

Editorial Office's comments:

- 1) Science Editor: Recommend for potential acceptance. 1 Scientific quality: B. The article is comparison of three administration modes of establishing a zebrafish seizure model induced by NMDA, within the scope of WJP. Summary of peer-review report: Overall, a competent study with some useful findings. The manuscript was well organized and provided some useful information. There were some mistakes in the manuscript. 5 figures, 49 references were cited, including 15 latest references from 2017-2020. No self-citation. 2 Language quality: B. Language certification is not qualified. 3 Academic norms and rules: Basic Study. Copyright license agreement, IRB and the ARRIVE Guidelines files are complete and qualified. The format of the Conflict-of-Interest statement is not correct. Being search and CrossCheck are eligible. 4 Others: With National Natural Science Foundation of China financial support. Corresponding author has not published articles in WJP. Invited manuscript.

Response: Thanks for your comments very much. We have corrected the mistakes in our manuscript. Due to the adjustment of structure in the discussion section, some cited documents have been deleted, from the original total of 49 references to 44. We invited a professional to polish our manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format and general readability, to meet your direct publishing needs. As for Conflict-of-Interest, we also reworked it. Revised portions are marked in **blue** throughout the paper, and changes to the manuscript are also highlighted with **red** text. Thanks again for your views and positive comments.

1 Please re-provide the original figure documents. All submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes; For line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes; Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. I have uploaded a sample document to the submission system.

This is our mistake that we did not understand those requirements for figures preparation. We have prepared the figures using PowerPoint with all graphs or arrows or text portions can be reprocessed.

2 Please re-write the article highlights section. Detailed writing requirements for "article highlights" can be found in the Guidelines and Requirements for Manuscript Revision which can be downloaded after you login to the submission.

Thanks for your kind reminder. We have re-written the highlights section in our article according to the Guidelines and Requirements for Manuscript Revision.

- 2) Editorial Office Director: Recommend for potential acceptance. 1 Scientific quality: I have checked the comments written by the science editor. I basically agree with the science editor. The topic of epilepsy is in the scope of WJP. Reviewer 03887097 showed that this is a competent study with some useful findings, but there are some following comments for the authors to contemplate. The questions raised by the reviewer should be answered. There are 5 figures in the manuscript. Forty-five references were cited, including fourteen references published in the last three years. No self-citation. 2 Language evaluation: I agree with the comments written by the science editor. There is no any Language editing certificate. 3 Academic norms and rules: I have checked the documents including the copyright license agreement, biostatistics review certificate, and The ARRIVE Guidelines, which are qualified. The institutional review board approval, Institutional Animal Care and Use Committee Approval Form or Document and conflict-of-interest

disclosure form are not qualified. The authors must provide the official Institutional Animal Care and Use Committee Approval Form or Document. No academic misconduct was found in the CrossCheck investigation and the Bing search. 4 Others: (1) Supported by National Natural Science Foundation of China. The authors should provide the grant application form(s). (2) Invited manuscript.

Response: Very grateful for your comments on our manuscript. The questions raised by the reviewer will be answered in the following part. We have re-edited your above-mentioned unqualified material documents. We make sure supplements official after our revision. Thanks again!

- 3) Company Editor-in-Chief: I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions.

Response: Thank you very much for meaningful comments on our work. We have revised our article and added information to address the critiques of the reviewer. Look forward to the publication of our article.

Specific Comments from Reviewer to Authors:

Overall, a competent study with some useful findings.

Response: Thanks for your time and consideration of our manuscript. Each of your comments below will be responded point by point.

Question 1: In the discussion section, authors should also mention that while zebrafish seizure models are appropriate for anti-convulsant discovery and study ictogenesis, they do not capture the entire disease process. When studying epilepsy and screening for anti-epileptic drugs, epilepsy models that truly reflect the pathogenesis and characteristics of the different human epilepsies are still lacking.

Response 1: Thanks for your tips and we have added the concern to the discussion section. But there are several points we want to explain. First, epilepsy research largely relies on models in vivo such as the acute seizure models induced by PTZ and MES models, which have a relatively high efficiency of screening anti-epileptic compounds^[1-2]. But with the deepening of research, it has been found that such models cannot mimic the entire development process of human epilepsy, nor can they simulate the pathophysiological process of refractory epilepsy and drug-resistant epilepsy^[3]. Second, chronic epilepsy models provide a basis for the genesis and development of epilepsy in order to make up for the shortcomings of acute ones. But such models cannot represent pathophysiological changes of epilepsies caused by specific factors such as trauma and stroke^[4]. Besides, genetic epilepsy models and drug-resistant epilepsy models have been built. We believe that the constructions of more and more models will make us discover more about the underlying pathogenesis and characteristic of different human epilepsies.

References:

- 1 **Torres-Hernández BA**, Del Valle-Mojica LM, Ortiz JG. Valerianic acid and valeriana officinalis extracts delay onset of pentylenetetrazole (PTZ)-induced seizures in adult danio rerio (Zebrafish). *BMC Complement Altern Med*. 2015 Jul 14;15:228 [PMID: 261689 DOI:10.1186/s12906-015-0731-3]
- 2 **Saadabadi A**, Kohen B, Irandoust M, Shafaroodi H, Mohammadpour T, Rezayat M, Davood A. 2, 5-Disubstituted Phthalimides: Design, Synthesis and Anticonvulsant Activity in scPTZ and MES Models. *Curr*

Comput Aided Drug Des. 2018;14(4):310–321[PMID:29766822 DOI:10.2174/1573409914666180516115450]

- 3 **Hui YY**, Ahmad N, Makmor-Bakry M. Pathogenesis of epilepsy: Challenges in animal models. *Iran J Basic Med Sci* 2013; 16(11):1119-32 [PMID:24494063 PMCID: PMC3909622]
- 4 **Verny M**, Greffard S. Contribution of geriatric model to the management of an epileptic seizure or epilepsy. *Geriatr Psychol Neuropsychiatr Vieil* 2019;17(S1):21–24 [PMID:30916647 DOI:10.1684/pnv.2019.0791]

Question 2: what is the advantage of the prodes method compared to current genetic zebrafish epilepsy models generated using MOs or hyperthermia-induced zebrafish seizure models? This did not come through in your manuscript.

Response 2: This is a great suggestion for our study. We have added this information to the discussion section to clarify the advantages of our methods as follows:

“Antisense morpholine oligonucleotides (MOs) and hyperthermia have been used to construct zebrafish epilepsy models in previous studies. Although MOs can effectively interfere with protein synthesis of target genes, it can induce p53-dependent apoptosis and non-targeted cell-specific effects in gene expression, which in turn affect behavioral phenotype analysis^[5]. Hyperthermia-induced zebrafish seizure model is more suitable for studying the mechanism of epileptic seizures in vivo and for acute seizure of chronic processes, but it does not show any persistence^[6]. Both methods are appropriate for studying the mechanism of zebrafish seizures during innate or embryonic development. However, the methods we use apply to study the process of seizures in adulthood. Not only can they induce characteristic seizures which are similar to the reactions observed in the case of mammalian seizures, but they also emphasize the role of the zebrafish model in glutamate excitatory neurotransmission by using them. In addition, the methods we proposed can screen out the effect of psychotropic drugs and toxicity to the animal at a glance, and reduce the rate twist and turn in the process of drug development.”

References:

- 5 **Mayu H**, Tomoya K. Formation of mos RNA granules in the zebrafish oocyte that differ from cyclin B1 RNA granules in distribution, density and regulation[J]. *Eur J Cell Biol* 2016;95(12):563-573[PMID: 27756483 DOI: 10.1016/j.ejcb.2016.10.001]
- 6 **Robert F**, Hunt RF, Hortopan GA, Gillespie A, Baraban SC. A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. *Exp Neurol* 2013;240:108-11 [PMID: 23178581 DOI: 10.1016/j.expneurol.2012.06.013]

Question 3: The use of the scn1lab mutant zebrafish model that mimics Dravet syndrome, a severe treatment-resistant epilepsy syndrome that starts within the first year of life, already led to the discovery of clemizole as a potential treatment (citation:ncbi.nlm.nih.gov/pubmed/24002024). We must study epilepsy as a syndrome rather than seizures as isolated symptoms.

Response 3: This is a great reference for our study. In this paper, it was introduced that Dravet syndrome (DS) is a catastrophic pediatric epilepsy with severe intellectual disability, impaired social development, and persistent drug-resistant seizures. One of its primary monogenic causes is mutations in Nav1.1 (SCN1A), a voltage-gated sodium channel. And it was found that etogenic diet, diazepam, valproate, potassium bromide and stiripentol attenuate mutant seizure activity; seven other antiepileptic drugs have no effect. And clomizole (a histamine receptor antagonist) is effective for the gene-induced epilepsy of SCN1lab zebrafish(a model of DS caused by SCN1a mutation), persistent drug-resistant epilepsy^[7]. Based on this paper, we consulted psychiatry textbooks and corrected the description of epilepsy as follows:

“Epilepsy is a complex neurological disorder characterized by recurrent, unprovoked seizures resulting from the sudden abnormal discharge of brain neurons. It leads to transient brain dysfunction, manifested by abnormal

physical movements and consciousness. And it can occur at any age, affecting approximately 65 million worldwide, one-third of which are still estimated to suffer from refractory seizures. There is an urgent need for further establishment of seizure models in animals, which provides an approach to model epilepsy and could be used to identify novel anti-epileptic therapeutics in the future.”

What’s more, in the introduction section, we introduced in more detail that epilepsy is a chronic brain disorder caused by abnormal, excessive and synchronous neuronal activities in the brain. The clinical manifestations are characterized by paroxysmal, transient, repetitive and stereotyped. The location of abnormal discharge neurons and the range of abnormal discharge spread are different, leading to different forms of seizure, which can be manifested as sensory, motor, conscious, mental, behavioral, autonomic dysfunction or a combination of multiple dysfunctions^[8]. According to the WHO report, there are many causes of epilepsy, such as stroke, brain trauma, and central nervous system infection^[9]. Although it is generally believed that about two-thirds of epilepsy is idiopathic, most of which are now considered to be hereditary. And it has various psychiatric complications such as depression, anxiety and cognitive defects^[10,11]. The molecular mechanism of epilepsy syndrome is still not fully understood^[3,12]. Based on the above considerations, it is accurate that epilepsy is defined as a syndrome rather than a disease. Therefore, the research on epilepsy needs to be considered from many aspects in the future, not just an independent manifestation.

References:

- 7 **Baraban SC**, Dinday MT, Hortopan GA. Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. *Nat Commun* 2013;4:2410[PMID:24002024 DOI: 10.1038/ncomms3410]
- 8 **Liao MJ**, Kundap U, Rosch RE, Burrows DRW, Meyer MP, Ouled ABB, Cossette P, Samarut É. Targeted knockout of GABA receptor gamma 2 subunit provokes transient light-induced reflex seizures in zebrafish larvae. *Dis Model Mech* 2019; Nov 11;12(11). [PMID: 31582559 DOI:10.1242/dmm.040782]
- 9 World Health Organization. WHO’s first Global Epilepsy Report. Available from: <https://www.who.int/en/news-room/fact-sheets/detail/epilepsy>
- 10 **Canzian J**, Müller TE, Franscescon F, Michelotti P, Fontana BD, Costa FV, Rosemberg DB. Modeling psychiatric comorbid symptoms of epileptic seizures in zebrafish. *J Psychiatr Res* 2019;119,14-22[PMID: 31542703 DOI:10.1016/j.jpsychires.2019.09.007]
- 11 **Wang ZL**, Luo ZW, Li SH. Anxiety screening tools in people with epilepsy: A systematic review of validated tools. *Epilepsy Behav* 2019;99:106392 [PMID: 31521915 DOI: 10.1016/j.yebeh.2019.06.035]
- 12 **Alsharafi WA**, Xiao B, Li J. MicroRNA-139-5p negatively regulates NR2A-containing NMDA receptor in the rat pilocarpine model and patients with temporal lobe epilepsy. *Epilepsia* 2016;57(11):1931-1940[PMID: 27731509 DOI:10.1111/epi.13568]