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OPEN PEER REVIEW

Effects of Chronic Pain Following Unilateral Ureteral Obstruction on Hippocampal CA1 Pyramidal Neurons in Male Wistar Rats

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1 Original submission

4 comments · 25 Feb 2024 – 5 Mar 2024

Referee 1 Reviewer

MAJOR REVISION 25 Feb 2024

1- The title is wrong:
It is unilateral ureteral obstruction, not urethral obstruction.

2- How did the researchers define chronic pain?
In humans, chronic pain is usually defined as pain persisting for at least 6 months. However, the entire process from the operation to the death of the rats lasted just 6 days. How did they define it as chronic pain? And the most important point is “not every ureteral obstruction provokes pain”.
You can discuss the effect of unilateral ureteral obstruction on the measured variables and not the effect of chronic pain. How did you prove and measure their pain?

3- They reported the difference in the same measured variable in the abstract as $p<0.05$, in the results as $p<0.001$, and in the discussion and figure as $P<0.0001$.

4- Endogenous as well as exogenous opioids act as pain relievers. They used naloxone at a high dose (1 mg/kg), so it might have blocked the analgesic effect of endogenous opioids. However, they did not discuss this important effect.

5- Lines 58 and 85 and 145: It is not a renal but a ureteral obstruction.

6- They mentioned the weight of the rats in the abstract as 250-300 grams and in the materials and methods as 230-280 grams.

7- It is very important to know whether the ureteral obstruction was complete or partial. This important point should be mentioned.

8- Line 113: 4 cases from each group

9- Line 116: -80 degrees centigrade? The unit should be mentioned. And why this extremely low temperature? The optimal freezing point for rat brain tissue seems to be -3 degrees centigrade.

10- Most importantly, we need confirmation of pain and not confirmation of a swollen kidney.

11- Lines 118, 129: The kits' countries of manufacture should be mentioned.

12- Line 120: Are the detection range 82.3-2000 and the sensitivity <25 pg/ml?

13- Line 121: Were standards pipetted into the plate?

14- Lines 145 – 148: Unilateral ureteral obstruction does not necessarily cause weight loss.
If the rats in the intervention groups lost weight after the operation compared to the sham group, they have to mention the mean weight after the intervention and discuss this important bias.

15- Lines 153, 159: The mean numbers of neurons in the sham and lesion groups are wrong in the parentheses.

16- Line 183: NGF is increased in the UUO+NA compared to UUO and not decreased.

17- Line 236: What is 122? Line 271: What is 163?

18- The article needs grammatical editing.

19- The abbreviations used have not been defined at their first appearance.

20- The figures are not inserted in the correct order.

Referee 2 Reviewer — MAJOR REVISION — 5 Mar 2024

Dear AE,
The research article presents a well-conducted experimental study investigating the effects of chronic pain on hippocampal neurons in a rat model of ureteral obstruction. The study provides valuable insights into the neurobiological mechanisms underlying chronic pain-induced memory impairment and highlights the potential therapeutic role of naloxone in mitigating neurodegenerative processes.
Addressing the following limitations and enhancing methodological rigor would strengthen the validity and impact of the study in the field of neuroscience:
- The abstract provides a concise overview of the research, outlining the investigation into the effects of chronic pain following unilateral ureteral obstruction on hippocampal neurons in male Wistar rats. The objectives, methodology, and key findings are clearly presented. It would be beneficial to include specific numerical results in the abstract to provide readers with a clearer understanding of the study outcomes.
- Some sentences lack references (for example, the first sentence of the Introduction and the first sentence of the Discussion). All sentences quoted from other articles must have references at the end.
- The statistical analyses are described briefly, but additional details on specific statistical tests used, including assumptions and corrections for multiple comparisons, would enhance the transparency and rigor of the study.
- While the histological studies are described, details regarding staining protocols, quantification methods, and criteria for identifying neuronal populations are lacking. Providing explicit criteria for neuronal identification and quantification would enhance the reliability and reproducibility of the histological analyses.
- The use of a unilateral ureteral obstruction (UUO) model in rats to simulate chronic pain has limitations in mimicking the complexity and variability of chronic pain conditions in humans. Discussing the relevance and limitations of the UUO model for studying chronic pain and its effects on hippocampal neurons in the context of human clinical conditions would provide important context for interpreting the findings.

Associate Editor — MINOR REVISION — 5 Mar 2024

Dear EIC,
Thanks to the authors and appreciation to the researchers. The manuscript is a valuable piece of research. But some revisions are needed. The authors should revise it based on the reviewers' comments. Also, minor changes should be made in the abstract:
The study design should be mentioned in the first line of the methods section of the ABSTRACT.

Editor-in-Chief — MAJOR REVISION — 5 Mar 2024

Dear Author,
Thank you for submitting the article. It needs some revision. Please revise it based on the reviewers' and AE's comments.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear editor in chief,

Thank you very much for very useful comments from the reviewers and associate Editor. The authors tried their best to answer all questions and applied the necessary changes through the whole text and highlighted in yellow. It should be noted that we omitted some words from the manuscript due to words limitation of the journal. The comments are answered as follows;

Reviewer 1:

1- The title is wrong:

It is unilateral ureteral obstruction not urethral.

Response: It is corrected in the title now.

2- How did the researches define chronic pain?

Chronic pain in human is usually defined as a pain persisting for at least 6 months. However, all the process of operation through death of the rats were just 6 days. How they defined it as a chronic pain?

Response: We answered the question and some related lines are added to the text in the discussion sections, lines **246-250**; According to the International Classification of Diseases (ICD-11), chronic pain in humans is defined as pain that persists or recurs for more than 3 months (1). Animal models of pain are generated to mimic human pain experiences, aiming to investigate potential pain-relief methods and develop new pharmacological agents(2). Typically, small animals like mice and rats are utilized in these models. Due to their shorter lifespan compared to humans, the distinction between acute and chronic pain differs in these animals. Acute pain is described as lasting for seconds to hours, while chronic pain persists for several days in these animal models (2, 3). In our study, we induced complete ureteral obstruction in the UUO animal model to replicate the intense abdominal or flank pain caused by different disease conditions such as kidney stones in humans (4). Since in UUO the pain persisted for several days, this model matches to chronic pain.

And the most important is “not every ureteral obstruction provokes pain”.

You can discuss effect of unilateral ureteral obstruction on the measured variables and not the effect of chronic pain. How did you prove and measured their pain?

Response: Visceral pain model is typically developed by irritating the peritoneum or hollow organs of the pelvis, urogenital and abdomen (2). The injuries are meant to mimic disease states such as irritable bowel syndrome (IBS) and colic pain (23, 24). We induced complete ureteral

obstruction in the UUO animal model to simulate the severe abdominal or flank pain induced by some issues such as kidney stones in humans (4). The presence of pain in this animal model is proved by symptoms such as weight loss, anorexia and reduced social exploration in animals (5, 6). Given that the pain persists for six days in our study, the UUO model induces severe and prolonged pain, aligning with the chronic pain category.

As our study did not prioritize assessing pain intensity, this aspect was omitted. It should be mentioned that measuring pain in visceral organs poses challenges due to the absence of a precise brain map for these areas, presenting a complication in studying visceral pain (7-9). Moreover, by inducing complete rather than partial ureteral obstruction, we ensured a consistent level of painful stimulus across all animals. Consequently, our focus was on evaluating neurodegeneration in the hippocampus following the induction of visceral pain in the UUO animal model.

3- They reported the difference in the same measured variable in abstract as $p<0.05$, in results as $p<0.001$, and in the and discussion and figure as $P<0.0001$.

Response: all p-values are corrected in the abstract and highlighted in yellow.

4- Endogenous as well as exogenous opioids act as pain relievers. They used naloxone at a high dose (1mg/ kg) so it might have blocked the analgesic effect of endogenous opioids. However, they did not discuss this important effect.

Response: The endogenous opioid system is the innate pain-relieving system and includes widely distributed neurons that produce different opioids; beta-endorphin, met-enkephalins, leu-enkephalins, and dynorphins (10). These opioids act as neurotransmitters and neuromodulators at the three famous receptors named mu, delta, and kappa. Like their endogenous counterparts, the opioid drugs, act at the same receptors to produce analgesia or pain relief effects (11). Naloxone hydrochloride, a synthetic N-allyl derivative of oxymorphone, is essentially a pure opioids antagonist, relatively safe and can block the effect of endogenous and exogenous opioids (12). It seems that naloxone performs its antagonistic activity in all three opioid receptors. Some sentences are added to the discussion part, lines **253-260**, page 10.

5- Lines 58 and 85 and 145: It is not a renal but a ureteral obstruction.

Response: all are corrected now.

6- They mentioned the weight of rats in the abstract 250-300 grams and in the materials and methods 230-280 grams.

Response: 230-280 g is correct and added to the text.

7- It is very important whether the ureteral obstruction was complete or partial? This important point should be mentioned.

Response: it is complete obstruction and this is added to the line **76**, page **4**, in the Method part.

8- Line 113: 4 cases from each group.

Response: it is corrected in line **123**, page **5**.

9- Line 116 : -80 degrees centigrade? The unit should be mentioned. And why this extreme low temperature? The optimal freezing point for rat brain tissue seems to be -3 degrees centigrade.

Response: The unit for -80°C is corrected in the text. It should be noted that, fast freezing is very important in keeping protein safe in the brain tissue. There are some documents mentioned that some markers are actively lost over few months in rat tissue which stored at -20°C, while there was little or no such lost at -70°C or even -90°C (13, 14).

10-The most important we need confirmation for pain and not confirmation for swollen kidney.

Response: As we answered in question 2, Visceral pain model is typically produced/studied by irritating the peritoneum or hollow organs of the pelvis, urogenital and abdomen (2). The injuries are meant to mimic disease states such as irritable bowel syndrome (IBS) and colic pain (23, 24). We induced complete ureteral obstruction in the UUO animal model to simulate the severe abdominal or flank pain induced by kidney stones in humans (4). The presence of pain in this animal model is evident and indicated by symptoms such as weight loss, anorexia and reduced social exploration in animals (5, 6). Given that the pain persists for 6 days in our study, the UUO model induces severe and prolonged pain, aligning with the chronic pain category.

We added some sentences in the discussion part lines **246-249**, pages **10** “In the present study, with the induction of the UUO model, chronic visceral pain has developed. The presence of pain in the UUO animal model is proved by symptoms such as weight loss, anorexia, and reduced social exploration in animals (5, 6). Our results showed anorexia and approximately 11% weight loss in animals undergoing UUO”.

11- Lines 118, 129: the kits' countries of manufacturing should be mentioned.

Response: the country for both is USA and it is added to line **128** and **138**.

12- Line 120: Are the detection range 82.3-2000 and the sensitivity <25 pg/ml?

Response: the detection range is 82.3 -20000, and the sensitivity =25. It is corrected.

13- Line 121: Were standards pipetted into the plate?

Response: Standards and samples were pipetted in the different wells.

14- Lines 145 – 148: Unilateral ureteral obstruction does not necessarily need weight loss. If the rats had weight loss after the operation in the intervention groups compared to the sham group, they have to mention the mean of weight after intervention and discuss about this important bias.

Response: The presence of pain in UUO animal model is evident and indicated by symptoms such as weight loss, anorexia and reduced social exploration in animals as we mentioned in question-2 (5, 6). Our results showed anorexia and approximately 11% weight loss in animals with UUO. However, sham group animals did not show these symptoms, although they had gone under surgery. Weight loss occurs due to pain during the first 2 weeks after ureteral ligation and might disappear around 28 days later (5).

The mean of wight was 259.4± 4.36 g before surgery and changed to 230.2 ± 3.3 g in UUO animals. However, the mean of weight in sham-operated animals was 256 ± 4.5 g before the experiments and changed to 265 ± 4.7 g, these sentences are added to the results section lines **151-157**, and lines **246-250** in the discussion part.

15- Lines 153, 159: Mean numbers of neurons in sham and lesion groups are wrong in the parenthesis.

Response: Both are corrected in the text now.

16- Line 183: NGF is increased in the UUO+NA compared to UUO and not decreased.

Response: It is corrected now.

17- Line 236: what is 122? Line 271 what is 163?

Response: the both numbers are replaced by the proper references.

18- The article needs grammatical edition.

Response: the whole text is checked for grammar and spell mistakes.

19- The abbreviations used have not been defined on their first appearance.

Response: whole text was checked for this issue, and we tried to add abbreviations on their first appearance.

20- The figures are not inserted as their true order.

Response: there are 4 figures in our manuscript. We checked for the order. Fig 1. showed the area for injection. Fig 2. showed the mean number of neurons in groups. Fig 3. showed TNFα level and Fig 4. showed NGF level.

Reviewer 3:

Dear AE,

The research article presents a well-conducted experimental study investigating the effects of chronic pain on hippocampal neurons in a rat model of ureteral obstruction. The study provides valuable insights into the neurobiological mechanisms underlying chronic pain-induced memory impairment and highlights the potential therapeutic role of naloxone in mitigating neurodegenerative processes.

Addressing the aforementioned limitations and enhancing methodological rigor would strengthen the validity and impact of the study in the field of neuroscience:

-The abstract provides a concise overview of the research, outlining the investigation into the effects of chronic pain following unilateral ureteral obstruction on hippocampal neurons in male Wistar rats. The objectives, methodology, and key findings are clearly presented. It would be beneficial to include specific numerical results in the abstract to provide readers with a clearer understanding of the study outcomes.

Response: More numerical results are now added to the abstract in the result part and highlighted in yellow.

-Some sentences lack references(FOR EXAMPLE: first sentence of Introduction and first sentence of Discussion). All sentences quoted from other articles must have references at the end of themselves.

Response: the introduction and discussion sections are checked and references are added as necessary. The number of references is **38** now.

-The statistical analyses are described briefly, but additional details on specific statistical tests used, including assumptions and corrections for multiple comparisons, would enhance the transparency and rigor of the study.

Response: the following sentences are added to the statistical analysis “The data was analyzed using Prism Graph-pad (VI) software. Group comparisons were made using the Student t-test followed by the Mann-Whitney post-test. The results were presented as Mean ± SEM, with significance defined as $P<0.05$ at a 95% confidence level.” in lines **148-150**.

-While the histological studies are described, details regarding staining protocols, quantification methods, and criteria for identifying neuronal populations are lacking. Providing explicit criteria for neuronal identification and quantification would enhance the reliability and reproducibility of the histological analyses.

Response: cresyl violet solution preparation technique and criteria for neuronal counting are added to the text as separate sections in lines **96-99** and **116-119** as following
"**Cresyl violet acetate solution:** 0.1g cresyl violet added to 100 ml distilled water in order to prepare 0.1% solution. Some drops of glacial acid were added before use. Solution was warmed and filtered for optimum result".

Criteria for neural counting: Each cell with intact cell membrane and obvious nucleus were counted as a healthy preserved neuron and counted in the specified area."

Staining protocol section is completed in lines **101-108**.

-The use of a unilateral ureteral obstruction (UUO) model in rats to simulate chronic pain has limitations in mimicking the complexity and variability of chronic pain conditions in humans. Discussing the relevance and limitations of the UUO model for studying chronic pain and its effects on hippocampal neurons in the context of human clinical conditions would provide important context for interpreting the findings.

Response: Animal models of pain are generated to mimic human pain experiences (2). However, there are some limitations in pain animal models. First, according to (ICD-11), chronic pain in humans is defined as pain that persists for more than 3 months (1). compared to humans, rats and mice have shorter life span consequently, acute pain is described as lasting for seconds to hours, while chronic pain persists for several days (2, 3). Hence, in the current study with complete ureteral obstruction in the UUO animal model in order to mimic the intense colic pain in humans (4) induced pain persisted for several days which aligns with chronic pain. Second, renal colic pain resulted from obstruction in urine flow or stones, is frequently considered as the most excruciating pain experienced by patients (15). However, animals cannot communicate their pain experience like humans leading to difficulties in assessing pain levels.

Enduring pain in humans not only causes discomfort but also contributes to neuronal damage in the brain(16-18). In clinics opioids have been employed for pain relief in renal colic due to their potent effects (19). However, despite their effectiveness, they may lead to adverse effects such as respiratory depression and narcotic dependence (4, 19, 20). Our findings indicate that naloxone as an antagonist of opioid receptors can prevent neuronal toxicity induced by chronic pain. Although, human clinical studies and translational research approaches are necessary beside animal researches.

We added some sentences in the discussion part lines **225-232**, page **9** and **290-295**, page **11**.

Associate Editor's Comments:

Associate Editor 1:

Dear EIC,

Thanks to the authors and appreciation to the researchers. The manuscript is valuable research. But some revisions are needed. Authors should revise it based on reviewers' comments. Also, minor changes should be done in abstract:

Study design should be mentioned in first line of method section of ABSTRACT.

Response: our research is a case-control study and this is added to the first line of the Method section in the abstract.

All the best,

Corresponding author

References:

1. Treede RD, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, et al. Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). Pain. 2019;160(1):19-27.
2. Abboud C, Duveau A, Bouali-Benazzouz R, Massé K, Mattar J, Brochoire L, et al. Animal models of pain: diversity and benefits. Journal of neuroscience methods. 2021;348:108997.
3. Ma C. Animal models of pain. International anesthesiology clinics. 2007;45(2):121-31.
4. Fontenelle LF, Sarti TD. Kidney stones: treatment and prevention. American family physician. 2019;99(8):490-6.
5. Prieto-Carrasco R, Silva-Palacios A, Rojas-Morales P, Aparicio-Trejo OE, Medina-Reyes EI, Hernandez-Cruz EY, et al. Unilateral Ureteral Obstruction for 28 Days in Rats Is Not Associated with Changes in Cardiac Function or Alterations in Mitochondrial Function. Biology (Basel). 2021;10(7).
6. Pitzer C, Kuner R, Tappe-Theodor A. Voluntary and evoked behavioral correlates in inflammatory pain conditions under different social housing conditions. Pain Rep. 2016;1(1):e564.
7. Johns E, Tracey I. Neuroimaging of Visceral Pain. Rev Pain. 2009;3(2):2-5.
8. Song J, Kim YK. Animal models for the study of depressive disorder. CNS Neurosci Ther. 2021;27(6):633-42.
9. West C, Neufeld K-AM. Animal models of visceral pain and the role of the microbiome. Neurobiology of Pain. 2021;10:100064.
10. Hill RG. Endogenous opioids and pain: a review. J R Soc Med. 1981;74(6):448-50.
11. Holden JE, Jeong Y, Forrest JM. The endogenous opioid system and clinical pain management. AACN Clin Issues. 2005;16(3):291-301.
12. Handal KA, Schauben JL, Salamone FRJAoem. Naloxone. 1983;12(7):438-45.
13. Das GD, Houle JD, Brasko J, Das KG. Freezing of neural tissues and their transplantation in the brain of rats: technical details and histological observations. J Neurosci Methods. 1983;8(1):1-15.
14. Iwanowski L. [Ultrastructure of the choroid plexus]. Neuropatol Pol. 1986;24(3):291-303.
15. Bektas F, Eken C, Karadeniz O, Goksu E, Cubuk M, Cete Y. Intravenous paracetamol or morphine for the treatment of renal colic: a randomized, placebo-controlled trial. Annals of emergency medicine. 2009;54(4):568-74.
16. Tyrtyshnaia A, Manzhulo I. Neuropathic Pain Causes Memory Deficits and Dendrite Tree Morphology Changes in Mouse Hippocampus. J Pain Res. 2020;13:345-54.
17. Chen L, Qin Q, Huang P, Cao F, Yin M, Xie Y, et al. Chronic pain accelerates cognitive impairment by reducing hippocampal neurogenesis may via CCL2/CCR2 signaling in APP/PS1 mice. Brain Research Bulletin. 2023;205:110801.
18. da Silva MD, Guginski G, Sato KL, Sanada LS, Sluka KA, Santos AR. Persistent pain induces mood problems and memory loss by the involvement of cytokines, growth factors, and supraspinal glial cells. Brain, Behavior, & Immunity-Health. 2020;7:100118.
19. Golzari SE, Soleimanpour H, Rahmani F, Mehr NZ, Safari S, Heshmat Y, et al. Therapeutic approaches for renal colic in the emergency department: a review article. Anesthesiology and pain medicine. 2014;4(1).

20. Phillips E, Hinck B, Pedro R, Makhlouf A, Kriedberg C, Hendlin K, et al. Celecoxib in the management of acute renal colic: a randomized controlled clinical trial. Urology. 2009;74(5):994-9.

2 Revision 1

3 comments · 25 Mar 2024 – 26 Mar 2024

Referee 1 Reviewer

ACCEPT 25 Mar 2024

Thanks for the required corrections.

Associate Editor

ACCEPT 26 Mar 2024

Dear Editor in Chief,

Thanks for assigning me as associate editor to handle this manuscript. The authors have successfully addressed the peer-review comments. This version of the manuscript is acceptable for publication in Archives of Neuroscience Journal.
Regards,

Editor-in-Chief

ACCEPT 26 Mar 2024

Dear Authors,
I appreciate your effort in addressing the concerns. Your manuscript is acceptable now.