Author's response to reviews

Title: Association between extracellular signal-regulated kinase expression and the anti-allodynic effect in rats with spared nerve injury by applying immediate pulsed radiofrequency

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Author's response to reviews: see over
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RE: Submission of revised manuscript MS: 2646482641320574- “Association between extracellular signal-regulated kinase expression and the anti-allodynic effect in rats with spared nerve injury by applying immediate pulsed radiofrequency”

Dear Editor,

Please find our revised manuscript entitled “Association between extracellular signal-regulated kinase expression and the anti-allodynic effect in rats with spared nerve injury by applying immediate pulsed radiofrequency” for the editors' and referees' approval.

The authors are extremely grateful for valuable comments and suggestions provided by the reviewers. These insightful perspectives will unquestionably strengthen the quality of this manuscript. Accordingly, details of all revisions and replies have been made and highlighted in the revised manuscript, and also elaborated in the “Authors’ Responses to Reviewers’ Comments”.

We hope that the revisions and replies are satisfactory, and we thank you very much for your help.

Sincerely yours,

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Authors’ Responses to Reviewers’ Comments

Reviewer: 1

Reviewer's report:

This manuscript examines the effects of pulsed radiofrequency, applied adjacent to a site of injury (spared nerve injury or SNI), on manifestations of neuropathic pain (mechanical and cold allodynia) and ERK expression in ipsilateral the spinal cord. It examines two settings (45V and 60V), and observes similar behavioral effects for both settings. SNI induces an increase in ERK1/2 expression (over 2-fold) and PRF restores this to almost normal levels.

The observations in this study are original and address an aspect of the mechanism by which this procedure might lead to alleviation of neuropathic pain in humans. The paper is generally well written, and it adds to an emerging body of preclinical information on how this technique might potentially lead to pain relief. However, it does need some attention to specific details.

Response: We highly appreciate the reviewer’s supportive comments and have made necessary revisions, taking account of the suggestions for improvement. We herein provide a point-by-point description on the corresponding changes, which are elaborated below.

1) The data in the tables looks to supplementary information. It should be included in the main body of the manuscript.

Response: Thank you for your valuable suggestions. We made the tables into lined graphs (Fig. 2 and Fig. 3) and included them in the main body of the manuscript. We also add some additional descriptions to the result and discussion sections. The
Fig 2. Paw Withdrawal Test of Mechanical Allodynia

**Figure 2.** The paw withdrawal threshold in rats that were subjected to the sham operation, SNI without PRF (SNI group), or SNI plus either 45 V PRF or 60 V PRF (SNI+PRF-45V and SNI+PRF-60V groups, respectively). Mechanical allodynia was evaluated using dynamic plantar aesthesiometry. The data presented above were examined by ANOVA followed by Bonferroni test. D, day. #P < 0.05, ##P < 0.01 SNI, SNI+PRF-45V, and SNI+PRF-60V compared with the sham. *P < 0.05, **P < 0.01 SNI+PRF-45V and SNI+PRF-60V compared with SNI.

Fig. 3 Paw Withdrawal Test of Cold Alloodynia
Figure 3. Behavioral response in rats subjected to sham operation or SNI without PRF (SNI group) or SNI plus either 45 V PRF or 60 V PRF (SNI+PRF-45V and SNI+PRF-60V groups, respectively). Cold allodynia was evaluated by the acetone spray test. The data presented above were examined by ANOVA followed by Bonferroni test. D, day. \( \# P < 0.05 \), \( \## P < 0.01 \) SNI, SNI+PRF-45V, and SNI+PRF-60V compared with the sham. \( * P < 0.05 \), \( ** P < 0.01 \) SNI+PRF-45V and SNI+PRF-60V compared with SNI.

2) The authors apply the PRF immediately following nerve injury. However, in humans this procedure would be applied well after a pain condition has become chronic, and is likely quite remote from the inciting injury. The authors refer to unpublished observations (page 16, line 2) in which PRF 14 days after SNI produces less effect than applying it immediately. This biases the observations towards seeing an effect, yet may bias it against clinical relevance. At the very least, the authors need to discuss the issue of immediate vs delayed application of PRF.

Response: Thank you for your valuable suggestions.

The application of PRF for the management of neuropathic pain should be considered
carefully, as the mechanisms involved in the immediate versus the delayed phase may differ, and the immediate early phase of nerve injury is less understood. PRF for the management of clinical chronic neuropathic pain is common; however, sometimes it fails to provide a satisfactory effect, with the delayed intervention being one of the possible explanations for this finding. In the case of acute nerve injury, the acute inflammatory process without proper intervention often results in chronic neuropathic pain [Ref.1]. Lin et al. found that early PRF treatment suppressed the levels of proinflammatory cytokines via neuromodulation and immune modulation, such as the downregulation of spinal MAPK (ERK) activation [Ref. 2]. Therefore, we assumed that an immediate or early PRF intervention may result in less neuroinflammation compared with a delayed PRF intervention.

In clinical practice, the application of immediate PRF therapy for the management of acute nerve injury might encounter several difficulties. Most patients will choose to undergo nerve reconstruction operation to restore neurological function. For those who cannot or will not undergo surgery, we propose an alternative, immediate/early PRF, to achieve a better effect, even though this study is still in the animal experiment stage. Nevertheless, to our knowledge, there is no clinical research-based investigation of the difference in antiallodynic effect between immediate and delayed PRF therapy after acute nerve injury. To translate our findings into future treatment strategies for developing neuropathic pain, we may choose to apply PRF treatment immediately, or combine one immediate and one delayed application of PRF to provide optimal results for patients.

References:


Ref. 2: Lin ML, Lin WT, Huang RY, Chen TC, Huang SH, Chang CH, Tsai SY, Chiu

The above response to your comments will be added to the discussion part of the revised manuscript. (Please refer to page15, line 11~page 16, line 14)

3) In figure 2, why was there no data for the 60V condition shown?

**Response:** Thank you for your suggestions. The 60V condition data had not been shown because we observed no significant differences in the mechanical and cold hypersensitivity at any time between SNI+PRF-45V and SNI+PRF-60V groups. For more comprehensive understanding of our results, the 60V data are incorporated in Figure 4 of the revised manuscript.

Several minor details also need attention.
p.3, line 7: Neuropathic pain was achieved in a SNI model in Wistar rats by ligation and … (then delete “was ligation and cut” line 8-9); line 9: Rats were divided (Wistar strain is now mentioned in methodology sentence; see above); line 10: treatment conditions (both are the same” modality” but differ in intensity); line 14: measurements included mechanical; line 15: and were examined
p.4, line 8: in the ipsilateral spinal; line 9: in the SNI_PRF; line 11: application on the proximal…
p.5, line 5: reduction of pain, and frequently there is unsatisfactory; line 7: and has been used; line 10: shown to be safe and helpful; line 12: stimulation adjacent to
p.6, line 2: The oncogene information looks out of place (it is molecular) as the sentence is referring to anatomical sites, check this sentence carefully. Line 5: systems within the spinal cord which provide…; line 11: In the present study, we examine the… page 8, line 6: Antinociception (correct the spelling) Page 13. Line 20: in the ipsilateral spinal … and this was effectively reduced… Page 15. Line 8: Refs 34-35. There are other clinical studies that should be listed here. In preclinical studies, in a rabbit neuropathic pain model induced by….; line 16: P We observed 45V and PRF 60V … provided a (not an) similar; line 19: allodynia developed at day 1 Page 16. Line 1: …SNI and lasted up to 28 days. Line 5: Furthermore, an animal study… line 7: Our study is the first to apply; line 13-14. For future – this sentence does not make sense, revise the wording. Line 16: which interferes with sensory; line 17: In other studies, Page 17. Line 4: shown to be…; line 7: that SNI induces ERK; line 8: ipsilateral spinal dorsal horn; line 9: was found to be increased 2.3-fold compared to; line 12: we conclude that the ; line 13: Furthermore, in neuropathic; line 17: our results strongly suggest; line 19: rats, and this reduces …

Response: Thanks for your kind suggestions for several minor details needed to revise. We have amended them and represented in the revised manuscript.

We sincerely hope the revisions and replies are satisfactory, and thank you again for your valuable suggestions and comments.
Authors’ Responses to Reviewers’ Comments

Reviewer: 2

Reviewer's report:

Yeh et al. investigated the effects of pulsed radiofrequency (PRF) on neuropathic pain behaviours in a spared nerve injury rat model. They report that PRF attenuated mechanical allodynia and cold allodynia in nerve-injured rats. PRF was also found to suppress the activation of ERK1/2 activation in the spinal cord following nerve injury. The authors therefore conclude that PRF may be effective in managing neuropathic pain.

Major Compulsory Revisions:

A major concern that I have with this paper is the association with ERK. The authors report an upregulation of pERK in the spinal cord of nerve injured animals, and that PRF prevented this upregulation. From this finding, the authors conclude that inhibition of ERK activation in the spinal cord is a key mechanism of action for PRF attenuation of neuropathic pain. This is a very weak conclusion as PRF could have affected a number of other cellular targets that have also been implicated in neuropathic pain. The findings implicating ERK are correlative. Thus, the conclusion made by the authors fail to show causation.

Response: We highly appreciate the reviewer’s valuable comment regarding to the correlation between ERK and PRF. We agree the reviewer’s viewpoint in that the evidence in this study is not sufficient to make a conclusion that inhibition of ERK activation in the spinal cord is a key mechanism of action for PRF attenuation of neuropathic pain. We have revised the relevant sentence, which is rewritten as:

“Immediate application of PRF at 45 or 60 V on the proximal (0.3–0.4 cm) nerve injury site was an effective treatment for the management of neuropathic pain; the
treatment was associated with an inhibition of ERK activity. For clinical translation, well-designed randomized controlled trials are required to identify the beneficial effect of PRF treatment proximal to the nerve injury site. Additional studies of the effect of PRF on the expression of ERK should be performed to provide evidences in treating neuropathic pain.” (please see page 19, line 2-8)

In addition, for clarity, an elaborating discussion regarding to the correlation between ERK and PRF has been added into the main text, please see page 18, line 2~18.

“Accumulating evidence shows that the activation of MAPKs (p38, ERK, and JNK) can induce the synthesis of proinflammatory/pronociceptive mediators via distinct molecular and cellular mechanisms, resulting in the enhancement and prolongation of pain. Another study indicated that early application of PRF adjacent to the DRG significantly diminished nerve ligation-induced mechanical allodynia for 7 days and thermal hyperalgesia on postoperative days 3–7 by downregulating p38 and ERK activation [Ref.1]. However, the long-term effect of PRF on analgesia remains unknown. Interestingly, nerve injury activates ERK in microglia and astrocytes, as observed in the early (days) and late (weeks) phases, respectively [2]. Therefore, we investigated the long-term effects of PRF on ERK activation. Other investigators also found a similar long-term efficacy of continuous PRF-DRG in the treatment of chronic pain in humans, and reported that PRF-DRG treatment had an analgesic effect that ranged from weeks to over 6 months, affording over 50% pain relief [Ref.3-4]. Clearly, further investigation is required to understand the cell types in the dorsal horns that are involved in this effect and the role of ERK in the regulation of allodynia and hyperalgesia.”

References:

1. Lin ML, Lin WT, Huang RY, Chen TC, Huang SH, Chang CH, Tsai SY, Chiu HW,


It is still unclear how PRF may be producing its antinociceptive effects; this should be more explicitly outlined. Also, were the experiments conducted so that the experimenter was blinded to the treatment groups?

Response: Thanks for your kind suggestions. Indeed, the mechanism of how PRF achieves its antinociceptive effects remains unclear. The findings in our study only established preliminary correlation of ERK with the attenuation of neuropathic pain of PRF in animal experiment.

The statistics analyzer and the experimenters worked independently and were blinded to the treatment groups. We also gave acknowledgement to the statistics analyzer. Please refer to the acknowledgements part. (page 20, line 13)

Minor Essential Revisions:

Differences in nociceptive behaviours are difficult to glean from Tables 1 and 2. These would be better represented as line graphs.

Response: Thank you for your suggestion. We have adjusted Table 1 and 2 to Figure
2 and 3, respectively, in the revised manuscript. The changes are as followed:

Figure 2. The paw withdrawal threshold in rats that were subjected to the sham operation, SNI without PRF (SNI group), or SNI plus either 45 V PRF or 60 V PRF (SNI+PRF-45V and SNI+PRF-60V groups, respectively). Mechanical allodynia was evaluated using dynamic plantar aesthesiometry. The data presented above were examined by ANOVA followed by Bonferroni test. D, day. \( P < 0.05 \), \( \# \# P < 0.01 \) SNI, SNI+PRF-45V, and SNI+PRF-60V compared with the sham. \( \* P < 0.05 \), \( \* \* P < 0.01 \) SNI+PRF-45V and SNI+PRF-60V compared with SNI.
Figure 3. Behavioral response in rats subjected to sham operation or SNI without PRF (SNI group) or SNI plus either 45 V PRF or 60 V PRF (SNI+PRF-45V and SNI+PRF-60V groups, respectively). Cold alldynia was evaluated by the acetone spray test. The data presented above were examined by ANOVA followed by Bonferroni test. D, day. \(^{*}P < 0.05, \^{**}P < 0.01\) SNI, SNI+PRF-45V, and SNI+PRF-60V compared with the sham. \(^{*}P < 0.05, \^{**}P < 0.01\) SNI+PRF-45V and SNI+PRF-60V compared with SNI.

The writing is this manuscript leaves much to be desired. There are many parts that need revision in terms of grammar and language.

Response: We sincerely apologize for any nonproficiency in English writing and the revised version has been professionally edited before resubmission.