Animal Trial

Spinal Interferon Regulatory Factor 8 and Brain-derived Neurotrophic Factor in the Prefrontal Cortex are Involved in Pain-induced Depression Relief via Ultrasound-guided Pulsed Radiofrequency in a Rat Spared Nerve Injury Model

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Background: Pain-depression comorbidity has become a great burden to individuals and society. Nevertheless, the mechanisms underlying comorbid diseases have still not been fully revealed. Ultrasound-guided pulsed radiofrequency (PRF) on peripheral nerves, which produces remarkable analgesia via high-frequency electromagnetic energy, has become a main, minimally invasive treatment for chronic neuropathic pain.

Objectives: The aim of this study was to explore the effect of ultrasound-guided PRF on the sciatic nerve of spared nerve injury (SNI) rats to relieve pain-induced depression.

Study Design: Experimental trial in rats.

Setting: The research took place in the Laboratory of The First Affiliated Hospital of Wenzhou Medical University.

Methods: Sixty male Wistar rats were randomly divided into a sham group, an SNI group, an SNI + free-PRF group, and an SNI + PRF group. Ultrasound-guided PRF was applied to the sciatic nerve on day 7 after SNI. The basal paw mechanical withdrawal threshold (PMWT) was evaluated as a measure for pain-related behavior, and a sucrose preference test was performed as a measure for depression-related behavior. The expression levels of spinal interferon regulatory factor 8 (IRF8) and of brain-derived neurotrophic factor (BDNF) in the prefrontal cortex (PFC) were also studied on days 21 and 42.

Results: The results showed that the PMWT was significantly decreased in rats following SNI operation; the decreased levels of PMWT were reversed in the SNI + PRF group after the application of PRF on the sciatic nerve on day 7. There were no statistically significant differences among the groups in the sucrose preference rate on day 21 after SNI operation. The sucrose preference rate on day 42 was higher in the SNI + PRF group than in the SNI + free-PRF group. Western blot and reverse transcription polymerase chain reaction also demonstrated that ultrasound-guided PRF on the sciatic nerve downregulated overexpression of spinal IRF8 and increased the levels of BDNF in the PFC.

Limitations: This study was performed using only an SNI rat model which cannot represent all rodent neuropathic pain models. Only the short-term effectiveness of ultrasound-guided PRF on the sciatic nerve of SNI rats was investigated. The BDNF changes of other important brain areas were not taken into consideration in this study.

Conclusions: These findings suggest that ultrasound-guided PRF on sciatic nerve could alleviate pain-induced depression. The mechanisms of this treatment may be involved in the downregulated spinal IRF8 and the increased BDNF in PFC.

Key words: Neuropathic pain, depression, ultrasound, pulsed radiofrequency, interferon regulatory factor 8, brain-derived neurotrophic factor, spinal cord, prefrontal cortex

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Neuropathic pain is a type of refractory pain that arises as an injury to a peripheral nerve or the central nervous system affecting sensory, cognitive, and affective symptoms. A large number of patients with neuropathic pain are afflicted with anxiety and depression; this pattern is also seen in animal models of chronic pain (1-3).

Chronic neuropathic pain and depression often present together and interact. The common signaling pathways and the overlapping mechanisms in the central nervous system suggest that this shared neurobiological process may lead to cognitive disorders and mental illness. Although the cellular and neural mechanisms underlying chronic neuropathic pain have been uncovered extensively through animal and human studies, the comorbid relationship between chronic pain and depression remains incompletely understood.

An activated microglia is a well-known structure involved in processing information of chronic neuropathic pain. Wang et al (4) found that interferon regulatory factor 8 (IRF8) is a key transcription regulator of a microglia-activated program of gene expression and converted microglia to a reactive state. Our previous animal experiment showed that treatment with pulsed radiofrequency (PRF) adjacent to the dorsal root ganglion markedly reduced neuropathic pain, which confirmed that downregulation of IRF8 plays a critical role in regulating pain signal transmission in the spinal cord (5). Brain-derived neurotrophic factor (BDNF) released from activated microglia contributes to neural plasticity and neurogenesis in the central nervous system and hyperexcitability of the pain signaling pathway. Chronic stress-induced depression and cognitive impairment are associated with a downregulation of BDNF expression and neuronal apoptosis in the hippocampus and prefrontal cortex (PFC), which can be reversed by antidepressants (2,6).

Ultrasound-guided PRF is a minimally invasive treatment with multiple therapeutic applications (7-10). Recent studies show that PRF administrated on the dorsal root ganglion or the sciatic nerve in animals with peripheral nerve injury in order to inhibit microglial activation in the spinal cord, improves pain-related behaviors (11,12). In addition, electron microscopy has been applied to investigate the morphological changes of peripheral nerves in rodent studies; it found that 42°C PRF-induced damage was only moderate (13).

In this experiment, our aim was to explore not only the alterations in pain-associated and depression-associated behaviors caused by spared nerve injury (SNI) operation, but also the expressions of spinal IRF8 and BDNF in the PFC. We also investigated whether ultrasound-guided PRF on the sciatic nerve could improve pain-depression comorbidity via regulating the expressions of spinal IRF8 and BDNF in the PFC.

Methods

Animals

Healthy male Wistar rats, weighing 180 to 200 g, (Shanghai Slac Laboratory Animal Co., Ltd) were kept in a cages with a 12-hour light/dark cycle, with food and tap water ad libitum. Controlled room temperature and humidity were maintained at 22°C ± 1°C and 50% ± 5%, respectively. The rats were habituated to the environment for 7 days. All procedures were approved by and performed in compliance with the requirements of the Institutional Animal Ethics Committee (The First affiliated Hospital of Wenzhou Medical University Wenzhou, Zhejiang Province, China).

Treatment Groups and Design

A total number of 60 Wistar rats were randomly assigned to one of 4 groups (n = 15 each): 1) sham, 2) SNI, 3) SNI + free-PRF, and 4) SNI + PRF. The left sciatic nerve of the rats in the sham group was exposed without ligation, whereas in the SNI, SNI + free-PRF and SNI + PRF groups, a cut was performed in the common peroneal and tibial branches of the left sciatic nerve. An RF electrode under ultrasound guidance without PRF was used in the SNI + free-PRF group rats on day 7 after SNI, whereas the rats in the SNI + PRF group were treated by ultrasound-guided PRF applied to the sciatic nerve on day 7 after SNI. Basal paw mechanical withdrawal threshold (PMWT) was evaluated before the SNI operation and on days 3, 7, 10, 14, 21, 28, 35, and 42. Sucrose preference was tested and the expression levels of IRF8 in the lumbar (L5) spinal cord and of BDNF in the PFC were measured on days 21 and 42 (Fig. 1).

SNI Model

The SNI model of neuropathic pain and sham operation were performed by intraperitoneal chloral hydrate anesthesia (300 mg/kg) under aseptic conditions. Using the method described by Francesca (14), the 3 branches of the sciatic nerve—the common peroneal nerve, the tibial nerve, and the sural nerve—were exposed and isolated after skin disinfection. The common peroneal and tibial branches were transected and ligated, leaving the sural nerve intact. Then, the muscle

and skin were closed in 2 layers. In the sham group, the nerves were only exposed, without transection or ligation. In all cases, utmost care was taken to neither stretch the nerve or its branches nor affect the intact nerves.

Pulsed Radiofrequency Therapy

In this study, the SNI + PRF group underwent PRF on day 7. As in the SNI operation, intraperitoneal chloral hydrate (300 mg/kg) anesthesia was administered under aseptic conditions. Rats were placed in the right lateral position; the left lumbosacral area of the rats was shaved and the operative field was prepped with povidone iodine.

A high frequency 5–10MHz ultrasound probe (Mindray Bio-Medical Electronics CO., LTD.) was placed on the lateral side of the hind limb at the midthigh and gel was placed to facilitate sound transmission. The femoral trochanter served as a landmark to achieve orientation. The ultrasound transducer probe was then placed overlying the orientation. The left sciatic nerve was identified as an oval area under the gluteus muscle (Fig. 2). Then an RF electrode (type 20G, 5 cm long, 4-mm active tip) was inserted in an in-plane approach.

When the electrode tip was adjacent to the sciatic nerve, PRF treatment was administered to the rats in the SNI + PRF group by using a radiofrequency device (Cosman Medical, Inc.) with parameters previously described by Chen (13): pulse rate 2 Hz, voltage 45 V, maximum temperature 42°C, pulse width 20 milliseconds, and 120 seconds of stimulation time. The animals were allowed to recover from the anesthesia on a heating pad with a temperature maintained at 37°C.

Behavioral Testing

All behavioral tests were performed by observers who were blinded to the surgery paradigm.

Paw Mechanical Withdrawal Threshold (PMWT)

To measure mechanical sensitivity, rats were placed in individual plexiglass cells (10 × 15 × 20 cm) on a wire mesh floor and accommodated for 15 minutes. Von Frey filaments (bending force of 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, and 15.0 g; [Stoelting]), as described previously, were applied to the plantar surface of the left hind paw by using the up-and-down method. The filament was subjected to the bending force for 3–4 seconds.

Starting with one filament and a bending force of 2 g, the next lighter filament was treated if a paw withdrawal response was observed. The next stronger bending force filament was applied if a paw withdrawal response was not observed. The series of von Frey filaments had a bending force ranging from one to 15 g. The 50% PMWT was measured according to the methods reported by Yang (15).

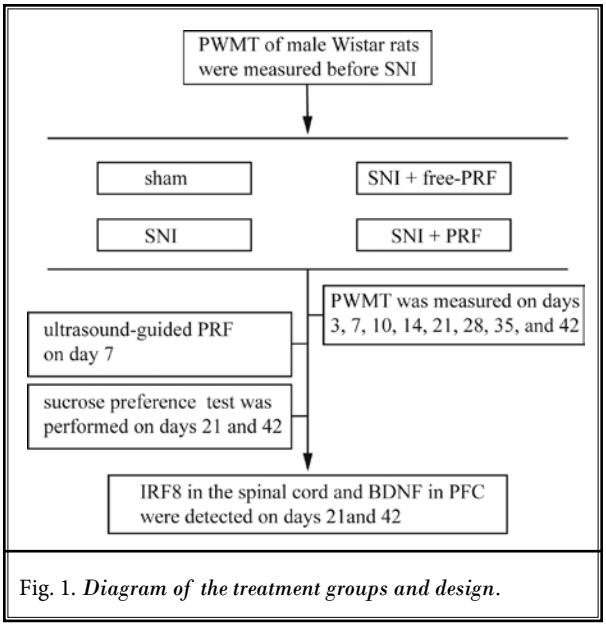

Fig. 1. *Diagram of the treatment groups and design.*

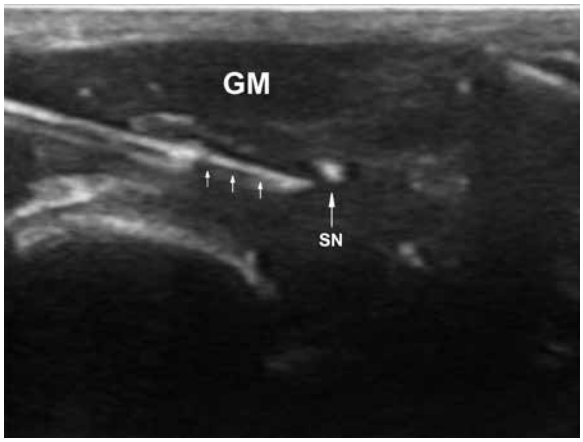

Fig. 2. *Representative image of ultrasound-guided in-plane PRF applied to sciatic nerve (SN). Gluteus muscle is above (GM).*

Sucrose Preference Test

A sucrose preference test was performed as a measure for depression-like behaviors in rats; a decrease in an animal's preference for sucrose suggests anhedonia.

To perform this test, rats were allowed a free

choice between 2 bottles for 48 hours; one bottle contained a 1% sucrose solution and the other contained tap water. After adaptation, the rats were food- and water-deprived for 23 hours, followed by the sucrose preference test, in which the rats were housed in individual cages for a one hour exposure to 2 preweighed test solution bottles. To avoid the possible effects of side preference, the position of the water and sucrose bottles was changed after 30 minutes.

At the end of the one hour test, fluid consumption was recorded by reweighing the bottles. The sucrose preference (%) was calculated according to the following equation: sucrose preference % = sucrose consumption/(sucrose consumption + water consumption) × 100%.

Western Blot

Animals were anesthetized by chloral hydrate and killed by decapitation. The L5 spinal segment was cut after laminectomy, and the bilateral PFC were excised by reference to Paxinos' atlas of the rat brain (16); both tissues were placed in a freezer at -80°C. Protein samples (50 mg) were resolved by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes.

The membranes were then blocked with 5% nonfat milk in phosphate-buffered saline at pH 7.4 (137 mmol/L NaCl [sodium chloride], 2.7 mmol/L KCl [potassium chloride], 10 mmol/L Na_2HPO_4 [disodium hydrogen phosphate], and 2 mmol/L KH_2PO_4 [potassium dihydrogen phosphate]) with Tween 0.05%, followed by incubation with IRF8 (AB2165981, 1:500; Abcam)and BDNF antibodies (AB108319, 1:500; Abcam). Horseradish peroxidase-conjugated secondary antibody (MD2142, 1:6000; Medical Discovery Leader Co.)was applied for detection of the primary antibody.

The signals of the IRF8 and BDNF were detected using an enhanced chemiluminescence detection system according to the manufacturer's instructions (170-8280, Bio-Rad Laboratories Inc.). Blots were stripped and incubated with a monoclonal antibody directed against β-actin, which was used as a loading control to normalize IRF8 and BDNF protein expression levels. Data analysis was carried out with Image J (National Institutes of Health Inc.).

Real-time Quantitative Polymerase Chain Reaction (PCR)

Total RNA was extracted from tissue with Trizol (Servicebo) and reverse transcribed to cDNA using a TIANScript RT KIT (TIANGEN). The following PCR cycles were used: 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. The primers utilized for IRF8 were as follows: (sense) 5′- GCAGGCAAGCAAGACTACAACC -3′ and (antisense) 5′-CGGGGACGATTCGGTAAACTT -3′. The primers used for BDNF were as follows: (sense) 5′-GTGTGACAGTATTAGCGAGTGGG-3′ and (antisense) 5′-ACGATTGGGTAGTTCGGCATT-3′. The primers employed for β-actin were as follows: (sense) 5′-TGCTATGTTGCCCTAGACTTCG-3′ and (antisense) 5′-GTTGGCATAGAGGTCTTTACGG -3′. The housekeeping gene β-actin was used as an internal control. The relative expression was calculated by the $2^{-\Delta\Delta CT}$ method: ΔC_T (test) = C_T (target, test) - C_T (ref, test), ΔC_T (calibrator) = C_T (target, cal) - C_T (ref, cal), $\Delta\Delta C_T$ = ΔC_T (test) - ΔC_T (calibrator).

Statistical Analysis

GraphPad Prism 6.0 (GraphPad Software, Inc.) was used for statistical analysis and graph generation. Data are presented as mean ± SD. To analyze the paw mechanical withdrawal threshold in the rats, we used a repeated-measures two-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. The sucrose preference and the expression levels of IRF8 and BDNF were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. The statistical significance was set at $P < 0.05$.

Results

PMWT of the Left Hindpaw of SNI Rats

PMWT was observed on days 0, 3, 7, 10, 14, 21, 28, 35, and 42 after SNI in this study (Fig. 3). A repeated-measure ANONA revealed that there was a significantly therapeutic effect ($F_{3, 402} = 3{,}467$; $P < 0.001$), along with the significant effect of trials ($F_{8, 402} = 293.9$; $P < 0.001$) and interaction of treatment trials ($F_{24, 402} = 88.86$; $P < 0.001$). No significant differences were detected among the sham, the SNI, and SNI + free-PRF groups ($P > 0.05$). The results of the multivariate tests indicated that PMWT was elevated in the SNI + PRF group after the application of PRF on the sciatic nerve on day 7 compared with those in the SNI + free-PRF group ($P < 0.01$).

Results of the Sucrose Preference Test in the SNI Rats

The sucrose preference rate was measured on days

21 and 42 after SNI operation (Fig. 4). One-way ANOVA did not find statistical differences in the sucrose preference rate among the groups on day 21 after SNI operation ($P > 0.05$). However, there were significant differences in the effect among the groups on day 42 ($F_{3, 27} = 8.607$, $P < 0.01$). Further analysis by the Tukey test demonstrated a significant decrease of the sucrose preference rate in the SNI and SNI + free-PRF groups on day 42 compared to the sham group ($P < 0.01$). The sucrose preference rate on day 42 was higher in the SNI + PRF group than in the SNI + free-PRF group ($P < 0.05$).

Protein Expression of IRF8 in the Spinal Cord and BDNF in the PFC

As depicted in Fig.5, the Western blot analysis of the IRF8 protein in the spinal cord and BDNF protein in the PFC was performed on days 21 and 42 after SNI. (Figs. 5A, 5B). Based on one-way ANOVA analysis results, we found significant differences in the effect among the groups on days 21 ($F_{3, 8} = 18.02$; $P < 0.01$) and 42 ($F_{3, 8} = 13.64$; $P < 0.01$]. Significantly enhanced expressions of the IRF8 protein were observed on days 21 and 42 in the SNI and SNI + free-PRF groups compared to the sham group ($P < 0.01$). An increased expression on days 21 and 42 in the SNI + PRF group was also observed compared to the sham group ($P < 0.05$), but was lower than that in the SNI + free-PRF group ($P < 0.05$) (Fig. 5C, Fig. 5D) No statistical differences in BDNF expression were found among the groups on day 21 ($P > 0.05$). One-way ANOVA analysis results showed significant differences in the effect among the groups on day 42 ($F_{3, 8} = 18.22$; $P < 0.01$). Lower expression of the BDNF protein was observed on day 42 in the SNI and SNI + free-PRF group compared to the sham group ($P < 0.05$). The expression of the BDNF protein was still lower in the SNI + PRF group than in the sham group ($P < 0.05$), but was higher than the SNI + free-PRF group ($P < 0.05$).

The mRNA Levels of IRF8 in the Spinal Cord and BDNF in the PFC

As illustrated in Fig. 6, the reverse transcription polymerase chain reaction analysis of IRF8 in the spinal cord and BDNF in the PFC was conducted on days 21 and 42 after SNI (Fig. 6A). The one-way ANOVA analysis results revealed significant differences in the effect among the groups on days 21 ($F_{5, 12} = 17.73$; $P < 0.01$] and 42 ($F_{5, 12} = 15.17$; $P < 0.01$). The expression of IRF8 on days 21 and 42 in the SNI and SNI + free-PRF groups was significantly higher than in the sham group ($P <$

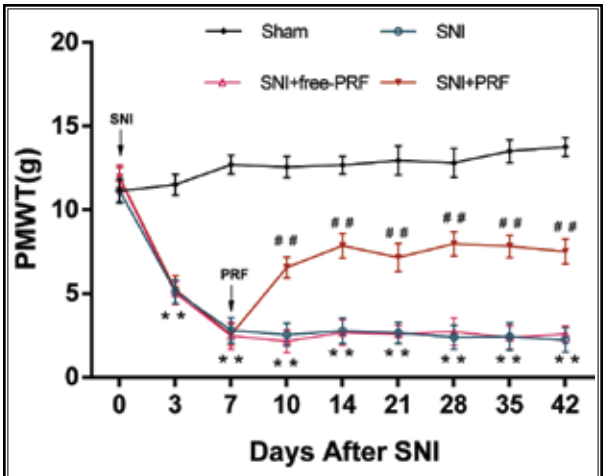

Fig. 3. *The decreased PMWT induced by SNI was reversed by the application of therapy by PRF on the sciatic nerve. The values are expressed as means ± SD. **P < 0.01: the SNI group vs the sham group; ##P < 0.01: the SNI + PRF group vs the SNI+ free-PRF group.*

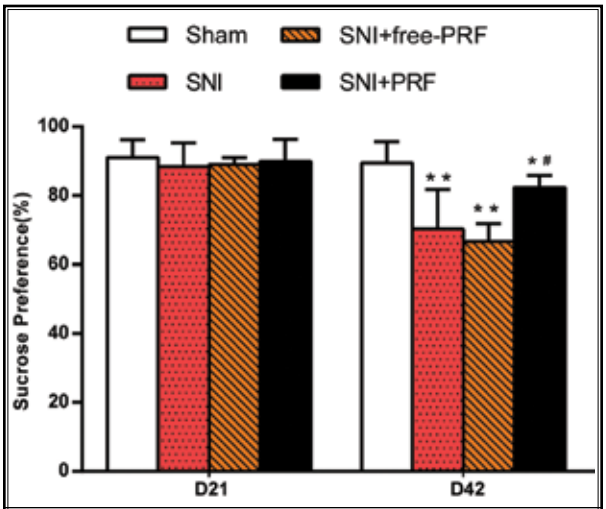

Fig. 4. *The reduced sucrose preference rate induced by SNI on day 42 was reversed by PRF on the sciatic nerve in rats. The values are expressed as means ± SD. **P < 0.01: vs the sham group; *P < 0.05: vs the sham group; #P < 0.05: vs the SNI + free-PRF group.*

0.01). In the SNI + PRF group, the mRNA expression of IRF8 remained higher than in the sham group ($P <$ 0.05), but was lower than in the SNI + free-PRF group ($P < 0.05$) (Fig. 6B). No statistical differences were observed among the groups on day 21 ($P > 0.05$). One-way ANOVA analysis results showed the presence of significant differences in the effect among the groups on day

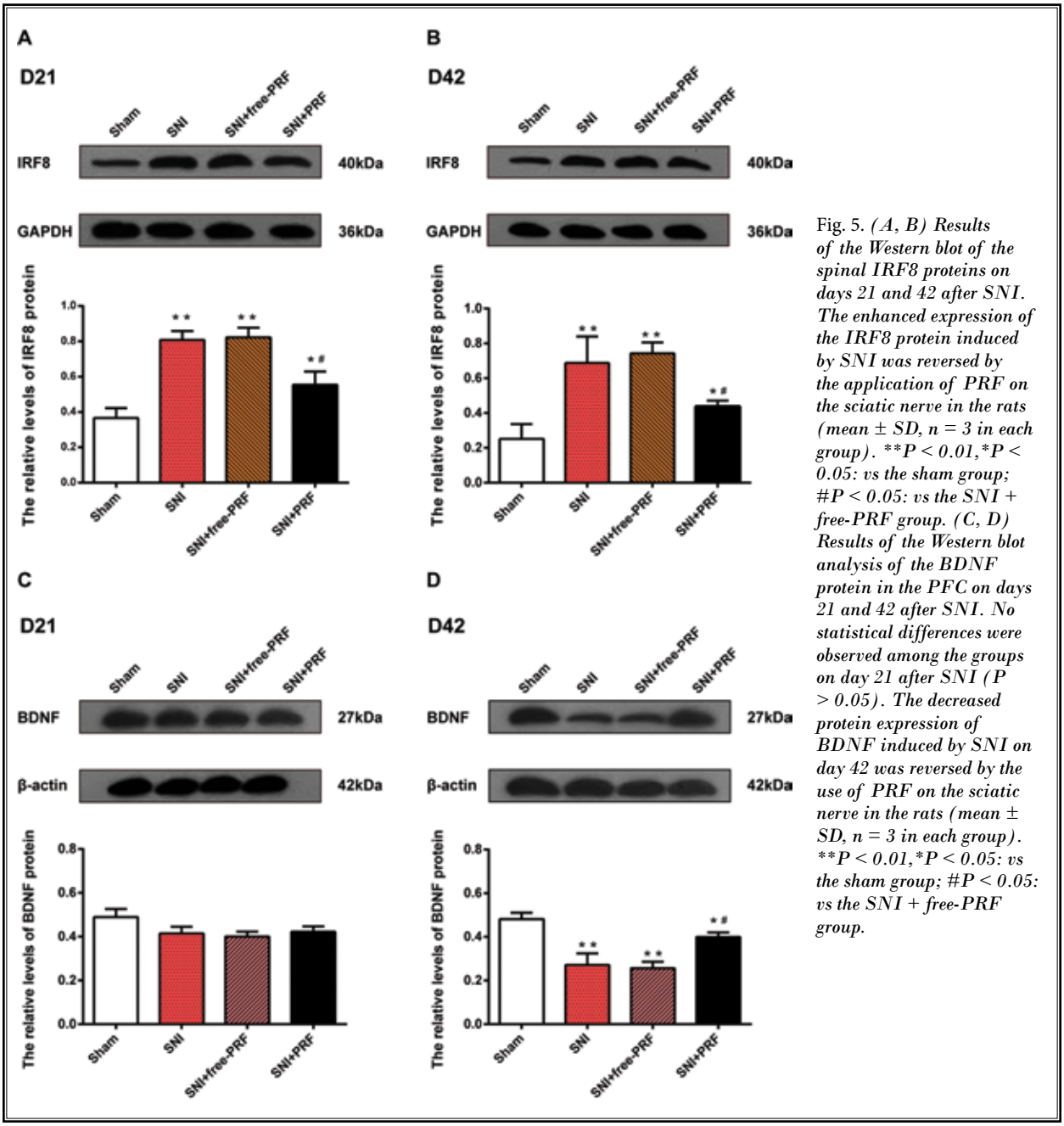

Fig. 5. *(A, B) Results of the Western blot of the spinal IRF8 proteins on days 21 and 42 after SNI. The enhanced expression of the IRF8 protein induced by SNI was reversed by the application of PRF on the sciatic nerve in the rats (mean ± SD, n = 3 in each group). **P < 0.01,*P < 0.05: vs the sham group; #P < 0.05: vs the SNI + free-PRF group. (C, D) Results of the Western blot analysis of the BDNF protein in the PFC on days 21 and 42 after SNI. No statistical differences were observed among the groups on day 21 after SNI (P > 0.05). The decreased protein expression of BDNF induced by SNI on day 42 was reversed by the use of PRF on the sciatic nerve in the rats (mean ± SD, n = 3 in each group). **P < 0.01,*P < 0.05: vs the sham group; #P < 0.05: vs the SNI + free-PRF group.*

42 ($F_{5, 12}$ = 38.74; P < 0.01). On day 42, the expression of BDNF was significantly lower in the SNI and SNI + free-PRF than in the sham group (P < 0.05). After the treatment of PRF on the sciatic nerve in SNI rats, the mRNA expression of BDNF showed a significant upregulation compared with that in the SNI group or the SNI + free-PRF group respectively (P < 0.05).

Discussion

The results of this study demonstrate that male Wistar rats with SNI developed nociceptive behavior that is reflected in a significant decrease in PMWT. We also used a sucrose preference test to measure depressive behavior, and found that the rats suffering from injury to the sciatic nerve decreased their sucrose consump-

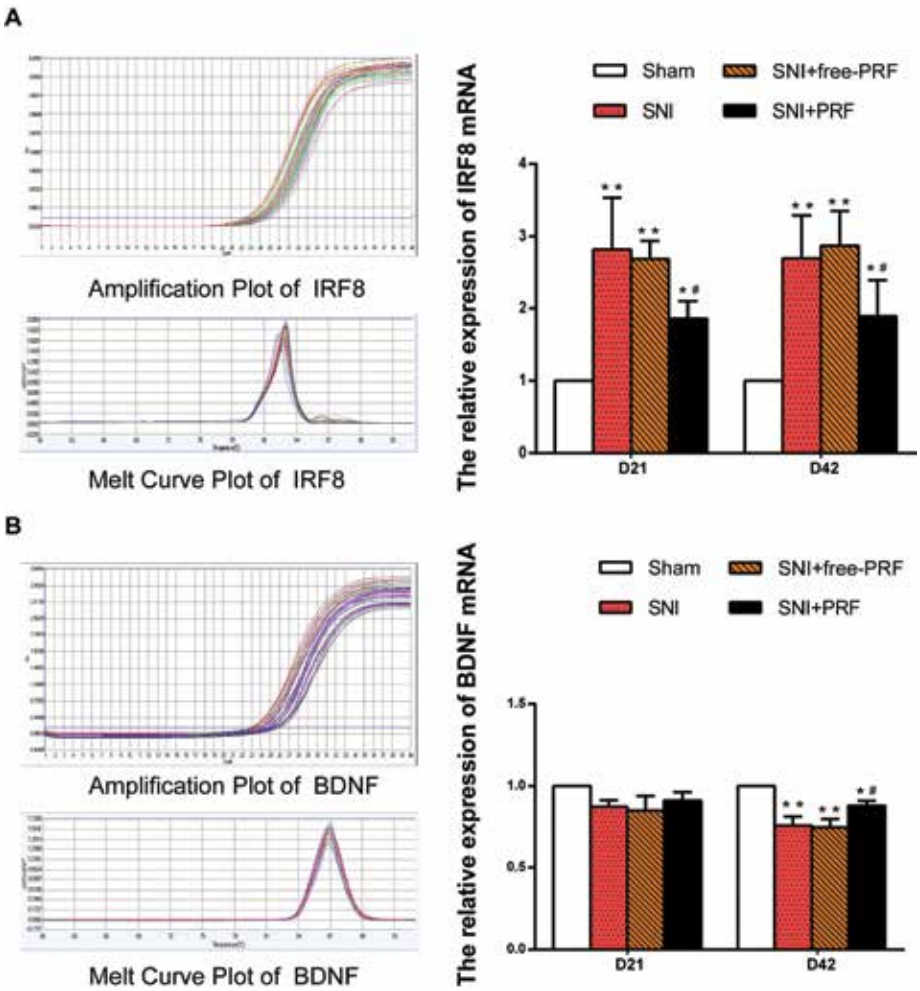

Fig. 6. *(A) PCR plots and results of IRF8 in the spinal cord and BDNF in PFC on days 21 and 42. The overexpressed IRF8 mRNA induced by SNI on days 21 and 42 was reversed by the application of PRF on sciatic nerve in rats (mean ± SD, n = 3 in each group). **P < 0.01, *P < 0.05: vs the sham group; #P < 0.05: vs the SNI + free-PRF group. (B) The reduced BDNF mRNA induced by SNI on day 42 was reversed by the application of PRF on sciatic nerve in the rats (mean ± SD, n = 3 in each group). **P < 0.01, *P < 0.05: vs the sham group; #P < 0.05: vs the SNI + free-PRF group.*

tion on day 42. Moreover, SNI operation increased the IRF8 expression in the spinal cord and decreased the BDNF levels in the PFC. On the contrary, ultrasound-guided PRF applied on the sciatic nerve significantly reduced the overexpression of IRF8 in the spinal cord and increased the expression of BDNF in the PFC. Meanwhile, to the best of our knowledge, this is the first study that employs ultrasound-guided PRF on the sciatic nerve to treat neuropathic-induced depression.

Neuropathic pain is a type of refractory condition that is a consequence of nerve lesion or diseases such as cancer, herpes zoster, Alzheimer disease, diabetes, and infection, which is involved in sensory discrimination, affective motivation and cognitive evaluation, with more than half showing comorbid anxiety and depression (9,17-19).

Microglial cells are a type of widespread immune cell in the central nervous system. Evidence has shown that microglial cells play a key role in neuropathic pain development (12,20-24). IRF8 belongs to a critical member of the transcription factor superfamily (IRF1-9). Recent experiments have reported that IRF8 is indispensable for microglial activation in the spinal dorsal horn of a murine pain model. The upregulation of IRF8 in the spinal dorsal horn promotes the reactive states of microglia. In contrast, an IRF8-knockout murine pain model have shown resistance to pain-induced tactile allodynia (4).

The IRF8 expression of spinal cord, therefore, was tested on days 21 and 42 after SNI operation in this experiment. We found that the IRF8 was significantly increased in SNI and free-PRF group on day 21 and 42. Conversely, ultrasound-guided PRF on the sciatic nerve downregulated the IRF8 overexpression in the PRF group on days 21 and 42; we also found that an increasing trend of PMWT from day 10 to day 42. Therefore,

the results of this study are consistent with the previous reports and suggest that IRF8 is critically involved in development and maintenance of neuropathic pain.

Neuropathic pain is processed via a complex multidimensional neuronal system involving many structures associated with anxiety and depression. Recent studies have shown that the PFC is not only important in executive functions like planning, working memory, and judgment, but also biopsychosocial pain management (25,26). Gene expression and glial cells are differentially modulated in the PFC during neuropathic pain that result in changes to its structure, neural activity, and connectivity (11). Luchtmann et al (27) used magnetic resonance image data analysis to find that the gray matter volume of the anterolateral PFC in patients suffering from sciatica and chronic low back pain due to prolapsed intervertebral discs was significantly decreased. Several postmortem studies have found that BDNF expressions are significantly suppressed in the prefrontal cortex of those who committed suicide. In comparison, it has been found that patients receiving antidepressant therapy restored BDNF expressions in their brain to normal levels (28).

BDNF is a member of the neurotrophin superfamily, which is widely expressed in the developing and adult mammalian brain. BDNF is likely involved in neurogenesis, neural regeneration, synaptic transmission, and cell survival and death. Several recent animal and clinical studies have indicated that BDNF plays a critical role in the pathophysiology of anxiety and depression (29-32).

Since the important role of BDNF in the PFC in the onset of pain-induced depression comorbidity, our object was to test the hypothesis that the abnormal expressions of BDNF in the PFC might be attributed to neuropathic pain-induced depression disorders, and might be restored to a normal level by PRF on the sciatic nerve. Our experiment showed that the BDNF expressions of day 42 in the SNI group and in the free-PRF group were significantly lower than that of day 42 in the sham group. In addition, the BDNF expressions of day 42 in the SNI group and in the free-PRF group were lower than that of day 21. The downregulation of the sucrose preference is also consistent with decreased BDNF expressions in the PFC, which are also in accordance with previous investigations (11,32). These results further confirm that neuropathic pain lasting 4 weeks promotes depression-like behaviors. In comparison, the sucrose preference and BDNF expressions in the PFC on day 42 were reversed by using ultrasound-guided PRF on the sciatic nerve. It is noteworthy that the downregulated expression of spinal IRF8 and upregulated BDNF in the PFC on day 42 in the PRF group exhibited improvement of pain-related behavior and depression-related behavior. This result demonstrates an important relationship between spinal IRF8 and BDNF in the PFC, and that their regulation may play a critical role in pain-induced depression.

PRF is a variation of thermal radiofrequency in which a radiofrequency electrode conveys electromagnetic fields to the target tissue. The temperature of the electrode tip did not exceed 42°C to avoid thermal injury to the tissue (33). Recently, there are have been an increasing number of clinical and laboratory studies indicating that PRF on peripheral nerve fibers under ultrasound guidance could effectively relieve neuropathic pain. Lee et al (9) reported that ultrasound-guided PRF stimulation treatment targeting cervical spinal nerves could provide significant pain relief for refractory chronic cervical radicular pain. Lin et al (34) demonstrated that bipolar high-voltage PRF on the cervical sympathetic chain under ultrasound guidance could effectively treat acute herpetic neuralgia of the oral, maxillofacial, neck, and upper limb regions. In addition, using PRF under ultrasound guidance on the saphenous nerve was proved to be effective for at least 12 weeks in patients with osteoarthritis-associated knee pain.

Ultrasound guidance has some technical advantages since it can confirm critical vessels, peripheral nerves and soft tissue and obtain high distinct real-time ultrasonic imaging with great convenience (8,35,36). The performance of PRF under ultrasound guidance is not a challenging technique for physicians and could be operated by individuals with experience in in-plane ultrasound-guided techniques (37). Furthermore, ultrasound-guided operations could avoid exposure to radiation. Therefore, the procedure of ultrasound-guided PRF on the sciatic nerve was chosen to be applied in this study to treat neuropathic pain-induced depression comorbidity.

The downregulation of IRF8 in the spinal cord and increased levels of BDNF in the PFC have been proved to be important factors in relieving neuropathic pain and depression in rodent peripheral nerve injury models (5,32). Our experiment further explored if PRF on the sciatic nerve under ultrasound guidance could inhibit the overexpression of IRF8 in the spinal cord and increase BDNF levels in the PFC in order to alleviate pain-depression comorbidity. The expressions of spinal

IRF8 and BDNF in the PFC were tested by Western blot and reverse transcription polymerase chain reaction in all our groups. It was found that ultrasound-guided PRF on the sciatic nerve significantly decreases the overexpression of spinal IRF8 and raised the levels of BDNF in the PFC, and that these changes are consistent with the alternation of behaviors in PMWT and sucrose consumption.

Limitations

This study has some limitations. First, this experiment was conducted using only an SNI rat model which cannot represent all rodent neuropathic pain models. Second, only the short-term effectiveness of ultrasound-guided PRF applied to the sciatic nerve was investigated. Third, the BDNF changes of other important brain areas (such as the hippocampus, amygdala, and anterior cingulated cortex) were not taken into consideration in this study.

Conclusion

These results indicate that ultrasound-guided PRF therapy applied to the sciatic nerve can alleviate SNI neuropathic pain-induced depression comorbidity. The mechanisms underlying this treatment may be involved in the downregulated expressions of spinal IRF8 and the increased levels of BDNF in the PFC.

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