Swimming Training Reduces Neuroma Pain by Regulating Neurotrophins

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ABSTRACT

TIAN, J., X. ZHANG, T. YU, Y. XU, S. PU, Y. LV, and D. DU. Swimming Training Reduces Neuroma Pain by Regulating Neurotrophins. *Med. Sci. Sports Exerc.*, Vol. 50, No. 1, pp. 54–61, 2018. **Introduction**: Neuroma formation after peripheral nerve transection leads to severe neuropathic pain in amputees. Previous studies suggested that physical exercise could bring beneficial effect on alleviating neuropathic pain. However, the effect of exercise on neuroma pain still remained unclear. In addition, long-term exercise can affect the expression of neurotrophins (NT), such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which play key roles in nociceptor sensitization and nerve sprouting after nerve injury. Here, we investigated whether long-term swimming exercise could relieve neuroma pain by modulating NT expression. **Methods**: We used a tibial neuroma transposition (TNT) rat model to mimic neuroma pain. After TNT surgery, rats performed swimming exercise for 5 wk. Neuroma pain and tactile sensitivities were detected using von Frey filaments. Immunofluorescence was applied to analyze neuroma formation. NGF and BDNF expressions in peripheral neuroma, dorsal root ganglion, and the spinal cord were measured using enzyme-linked immunosorbent assay and Western blotting. **Results**: TNT led to neuroma formation, induced neuroma pain, and mechanical allodynia in hind paw. Five-week swimming exercise inhibited neuroma formation and relieved mechanical allodynia in the hind paw and neuroma pain in the lateral ankle. The analgesic effect lasted for at least 1 wk, even when the exercise ceased. TNT elevated the expressions of BDNF and NGF in peripheral neuroma, dorsal root ganglion, and the spinal cord to different extents. Swimming also decreased the elevation of NT expression. **Conclusions**: Swimming exercise not only inhibits neuroma formation induced by nerve transection but also relieves pain behavior. These effects might be associated with the modulation of NT. **Key Words**: EXERCISE, NEUROPATHIC PAIN, NEUROMA, NGF, BDNF

The increasing incidence of trauma, peripheral vascular/nerve disease, and limb tumors has led to an increase in limb amputation. Postamputation pain is a common complication and has received widespread attention in clinical and basic research. Phantom limb pain occurs in 50%–80% of amputees, and approximately 61% of amputees report residual limb pain (1–3). Although the underlying mechanism is not fully understood, previous studies suggested that terminal neuromas might play an important role in phantom limb pain and residual limb pain (4).

Terminal neuromas usually form at the end of transected nerves. After injury, a transected nerve attempts to regenerate to promote functional recovery. However, abnormal regeneration caused by scar tissue, large gaps, or absence of distal dominative limbs often twines axons into a bulbous neuroma. Neuroma consists of an unorganized network of connective tissue intermingled with spouting fibers, Schwann cells, macrophages, and fibroblasts. Several mechanisms may be involved in the process of neuroma-induced pain, including ectopic discharge of axons, altered sodium iron channels, transient receptor potential cation channel subfamily A1, and nerve growth factor (NGF) (4,5). However, the exact mechanisms are not fully elucidated.

Neuroma pain always resists to traditional treatment, severely reduces life quality, and presents a heavy financial burden to society. Physical activity is becoming an important part of rehabilitative treatments for patients suffering from acute or chronic pain. The advantage of physical activity is its effectiveness and fewer side effects. Exercise alleviates various types of chronic pain, including pain after spinal cord

contusion, lower back pain, diabetic neuropathy, chronic musculoskeletal pain, complex regional pain syndrome type I, and noninflammatory chronic muscle pain (6–8). Rodent models have been used to investigate the analgesic effects of exercise. For example, swimming therapy reduced hyperalgesia after chronic constriction injury of the sciatic nerve (9), streptozotocin-induced diabetic neuropathy (10), and formalin-induced inflammatory pain in rats and mice (11). Running on an exercise wheel also alleviated neuropathic pain caused by prediabetes in mice (12). However, the effect of exercise on neuroma pain has not been investigated.

Neurotrophins (NT) are not only important mediators of neuronal survival and growth but also responsible for the transmission of neuropathic pain (13–15). Almeida et al. (16) reported that exercise alleviated hyperalgesia after partial nerve injury by reducing NGF and brain-derived neurotrophic factor (BDNF) expressions in dorsal root ganglion (DRG). However, the role of NT in neuroma pain remains unknown.

In this study, we used a tibial neuroma transposition (TNT) model (including neuroma pain and mechanical allodynia) to investigate the effect of swimming therapy on pain relief and neuroma formation. In addition, we measured the expression of NT and tried to address whether NT plays key roles in neuroma pain modulation.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (200–250 g) were purchased from the Shanghai Laboratory Animal Center at Chinese Academy of Science (Shanghai, China). All experiments were approved by the Animal Care and Use Committee of Shanghai Sixth People's Hospital affiliated to Shanghai Jiaotong University (SYXK (Shanghai, China) 2011–0128). This study was conducted according to National Institutes of Health guidelines for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80–23, revised 1996). Animals were housed in cages, allowed free access to food and water, and maintained on a 12/12 h light/dark cycle (lights on 8:00 AM–8:00 PM).

TNT Model

For surgery, rats were deeply anesthetized with pentobarbital intraperitoneally (50 mg·kg^{-1}). TNT was performed according to the method described by Dorsi et al. (17). The tibial nerve of the right limb was exposed and ligated at the proximity of the plantar bifurcation with a 6-0 silk suture. The nerve was then transected at the distant side of the suture with scissors. The transected nerve terminal was pulled toward the lateral malleolus through a subcutaneous tunnel, and the nerve terminal was just embedded beneath the skin. The nerve stump was fixed 10 mm superior to the lateral malleolus. After surgery, a subcutaneous terminal neuroma formed at the lateral malleolus area. The suture material was visible and served as a marker for mechanical stimuli, which induced neuroma pain. In addition, stimulating the denervated hind paw plantar could evoke nerve injury–induced alloynia. In the sham group, the tibial nerve was dissected and kept intact. A small piece of connective tissue was ligated and passed through the subcutaneous tunnel according to the method described previously.

Swimming Exercise Protocol and Experimental Design

The swimming pool was a 70 × 110 × 60-cm glass container filled with warm water (30°C ± 2°C) and divided into seven lanes. Animals were monitored for floating behavior or distress during the whole swimming exercise. If animals began floating, they were encouraged to swim by stirring the water with a stick. Tired rats that appeared distressed were allowed to take a 5-min rest until finishing the whole exercise. After each swimming session, rats were gently dried with a cotton cloth. In the first week, rats performed 10-min swimming exercise at the first day, then swimming period was gradually increased until animals could swim continuously for 60 min. In the following 4 wk, rats swam daily for 5 d followed by 2-d rest (Fig. 1).
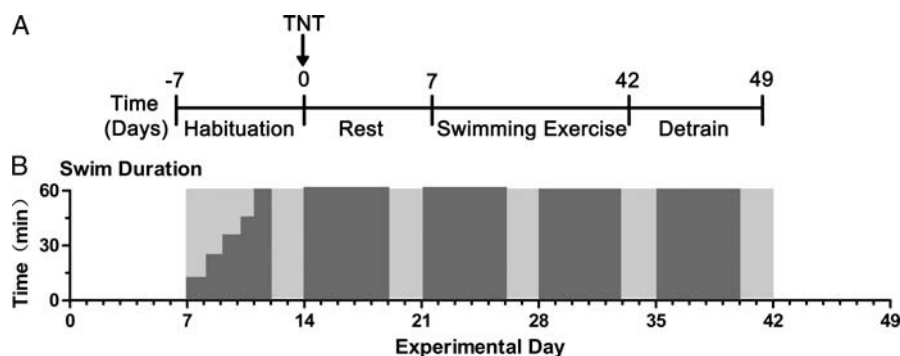
FIGURE 1—Experimental design and swimming protocols. A, Flow diagram showing experimental design of swimming exercise. Rats in the no-swim group were kept in a sedentary environment during swimming sessions. B, Time course of swimming exercise. The *dark gray area* indicates swimming exercise, and *light gray area* means intermittent period among the swimming section. To assess the effects of long-term exercise on pain, some rats were submitted to detrain and kept in a sedentary condition for additional 1 wk.

First, we investigated whether swimming exercise could reduce pain behavior and inhibit neuroma formation in rats. Animals were randomly divided into four groups: group 1, sham operation without swimming ($n = 6$); group 2, sham operation with swimming ($n = 6$); group 3, TNT without swimming ($n = 6$); and group 4, TNT with swimming ($n = 6$). A 7-d habituation period was performed before the operation. One week after surgery, rats began a 5-wk swimming exercise. After the swimming session, rats were detrained for additional 1 wk. Nerve tissues were collected after detraining. During the observation period, pain behaviors were measured on days 0, 3, 5, 7, 14, 21, 28, 35, 42, and 49. The experimenter was blinded to the treatment groups.

Next, we investigated whether swimming affected the expressions of NGF and BDNF in the spinal cord, DRG, and peripheral neuroma of rats after TNT. Forty-eight rats were separated into three groups: Group 1, control ($n = 6$); group 2, 24 no-swimming TNT rats (tissue were collected on days 7, 21, 42, and 49; $n = 6$); group 3, 18 swimming TNT rats (tissue were collected on days 21, 42, and 49; $n = 6$).

Pain Behavior Test

Assessment of neuroma pain. To assess neuroma pain, the neuroma was stimulated with a 15-g von Frey filament on the lateral malleolus area of the hind limb 10 times. Each time, a von Frey hair was applied to probe the area for 2–3 s, with a 1- to 2-min interval. A positive response was defined as slow withdrawal of the hind paw, or a rapid withdrawal with vocalization, licking, or shaking. The behavioral response frequency was defined as the percentage of positive trials. A grading system was used to qualitatively evaluate neuroma pain on the basis of the animal's response. A grade of 0 indicated that the animal did not respond to the stimuli. A grade 1 response presented as a slow withdrawal of the hind paw, and a grade 2 response was defined as a quick withdrawal with shaking, licking, or vocalization. The behavioral response score was defined as the sum of response grades for 10 trials, ranging from 0 to 20. An examiner who was blinded to treatments performed all the behavioral tests.

Assessment of nerve injury–induced pain. To evaluate the behavioral response to mechanical stimuli on hind paw plantar, we measured the 50% paw withdrawal threshold (PWT) using the up–down method described by Chaplan et al. (18). Animals were placed into transparent plexiglass cages with a wire mesh floor. After 30-min adaptation, the von Frey filaments (0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, and 15.0 g; Stoelting, Wood Dale, IL) were applied to the lateral plantar surface in an up-and-down order. Each filament was applied to the foot until it began to bend. A positive reaction was defined as a quick paw withdrawal or hind paw licking.

Nerve histology analyses. Rats were deeply anesthetized with pentobarbital and transcardially perfused with normal saline followed by ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS) buffer (pH 7.4). After perfusion, neuromas or tibial nerves were rapidly removed (at least 0.5 cm) and fixed in 4% paraformaldehyde for 24 h for immunohistochemistry staining. To analyze regeneration of the neuroma after swimming exercise, longitudinal sections of injury tibial nerve samples were acquired on day 49. The nerve segments were kept straight and cut in a cryostat. Then, sections were incubated in 3% goat serum in 0.01 M PBS (pH 7.4) for 1 h at room temperature. After blocking, sections were labeled with an anti–neurofilament-200 primary antibody (1/1000, chicken monoclonal IgG; Abcam, Cambridge, MA) overnight 4°C. Sections were washed with PBS and incubated in appropriate secondary antibody (goat antichicken IgG Alexa Fluor 488; 1/500; Abcam) for 1 h at room temperature. Then, sections were washed with PBS and coverslipped with Aqua Poly mount (Polysciences, Warrington, PA). Images were acquired using a light microscope (Sclipse 80i; Nikon, Tokyo, Japan). The maximal diameter of the nerves or neuromas was measured using Photoshop software (Adobe systems Inc., San Jose, CA).

Enzyme-linked immunosorbent assay. Neuromas and ipsilateral L4–L5 DRG samples were harvested and cut into pieces using ophthalmic scissors and homogenized by sonication. Homogenates were centrifuged at 13,000 rpm for 10 min, and the supernatants were collected for further protein analysis. NT concentration was determined using enzyme-linked immunosorbent assay (ELISA; Boster Biotechnology Corporation, Wuhan, China) following the recommended protocols provided by the manufacturers. Total protein content of each sample was measured using a BCA Protein Assay Kit (Pierce Thermo Fisher Scientific Inc, Rockford, IL). NGF and BDNF concentrations were standardized to the total amount of protein in each sample.

Western blotting. The L4–L6 spinal cords ipsilateral to the injury site were cut into pieces using scissors and homogenized in tissue protein extraction buffer (Pierce Thermo Fisher Scientific Inc) containing protease inhibitor cocktail (Complete, Roche Diagnostics Ltd, Switzerland). A total of 60 μL protein was separated by electrophoresis on a 12.5% sodium dodecyl sulfate–polyacrylamide gel then transferred to polyvinylidene difluoride membranes (Merck Millipore Company, Billerica, MA). The membranes were blocked with 5% bovine serum albumin (Sigma-Aldrich Inc, St Louis, MO) in Tris-buffered saline and 0.1% Tween 20 (TBS-T) for 2 h at room temperature and then incubated with anti-NGF (1/700, rabbit monoclonal IgG; Abcam), anti-BDNF (1/700, rabbit monoclonal IgG; Abcam), and anti–α-tubulin (1/2000, mouse monoclonal IgG; HuaAn Biotechnology, Hangzhou, China) antibodies overnight at 4°C. After TBS-T washing, the blots were incubated with horseradish peroxidase–conjugated goat antirabbit or goat antimouse secondary antibody (1/5000; HuaAn Biotechnology) for 2 h at room temperature. Membranes were washed three times in TBS-T, and labeled protein bands were detected using Image Quant Software (ImageQuant LAS 4000 mini; GE Healthcare, Chicago, IL) using enhanced chemiluminescence substrate solution (Merck Millipore Company,

Billerica, MA). The relative levels of NGF and BDNF were normalized to α-tubulin, which were presented as NGF/α-tubulin and BDNF/α-tubulin.

Statistical Analysis

To analyze PWTs, behavioral response frequency, and scores in different groups at respective time points, a two-way ANOVA followed by Bonferroni *post hoc* test was used. To analyze the neuroma diameter, Western blot data, and ELISA data between different groups, one-way ANOVA followed by a *post hoc* Bonferroni test for multiple comparisons was conducted. All data are presented as mean ± SEM. In each case, statistical significance was considered as a P value < 0.05. All analyses were performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA).

RESULTS

Swimming exercise attenuated neuroma pain at the lateral malleolus and nerve injury–induced pain at the hind paw plantar after TNT surgery. First, we examined changes of pain behavior after TNT and whether swimming exercise could relieve pain behavior. After TNT, rats exhibited obvious pain behavior at the lateral malleolus (neuroma pain) and hind paw plantar (nerve injury–induced pain). The pain response to mechanical stimuli on the neuroma area increased gradually and maintained at the high level during the observation period (days 7–49, $P < 0.01$; Fig. 2A, B). Similarly, 50% PWT after mechanical stimuli on the hind paw plantar decreased significantly after TNT and maintained at low level for 6 wk (day 3–49, $P < 0.01$; Fig. 2C). After swimming exercise, the TNT-induced neuroma pain behaviors were attenuated significantly, and the effect lasted until day 49, 1 wk after swimming (day 35, $P < 0.05$; days 42–49,

$P < 0.01$; Fig. 2A, B). Interestingly, swimming also increased the 50% PWT in the denervated hind paw plantar area in TNT rats (days 21–42, $P < 0.01$), and this effect also lasted until day 49 (day 49, $P < 0.01$; Fig. 2C).
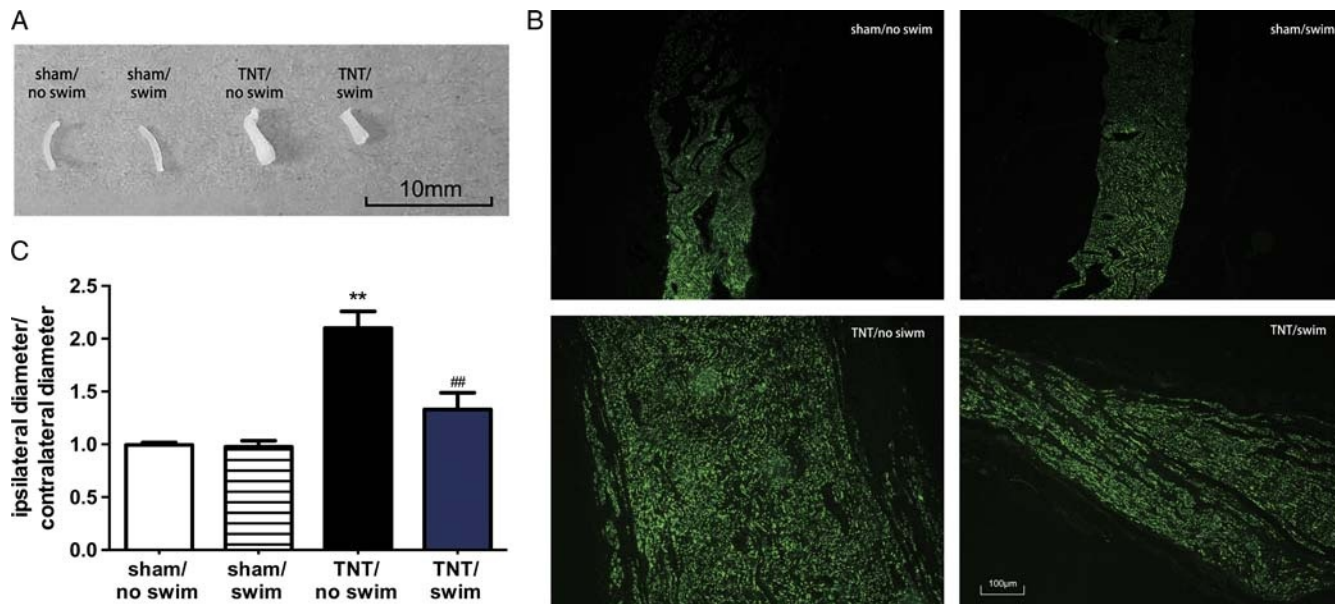
Swimming exercise inhibited neuroma formation. Next, from these peripheral neuromas, we found that neuromas in TNT/no-swim rats were obviously larger than those in TNT/swim rats (Fig. 3A). This observation was confirmed by immunofluorescence labeled by neurofilament-200, a marker of myelinated nerve fibers (Fig. 3B). The maximal neuroma diameter in TNT/swim rats was smaller than those in TNT/no-swim rats. However, the diameter of the tibial nerve did not change after the 5-wk swimming session in the sham group. As shown in Figure 3C, we measured the ratio of the ipsilateral neuroma diameter to contralateral nerve diameter from different groups. The ratios in the TNT/no-swim group were significantly higher than those in the sham/no-swim group ($P < 0.01$). Excitedly, the ratios in the TNT/swim group were lower than those in the TNT/no-swim group ($P < 0.01$).

Swimming exercise reduced TNT-induced increase of NT expression in ipsilateral neuroma, DRG, and the spinal cord. NT, NGF, and BDNF regulate neuronal survival, neuronal growth, and neuroma formation (5). After getting these striking behavioral and histological results, we investigated how NT expression changed in TNT rats and whether swimming exercise could influence NT expression. We examined protein expression in the spinal cord, DRG, and peripheral neuroma at different time points (days 7, 21, 42, and 49).

We measured NGF and BDNF protein levels in the ipsilateral spinal cord by using Western blotting. As shown in Figure 4, TNT increased NGF and BDNF protein levels on days 7, 21, 42, and 49 (NGF: days 7–49, $P < 0.01$; BDNF: days 7–49, $P < 0.01$; compared with the control). Swimming attenuated the TNF-induced increase of BDNF and NGF;
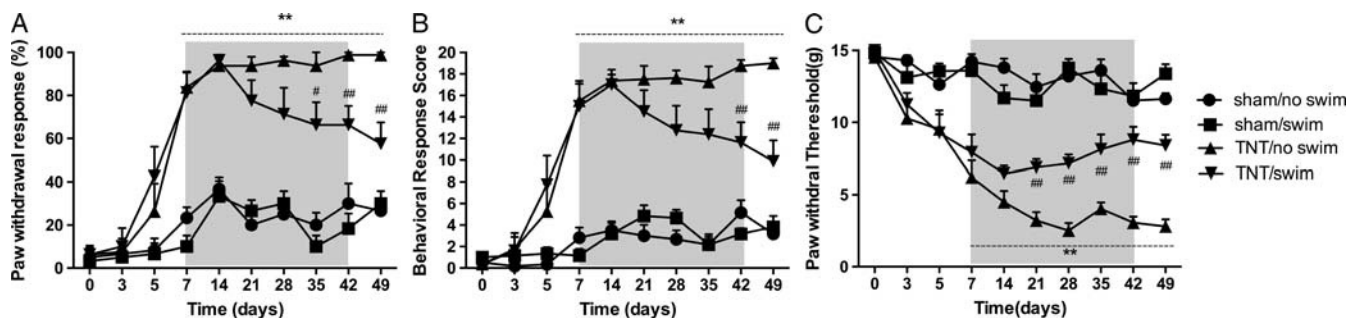
FIGURE 2—Swimming exercise relieved pain behavioral in response to mechanical stimuli on the lateral malleolus area and increased mechanical allodynia after mechanical stimuli on the paw plantar area. A, The paw withdrawal frequency in response to mechanical stimuli on the lateral malleolus area. After TNT, the paw withdrawal frequency increased gradually and reached a peak at about day 7, then maintained at the high level during the observation period. After swimming, the paw withdrawal frequency of TNT rats decreased gradually, and this effect lasted until 1 wk after swimming exercise ($n = 6$; **$P < 0.01$, TNT/no-swim group vs sham/no-swim group; #$P < 0.05$, ##$P < 0.01$, TNT/swim group vs TNT/no-swim group). B, The pain behavioral score in response to mechanical stimuli on the lateral malleolus area. After TNT, the behavior response score increased gradually, reaching a peak at about day 7, then maintained at the high level during the observation period. Five-week swimming exercise reduced the behavior response score of TNT rats, and this effect lasted until 1 wk after swimming exercise ($n = 6$; **$P < 0.01$, TNT/no-swim group vs sham/no-swim group; ##$P < 0.01$, TNT/swim group vs TNT/no-swim group). C, The hind PWT after mechanical stimuli on the paw plantar. TNT reduced the PWT gradually. Swimming elevated the TNT-induced decrease of PWT, and this effect lasted until 1 wk after swimming ($n = 6$; **$P < 0.01$, TNT/no-swim group vs sham/no-swim group; ##$P < 0.01$, TNT/swim group vs TNT/no-swim group). Swimming had no effect on pain behavior in sham rats ($P > 0.05$). Data were represented as the means ± SEM.

FIGURE 3—Swimming exercise inhibited neuroma formation after TNT. A, Representative tibial nerve terminals obtained from different groups on day 49. Neuroma in the TNT/swim group was obviously smaller than that in the TNT/no-swim group. B, Neurofilament-200 immunofluorescence of terminal tibial nerve sections (scale bar, 100 μm). After TNT, neuromas formed at the end of the transected tibial nerve. Neuromas in the TNT/no-swim group were obviously smaller than those in the TNT/swim group. C, Ratio of ipsilateral to contralateral diameter of nerves or neuromas. The ratio increased significantly after TNT surgery, and swimming exercise inhibited TNT-induced neuroma growth (n = 6; **P < 0.01, TNT/no-swim group vs sham/no-swim group; ##P < 0.01, TNT/swim group vs TNT/no-swim group). Data were presented as means ± SEM.

this effect lasted until day 49, 1 wk after exercise (NGF: days 42, P < 0.01; days 49, P < 0.05; BDNF: days 42 and 49, P < 0.01; compared with the TNT/no-swim group).

We used ELISA to measure NGF and BDNF protein expression in ipsilateral DRG (L4–L5) and neuroma. In ipsilateral DRG, NGF expression increased on day 21 (P < 0.01, compared with the control) and BDNF increased on day 7 (P < 0.01, compared with the control) after TNT surgery. Swimming exercise attenuated the TNT-induced increase of NT expression (NGF: day 21, P < 0.01; BDNF: day 21, P < 0.01;

compared with the TNT/no-swim rats) (Fig. 5A, B). Consistently, in ipsilateral neuroma, TNT induced the increase of NGF and BDNF protein expression (NGF: days 7 and 21, P < 0.01; BDNF: day 7, P < 0.01; compared with the control; Fig. 5C, D). TNT-induced elevation of NGF and BDNF expression was significantly reduced after swimming exercise (NGF: day 21, P < 0.05; BDNF: day 21, P < 0.01; compared with the TNT/no-swim group). However, on days 42 and 49, NGF and BDNF expressions in TNT rats were not different from those in the controls. In addition, swimming exercise
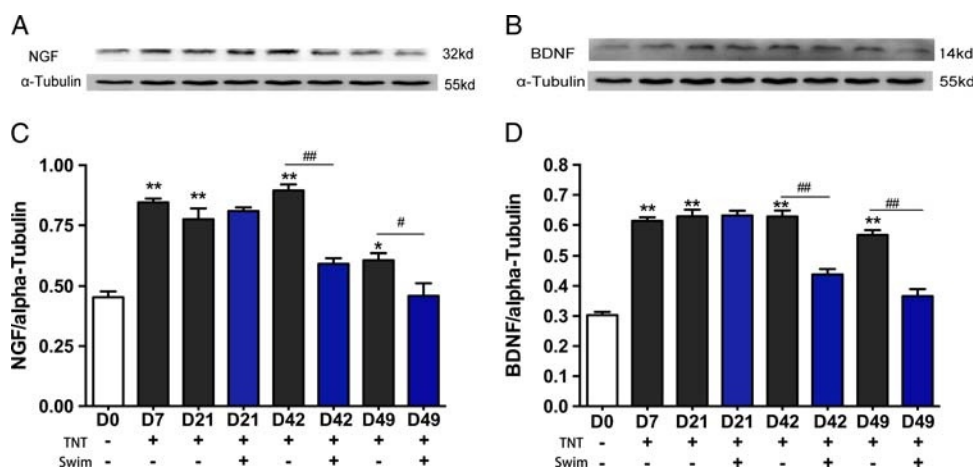
FIGURE 4—Swimming reduced TNT-induced increase of NT expression in the ipsilateral spinal cord. A, Western blotting band of NGF protein in the ipsilateral spinal cord. B, Western blotting band of BDNF protein in the ipsilateral spinal cord. C, Quantification of NGF expression from different groups. Data were presented as the ratio of NGF to α-tubulin. D, Quantification of BDNF expression from different groups. Data were represented as the ratio of BDNF to α-tubulin. TNT increased NT expression in the spinal cord on days 7, 21, 42, and 49 (**P < 0.01, vs control). Swimming exercise attenuated TNT-induced increase of NT expression; the effect lasted until 1 wk after swimming section (##P < 0.01, TNT/no-swim group vs TNT/swim group).
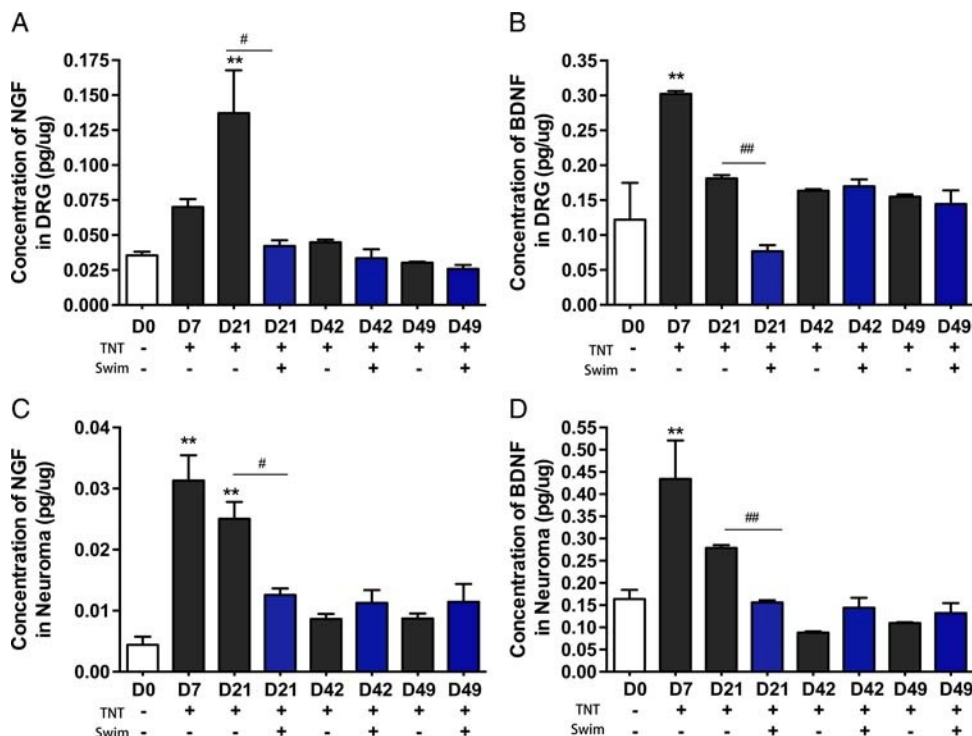
FIGURE 5—Swimming reduced TNT-induced increase of NT expression in the ipsilateral DRG and neuroma. A, NGF protein levels in the ipsilateral DRG from different groups. TNT increased NGF expression in DRG, and swimming exercise reduced the increased NGF expression (day 21: **$P < 0.01$, vs control; day 21: #$P < 0.05$, TNT/no-swim group vs TNT/swim group). **B,** BDNF protein levels in the ipsilateral DRG from different groups. TNT increased BDNF expression in DRG, and swimming exercise reduced the increased BDNF expression (day 7: **$P < 0.01$, vs control; day 21: ##$P < 0.01$, TNT/no-swim group vs TNT/swim group). **C,** NGF protein levels in neuromas from different groups. TNT increased NGF expression in neuroma, and swimming exercise reduced the increased NGF expression (days 7 and 21: **$P < 0.01$, vs control; day 21: #$P < 0.05$, TNT/no-swim group vs TNT/swim group). **D,** BDNF protein levels in neuromas from different groups. TNT increased BDNF expression in neuroma, and swimming exercise reduced the increased BDNF expression (day 7: **$P < 0.01$, vs control; day 21: ##$P < 0.01$, TNT/no-swim group vs TNT/swim group).

showed no effect on NT expression on days 42 and 49 ($P > 0.05$; Fig. 5).

Collectively, these findings demonstrated that TNT increased NGF and BDNF expressions in the spinal cord, DRG, and neuroma tissue, and swimming exercise could attenuate the TNT-induced increase of NT expression.

DISCUSSION

The purpose of this study was to investigate whether long-term swimming could relieve neuroma pain. We found that 5-wk swimming attenuates neuroma pain in the lateral ankle area and nerve injury–induced pain in hind paw plantar. Even 1 wk after the swimming section, the affect still lasted. Interestingly, 5-wk swimming not only relieved pain behavior but also inhibited injury-induced terminal neuroma formation. The detail mechanism might be related to the modulation of NGF and BDNF expression in neuroma, DRG, and the spinal cord.

Many animal models have been developed to mimic nerve injury–induced neuropathic pain, and neuroma pain model was developed in 2008 (17), when Dorsi et al. generated a TNT model. In this model, neuroma forms at the lateral ankle, where mechanical stimuli could be performed easily to induce a paw withdrawal response, mimicking neuroma

pain. Moreover, mechanical allodynia can also be evoked at the hind paw plantar, which mimics nerve injury–induced pain. Two different types of neuropathic pain (neuroma pain and mechanical allodynia) can be investigated in the same model (19,20).

Exercise can alleviate different kinds of chronic pain. For instance, swimming for 5 consecutive days for 5 wk decreased thermal hyperalgesia and mechanical allodynia in a Baba/c mouse model of neuropathic pain (16). Similarly, 5 wk of regular swim therapy had significant antinociceptive effects in rats with chronic constriction nerve injury (9). However, another controversial study found that treadmill running after constriction nerve injury promoted neuropathic pain and impaired functional recovery in CD1 mice (21). Inconsistency of difference findings might be caused by differences of pain model, exercise type, exercise duration, and exercise intensity. There are still some different voices about the effect of swimming on pain behavior. Rats displayed continuous thermal and chemical hyperalgesia after forced swimming in cool water for 3 d (22). On the contrary, swimming in 37°C water could decrease injury-induced behavioral hypersensitivity in rats and mice (11). These different findings might be related to stress, temperature change, skin moisture, or timing (23,24). In our study, we investigated whether 5-wk swimming session could relieve neuroma pain in a rat neuroma

model. Animals swam in a swimming pool filled with warm water, and all behavioral tests were conducted 24 h after swimming exercise to avoid stress-related effects.

We found that 5-wk swimming section attenuated pain behavior and inhibited neuroma formation; the finding is consistent with some other studies. Sabatier et al. (25) observed nerve regeneration using fluorescence microscopy in thy-1–YFP-H mice and found that continuous intense exercise increased axon elongation but reduced axon sprouting and in transected fibular nerves. Kater and Mills (26) reported that wheel running impaired axon spouting by increasing intracellular calcium concentrations. Inhibited sprouting of injured nerves may be associated with the increasing neuromuscular activity during exercise in rats (27). Although exercises were proved to inhibit nerve sprouting and neuroma formation, the detail mechanism remained unclear. As we all know, NT plays crucial roles in the regeneration of injured nerves. Endogenous BDNF is required for myelination and sciatic nerve regeneration in rodents (28). Injection of *BDNF* siRNA or BDNF antibody prevented intrinsic neuronal growth in injured sensory neurons (29). NGF-transduced Schwann cells promote regeneration of damaged peripheral nerves by increasing the number of myelinated fibers and CGRP (calcitonin gene-related peptide)-positive sensory fibers (30). In addition, several studies suggested that NT plays key roles in the development of and maintaining a period of neuropathic pain (13,31). Here, we hypothesized that NT might be involved in the process of swimming modulating pain behaviors and neuroma formation. Our experiments showed that TNT increased NT expression in neuroma, DRG, and the spinal cord, and swimming exercise reduced the TNT-induced increase of NT expression. Our finding was consistent with the study by Almeida et al. (16), which found that swimming exercise normalized nerve injury–induced NGF, and BDNF enhanced expression in the DRG in nerve injury model. Interestingly, Macias et al. (32) suggested a different finding showing that exercise increased the expression of BDNF and its receptor TrkB in the spinal cord and improved functional motor and sensory recovery. The discrepancy may be caused by the difference of animal model. Variations between studies could also be explained by differences in type of exercise, animal models, animal species, and duration and timing of exercise. We found that NT expression increased in neuroma and DRG tissue during the early and middle phases of the study, whereas NT expression in spinal cord dorsal horn was increased throughout the whole study. These findings suggested that swimming-induced changes in pain-like behavior might be related to NT expression in the peripheral and central nervous systems during the early and middle phases of the experiment, and in the central nervous system during later stages.

Our study was the first to find that long-term swimming therapy attenuated neuroma formation and neuroma pain, in which NT expression might be involved in the process. Further investigations are still needed to address the relationship between nerve regeneration and neuropathic pain. In conclusion, our findings provide evidence that swimming exercise can reduce neuroma pain, and treatments targeted toward NT may prove useful in the management of postamputation pain.

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