Author's response to reviews

Title: Melatonin prevents morphine-induced hyperalgesia and tolerance in rats: role of protein kinase C and N-methyl-D-aspartate receptors

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Author's response to reviews: see over
Dear Editor,

Thank you very much for the email and the attached reviewers’ comments. We greatly appreciate that you give us this opportunity to revise the manuscript.

We entirely agree with the comments and have revised the manuscript accordingly.

A point-by-point response to reviewers’ comments is attached in separate pages. Changes have been made in the revised manuscript. We greatly appreciate your consideration. Many thanks.

Best regards

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A point-by-point response to the comments
We thank the reviewers for their valuable comments. The following is a point-by-point response to each comment and changes have been made in the revised manuscript with red characters. The reviewers’ comments are in an italicized font and our responses in a bold font.

Reviewer #1’ comments:
1. In Fig. 1 and Fig. 2, the dash-lines can be changed to solid since the different colors are used.
Response: We agree with the comments and have revised the lines in Fig. 1 and Fig.2.

Reviewer #2’ comments:
1. Morphine analgesic tolerance was induced as injected subcutaneously (10mg/kg) each day for consecutive 14 days. Why this regimen was used? As commonly accepted morphine analgesic tolerance could be induced by injection of morphine (s.c, i.p., and i.t.) twice or once per day for 5 or 7 days. Could the authors comment on this?
Response: Thanks for the detailed comments. We used the regimen and injection method according to our previous study [15]. In the manuscript, this paragraph reads: Based on our previous study, repetitive morphine treatment (10 mg/kg, s.c) given once daily for 14 days can produce the morphine tolerance to the analgesic effect [15].

2. Why the melatonin was administered 10 min after morphine injection? As antagonists or inhibitors which were used to affect the development of morphine-induced analgesic tolerance commonly were administered 30 min or longer before morphine injection. What is the theoretical basis of this?
Response: Thanks for the detailed comments. According to Wei’s study [3], the peak effect time of melatonin was about 20 min. And the peak effect time of morphine was about 30 min. So in order to study the interaction of melatonin and morphine, we use this method to administration.

3. Morphine-induced hyperalgesia and tolerance are two different phenomenons, as well as the mechanisms. Could the authors discuss these distinctively in the discussion section?
Response: We agree with the comments and have added the required content in P11L17-28.

4. The dose of melatonin used in this work is 10 mg/kg. Why this dose was selected?
Response: According to Raghavendra’s study, co-administration with morphine, melatonin in dose of 10 mg/kg can significantly reverse the
morphine tolerance and dependent [26]. So we used 10 mg/kg melatonin in this study.

5. P6L13. "Mechanical allodynia and thermal hyperalgesia of all rats were measured on day 0 (before drug administration), 1, 3, 5, 7 and 14.” Is any morphine-induced allodynia and hyperalgesia on day 0, 1, 3...? Whether this statement is accurate?
Response: Thanks for the detailed comments. We have revised this sentence in P6L13. In the manuscript, this sentence reads: Mechanical withdrawal threshold and thermal withdrawal latency of all rats were measured on day 0 (before drug administration), 1, 3, 5, 7 and 14.

6. P6L18. "The morphine tolerance was measured at 60 min after co-administration (10 mg/kg...”, how to measure morphine tolerance? Maybe the author means the mechanical threshold was measured.....
Response: Thanks for the comments. The morphine tolerance have been explained in P7L12-18. Development of morphine analgesic tolerance was assessed by hindpaw thermal withdrawal latency test.

7. P6L21. "Rats were euthanized after the final behavioral test...” Which anesthesia or other method was used to euthanize the rats?
Response: “Rats in each experimental group (n=3/6) were deeply anesthetized with pentobarbital (80 mg/kg, i. p.)” in P7L20-21 and P8L18-19.

8. Two-way ANOVA should be used to analyze the repetitive behavioral data.
Response: Yes, we agree with this comment. We have revised this content with red characters in P9L16.

9. This description of morphine tolerance P9L16 is not correct and should be deleted, and keep the MPAE% of morphine there.
Response: We agree with the comments. And we have deleted “morphine tolerance” P9L16.

10. P10L11. Morphine tolerance should have a consistent expression throughout this ms, etc morphine analgesic tolerance.
Response: We agree with the comments. And we have revised this expression with red characters in the manuscript.

11. How the melatonin inhibits the PKCgamma and NR1, and influence the development of morphine-induced analgesic tolerance? The author should make a discussion and statement in Discussion.
Response: We agree with the comments and have added this content in
12. Some tidying and revision of English of the MS is required.
Response: We agree with the reviewer. Pro. Xuejun Song at Parker University in the United States, had revised this manuscript.

Editor’s comments
13. I suggest as well that the description in the legend for Fig. 3C, as well as the related description in the Results section, needs to be expanded to explain how the interpretation of colocalization of PKC gamma and NR1 was derived.
Response: We agree with the comments. And We have added the content with red characters in the legend for Fig. 3C (P18L10-15) and the Results section (P11L5-7). Meanwhile, previous Fig. 3C about immunohistochemistry of PKCy and NR1 was not very well, so we have changed another figure.