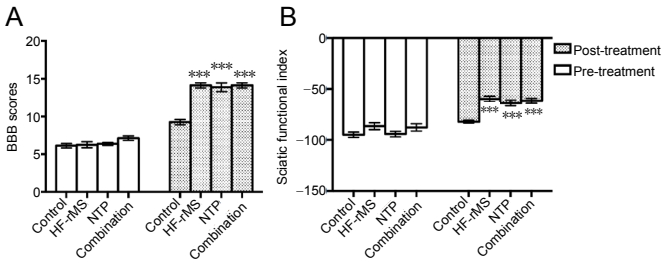


Figure 2 BBB score and sciatic functional index before and after treatment in each group.
(A) BBB scores before and after the treatment: The higher the score, the better the behavioral recovery. (B) Sciatic functional index before and after the treatment: The smaller the negative value, the better recovery of motor functions. ***P < 0.001, *vs.* control group. Data are expressed as the mean ± SEM (n = 8; one-way analysis of variance followed by Student-Newman-Keuls *post hoc* test). BBB: Basso-Beattie-Bresnahan scale; HF-rMS: high-frequency repetitive magnetic stimulation; NTP: neurotropin.

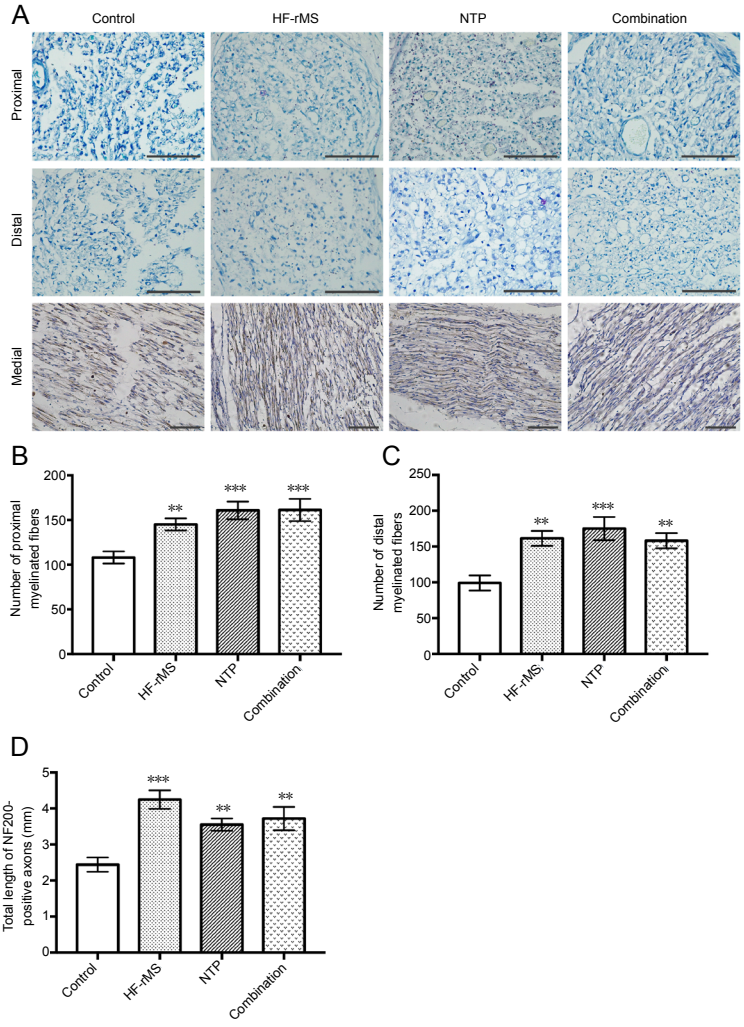


Figure 3 Effects of HF-rMS and NTP on morphological analysis of regenerated nerve.
(A) Representative images of the regenerated nerve: proximal: the myelinated fibers stained by toluidine blue in the transverse section of proximal end (toluidine blue staining); distal: the myelinated fibers stained by toluidine blue in the transverse section of distal end (toluidine blue staining); medial: the axons stained by NF200 in the longitudinal of intermediate regime (immunohistochemical staining with NF200). Scale bars: 100 μm. (B) The number of proximal myelinated fibers in the four groups. (C) The number of distal myelinated fibers in the four groups. (D) The total length of NF200-positive axons in the four groups; **P < 0.01, ***P < 0.001, *vs.* control group. Data are expressed as the mean ± SEM (n = 8; one-way analysis of variance followed by Student-Newman-Keuls *post hoc* test). HF-rMS: High-frequency repetitive magnetic stimulation; NTP: neurotropin.

lated neurotrophic factors and the regeneration of axons by direct stimulation of the injured nerve. Unlike traditional electrical stimulation, rMS appears to be more extensive. Simultaneous stimulation of other tissues (e.g. muscle, blood vessels, lymphatic vessels and soft tissue) can also promote the functional recovery of affected limbs. Additionally, rMS may increase the excitability of spinal reflexes and spinal reflexes in distal muscles by activating peripheral nerve fibers, and increase corticospinal excitability.

Isolated distal axons undergo Wallerian degeneration following peripheral nerve injury. Schwann cells distal to the injury location proliferate, dedifferentiate, and line the en-

doneurial tubes, guiding regeneration axons until re-differentiation to myelination once the axons are guided towards the target (Gaudet et al., 2011). Previous studies showed that NTP can promote neurite outgrowth in PC12 cells (Morita et al., 1988; Fukuda et al., 2015) and ameliorate demyelination in a chronic constriction injury model (Bryden et al., 2016). Furthermore, there is evidence that NTP can accelerate the differentiation of Schwann cells in both the myelinating and promyelinating states to treat peripheral nerve injury and demyelinating diseases (Matsuoka et al., 2018). Our study was an extension of previous studies that showed that NTP can be used to treat severe nerve injury.

Unfortunately, there were no differences between the combined treatment and the individual treatments, suggesting no additive or synergistic effects of the combined therapy. The following reasons might explain this. First, the treatment time may have been too short to observe any combined effects (Mohammadi et al., 2014; Mashhoudi et al., 2017). The regenerating fibers have to reach the peripheral targets first, re-innervating them to cause functional re-innervation. This process takes a relatively long time (Faroni et al., 2015). Second, two treatments were presented at the same time in our study. Thus we do not know whether a combined effect would be observed by presenting the two treatments at an appropriate interval. Third, the parameters of HR-rMS (such as intensity and frequency) and the dose of NTP might be further optimized. Fourth, the sample size in this study might have been too small to observe the significance. Notably, electrophysiological and biochemical analyses were not included in this study to support our observations, and require further investigation.

In summary, either peripherally administered HF-rMS or NTP may serve as new treatments for inducing peripheral nerve regeneration in a rat sciatic injury model, but the combination of the two treatments provides no further advantage.

Author contributions: *Study conception and design: XJZ, RBS, JC; experimental implementation: RBS, JC; paper writing: JC; manuscript reviewing and editing: XJZ, JC. All authors approved the final version of the paper.*
Conflicts of interest: *The authors declare that there are no conflicts of interest associated with this manuscript.*
Financial support: *This work was funded by the National Natural Science Foundation of China, No. 81471338 and No. 81671284 (to XJZ); the Changzhou Science & Technology Program of China, No. CE20145045 (to XJZ); the Changzhou High-Level Medical Talents Training Project of China, No. 2016CZLJ018 (to XJZ). The funding sources had no role in study conception and design, data analysis or interpretation, paper writing or deciding to submit this paper for publication.*
Institutional review board statement: *This study was approved by the Animal Ethics Committee of the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, China on December 23, 2014 (approval No. 2014keyan002-01). The experimental procedure followed the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).*
Copyright license agreement: *The Copyright License Agreement has been signed by all authors before publication.*
Data sharing statement: *Datasets analyzed during the current study are available from the corresponding author on reasonable request.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewer: *Ozgur Boyraz, Gulhane Military Medical Academy, Turkey.*
Additional file: *Open peer review report 1.*

References

Bain JR, Mackinnon SE, Hunter DA (1989) Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 83:129-138.

Banerjee J, Sorrell ME, Celnik PA, Pelled G (2017) Immediate effects of repetitive magnetic stimulation on single cortical pyramidal neurons. PLoS One 12:e0170528.

Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12:1-21.

Bervar M (2000) Video analysis of standing-an alternative footprint analysis to assess functional loss following injury to the rat sciatic nerve. J Neurosci Methods 102:109-116.

Bryden AM, Hoyen HA, Keith MW, Mejia M, Kilgore KL, Nemunaitis GA (2016) Upper extremity assessment in tetraplegia: the importance of differentiating between upper and lower motor neuron paralysis. Arch Phys Med Rehabil 97:S97-104.

Carvalho CR, Wrobel S, Meyer C, Brandenberger C, Cengiz IF, Lopez-Cebral R, Haastert-Talini K (2018) Gellan Gum-based luminal fillers for peripheral nerve regeneration: an in vivo study in the rat sciatic nerve repair model. Biomater Sci 6:1059-1075.

Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS (1997) Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nat Med 3:73-76.

Faroni A, Mobasseri SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: experimental strategies and future perspectives. Adv Drug Deliv Rev 82-83:160-167.

Fukuda Y, Fukui T, Hikichi C, Ishikawa T, Murate K, Adachi T, Mutoh T (2015) Neurotropin promotes NGF signaling through interaction of GM1 ganglioside with Trk neurotrophin receptor in PC12 cells. Brain Res 1596:13-21.

Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. J Neuroinflammation 8:110.

George MS, Lisanby SH, Avery D, McDonald WM, Durkalski V, Pavlicova M, Sackeim HA (2010) Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. Arch Gen Psychiatry 67:507-516.

Geuna S (2015) The sciatic nerve injury model in pre-clinical research. J Neurosci Methods 243:39-46.

Gordon T, English AW (2016) Strategies to promote peripheral nerve regeneration: electrical stimulation and/or exercise. Eur J Neurosci 43:336-350.

Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. Biomed Res Int 2014:698256.

Hausner T, Pajer K, Halat G, Hopf R, Schmidhammer R, Redl H, Nogradi A (2012) Improved rate of peripheral nerve regeneration induced by extracorporeal shock wave treatment in the rat. Exp Neurol 236:363-370.

Hosomi K, Shimokawa T, Ikoma K, Nakamura Y, Sugiyama K, Ugawa Y, Saitoh Y (2013) Daily repetitive transcranial magnetic stimulation of primary motor cortex for neuropathic pain: a randomized, multicenter, double-blind, crossover, sham-controlled trial. Pain 154:1065-1072.

Huang J, Zhang Y, Lu L, Hu X, Luo Z (2013) Electrical stimulation accelerates nerve regeneration and functional recovery in delayed peripheral nerve injury in rats. Eur J Neurosci 38:3691-3701.

Ikumi A, Hara Y, Yoshioka T, Kanamori A, Yamazaki M (2018) Effect of local administration of platelet-rich plasma (PRP) on peripheral nerve regeneration: an experimental study in the rabbit model. Microsurgery 38:300-309.

Jiang B, Zhang Y, She C, Zhao J, Zhou K, Zuo Z, Dong Q (2017a) X-ray irradiation has positive effects for the recovery of peripheral nerve injury maybe through the vascular smooth muscle contraction signaling pathway. Environ Toxicol Pharmacol 54:177-183.

Jiang B, Zhang Y, Zhao J, She C, Zhou X, Dong Q, Wang P (2017b) Effects of localized X-ray irradiation on peripheral nerve regeneration in transected sciatic nerve in rats. Radiat Res 188:455-462.

Jiang JL, Guo XD, Zhang SQ, Wang XG, Wu SF (2016) Repetitive magnetic stimulation affects the microenvironment of nerve regeneration and evoked potentials after spinal cord injury. Neural Regen Res 11:816-822.

Kawai H, Asaoka N, Miyake T, Nagayasu K, Nakagawa T, Shirakawa H, Kaneko S (2018) Neurotropin inhibits neuronal activity through potentiation of sustained Kv currents in primary cultured DRG neurons. J Pharmacol Sci 137:313-316.

Kudo T, Kushikata T, Kudo M, Kudo T, Hirota K (2011) Antinociceptive effects of neurotropin in a rat model of central neuropathic pain: DSP-4 induced noradrenergic lesion. Neurosci Lett 503:20-22.

Kumru H, Albu S, Rothwell J, Leon D, Flores C, Opisso E, Valls-Sole J (2017) Modulation of motor cortex excitability by paired peripheral and transcranial magnetic stimulation. Clin Neurophysiol 128:2043-2047.

Lee D, Beom J, Oh BM, Seo KS (2012) Effect of magnetic stimulation in spinal cord on limb angiogenesis and implication: a pilot study. Ann Rehabil Med 36:311-319.

Lefaucheur JP, Andre-Obadia N, Antal A, Ayache SS, Baeken C, Benninger DH, Garcia-Larrea L (2014) Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS) Clin Neurophysiol 125:2150-2206.

Malin D, Sonnenberg-Riethmacher E, Guseva D, Wagener R, Aszodi A, Irintchev A, Riethmacher D (2009) The extracellular-matrix protein matrilin 2 participates in peripheral nerve regeneration. J Cell Sci 122:995-1004.

Mashhoudi Barez M, Tajziehchi M, Heidari MH, Bushehri A, Moayer F, Mansouri N, Movafagh A (2017) Stimulation effect of low level laser therapy on sciatic nerve regeneration in rat. J Lasers Med Sci 8:S32-S37.

Masuguchi K, Watanabe H, Kawashiri T, Ushio S, Ozawa N, Morita H, Egashira N (2014) Neurotropin(R) relieves oxaliplatin-induced neuropathy via Gi protein-coupled receptors in the monoaminergic descending pain inhibitory system. Life Sci 98:49-54.

Matsuoka H, Tanaka H, Sayanagi J, Iwahashi T, Suzuki K, Nishimoto S, Yoshikawa H (2018) Neurotropin((R)) accelerates the differentiation of schwann cells and remyelination in a rat lysophosphatidylcholine-induced demyelination model. Int J Mol Sci doi:10.3390/ijms19020516.

Mohammadi R, Saadati A (2014) Influence of insulin-like growth factor I on nerve regeneration using allografts: a sciatic nerve model. J Craniofac Surg 25:1510-1514.

Morita S, Takeoka Y, Imai H, Yamamoto H, Suehiro S, Ueda S, Katoh S (1988) Differential action of nerve growth factor, cyclic AMP and neurotropin on PC12h cells. Cell Struct Funct 13:227-234.

Ohta S, Iwashita Y, Takada H, Kuno S, Nakamura T (2005) Neuroprotection and enhanced recovery with edaravone after acute spinal cord injury in rats. Spine (Phila Pa 1976) 30:1154-1158.

Okazaki R, Namba H, Yoshida H, Okai H, Taguchi K, Kawamura M (2013) Combined antiallodynic effect of neurotropin(R) and pregabalin in rats with L5-spinal nerve ligation. Life Sci 92:259-265.

Paradiso B, Simonato M, Thiene G, Lavezzi A (2018) From fix to fit into the autoptic human brains. Eur J Histochem doi:10.4081/ejh.2018.2944.

Prikryl R, Ustohal L, Prikrylova Kucerova H, Kasparek T, Venclikova S, Vrzalova M, Ceskova E (2013) A detailed analysis of the effect of repetitive transcranial magnetic stimulation on negative symptoms of schizophrenia: a double-blind trial. Schizophr Res 149:167-173.

Quan Q, Chang B, Meng HY, Liu RX, Wang Y, Lu SB, Zhao Q (2016) Use of electrospinning to construct biomaterials for peripheral nerve regeneration. Rev Neurosci 27:761-768.

Soundara Rajan T, Ghilardi MFM, Wang HY, Mazzon E, Bramanti P, Restivo D, Quartarone A (2017) Mechanism of action for rTMS: a working hypothesis based on animal studies. Front Physiol 8:457.

Stolting MN, Arnold AS, Haralampieva D, Handschin C, Sulser T, Eberli D (2016) Magnetic stimulation supports muscle and nerve regeneration after trauma in mice. Muscle Nerve 53:598-607.

Takahashi HK, Iwagaki H, Tamura R, Yagi T, Yoshino T, Mori S, Nishibori M (2005) Effect of antibodies against intercellular adhesion molecule-1, B7, and CD40 on interleukin-18-treated human mixed lymphocyte reaction. J Pharmacol Sci 97:447-450.

Thut G, Pascual-Leone A (2010) A review of combined TMS-EEG studies to characterize lasting effects of repetitive TMS and assess their usefulness in cognitive and clinical neuroscience. Brain Topogr 22:219-232.

Van Soens I, Struys MM, Van Ham LM (2010) Muscle potentials evoked by magnetic stimulation of the sciatic nerve in unilateral sciatic nerve dysfunction. J Small Anim Pract 51:275-279.

Varejao AS, Meek MF, Ferreira AJ, Patricio JA, Cabrita AM (2001) Functional evaluation of peripheral nerve regeneration in the rat: walking track analysis. J Neurosci Methods 108:1-9.

Wu CW, Chiu WT, Hsieh TH, Hsieh CH, Chen JJ (2018) Modulation of motor excitability by cortical optogenetic theta burst stimulation. PLoS One 13:e0203333.

Zhang LQ, Xu CG, Li ZY, Yao F, Zha XW, Qi L, Jing YH (2019) Low-frequency pulsed electromagnetic field promotes neurologic function recovery after delayed repair of perioheral nerve injury in rats. Zhongguo Zuzhi Gongcheng Yanjiu 23:1711-1716.

Zhang XQ, Li L, Huo JT, Cheng M, Li LH (2018) Effects of repetitive transcranial magnetic stimulation on cognitive function and cholinergic activity in the rat hippocampus after vascular dementia. Neural Regen Res 13:1384-1389.

Zhang Z, Bu X, Chen H, Wang Q, Sha W (2016) Bmi-1 promotes the invasion and migration of colon cancer stem cells through the downregulation of E-cadherin. Int J Mol Med 38:1199-1207.

Zhivolupov SA, Odinak MM, Rashidov NA, Onischenko LS, Samartsev IN, Jurin AA (2012) Impulse magnetic stimulation facilitates synaptic regeneration in rats following sciatic nerve injury. Neural Regen Res 7:1299-1303.

Zhou XB, Zou DX, Gu W, Wang D, Feng JS, Wang JY, Zhou JL (2018) An experimental study on repeated brief ischemia in promoting sciatic nerve repair and regeneration in rats. World Neurosurg 114:e11-21.

P-Reviewer: Boyraz O; C-Editor: Zhao M; S-Editors: Wang J, Li CH; L-Editors: Brooks W, Robens J, Qiu Y, Song LP; T-Editor: Jia Y