Reviewer's report

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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Reviewer: Wei Jiang

Reviewer's report:

Comments to the Author

I think with this study the authors are addressing an important clinical problem, that of opioid-induced analgesic tolerance and hyperalgesia. I also agree, our knowledge about this phenomenon is limited and hence more research is needed. I have the following comments to make in order of appearance rather than importance.

1. Morphine analgesic tolerance was induced as injected subcutaneously (10 mg/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?

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?

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?

4. The dose of melatonin used in this work is 10 mg/kg. Why this dose was selected?

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?

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......

7. P6L21. "Rats were euthanized after the final behavioral test...” Which anesthesia or other method was used to euthanize the rats?

8. Two-way ANOVA should be used to analyze the repetitive behavioral data.
9. This description of morphine tolerance P9L16 is not correct and should be deleted, and keep the MPAE% of morphine there.

10. P10L11. Morphine tolerance should have a consistent expression throughout this ms, etc morphine analgesic tolerance.

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.

12. Some tidying and revision of English of the MS is required.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.