Reviewer’s report

Title: Antinociceptive tolerance effects of NSAIDs microinjected into dorsal hippocampus

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Reviewer: Kabirullah Lutfy

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The manuscript by Gurtskaia and colleagues reports that microinjection of some NSAIDs into the dorsal hippocampus (DH) will induce antinociception and tolerance develops to this effect after repeated administration of each NSAID tested in the current study. Also, the effect of these drugs is blocked by naloxone showing the involvement of the endogenous opioid system in this response. Overall, the current research is of interest to the readers of the journal. However, there still remain several issues, in particular, the interpretation of data and experimental design, that need to be addressed.

Major:

1. The current study assess antinociception in the same animals on three consecutive days. On day 1, all NSAIDs studied in the present manuscript induce antinociception. On days 2 and 3, this response reduces, suggesting the development of tolerance. However, the tolerance could be because of the repeated testing and thus a control group where animals injected with saline or at least of the NSAIDs for three days and tested only on day 3 should be included to ensure that its tolerance but not hyperalgesia or any other mechanical damage to the tail or paw of the animals that is responsible for tolerance.

2. Naloxone blocks antinociceptive effect of NSAIDs rather than tolerance. If naloxone blocks tolerance, then NSAIDs should be effective in inducing antinociception on days 2 and 3. Thus, this should be corrected throughout the manuscript.

3. Naloxone is not a mu-selective antagonist and should be changed to naloxone, a non-selective opioid receptor antagonist throughout the manuscript.

4. The length of the cannula (12 mm) appeared too long for the dorsal hippocampus. Is it 2 mm or 12 mm? Also, the amount of time for the duration of injection should be provided.

5. Figures 5 and 6 are not informative and should be removed or the information should be presented in a table.

6. There are many changes that need to be made to make the paper read better. Here are some suggestions:

   a. The title of the article should be changed since "Antinociceptive tolerance..."
effects" does not seem a correct clause.

b. The Methods section of the Abstract should be changed. For example, the first two sentences can be replaced by "Male rats were implanted with a guide cannula in the DH and tested for antinociception following injection of NSAIDs into the DH in the tail flick and hot plate tests."

c. Please change the Results section of the Abstract as follows:

.......in the TF and HP tests compared to controls treated with saline into the DH. Subsequent tests on days 2 and 3, however, showed that the antinociceptive effect of NSAIDs progressively decreased, suggesting tolerance developed to this effect of NSAIDs. Both pretreatment and post-treatment with the opioid antagonist into the DH significantly reduced the antinociceptive effect of NSAIDs in both pain models.

d. Conclusion of the Abstract should be changed to "Our results indicate that microinjection of NSAIDs into the DH induces antinociception which is mediated via the opioid system and exhibits tolerance.

e. P. 3, Bkgd, Line 6. please include the reference after shown. Also, please change the next sentence as "Particularly, mice with spared nerve injury (SNI) neuropathic pain were unable..........

f. The next sentence should be changed to "Additionally, mice with SNI compared with sham........

g. P. 3, Third Paragraph, Line 2, the clause "(NRM) the microinjection" should be changed to " "(NRM), microinjection"

h. last line on this page, please remove the citation inside the bracket and just leave the number assigned to that reference.

i. P. 4, first sentence, please change this sentence to "......DH induces antinociception and whether tolerance would develop to this action of NSAID and whether this action is mediated via the endogenous opioid system".

j. P. 5, Line 2, should be changed to "......animals were tested for antinociception using the TF and HP tests."

k. anti-nociception should be changed to antinociception through the manuscript.

l. Result section, P. 6, "At the second and third days" should be changed to "On the second and third days"

m. P.6, last paragraph, lines 1 and 2, "prevents antinociception induced by NSAID microinjected into the DH".

n. P. 7, third paragraph, line 2, please delete extremely. Also, please change "........microinjection effects of dipyrone and aspirin....." to "........microinjection of dipyrone and aspirin....."
p. Last line, first paragraph on p. 8, should be changed to CTOP blocks.

q. Next sentence, please changed "As we mentioned above" to "As stated above"

r. Conclusion (P. 9), Lines 2-4, should be changed to "......DH induce antinociception that is opioid mediated. The finding confirms.................NSAIDs may be mediated via the endogenous opioid system, as its blocked by naloxone and exhibits tolerance."

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

Quality of written English: Not suitable for publication unless extensively edited

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

Declaration of competing interests:

I have no competing interest with the content of this manuscript.