Review of: "Impending role of hippocampal neurogenesis in the development of chronic epilepsy following seizures after Kainic acid and Pentylenetetrazol treatment"

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Potential competing interests: No potential competing interests to declare.

Comments to the Author

Research deals with a current topic: Generation of Neurons and Their Integration in Pre-Existing Circuits in the Seizure Brain. This study provided data to assess how seizures contribute to the generation of newly formed cells in the hippocampus and the potential of these cells on seizure activity (in 48 hours and chronically - 8 weeks), attempting to determine whether seizure activity-induced neurogenesis is a contributing factor or a protective mechanism against the development of epilepsy. Neuronal activity is a major regulator of adult neural stem cells (NSCs), which undergo a profound alteration of their neurogenic program in response to different levels of neuronal activity. It could be argued that the *glutamate receptor agonist* kainic acid (KA) causes seizures and has strong effects on the neurogenic niche. The authors used a chronic epileptic systems - KA-induced status epilepticus model and a pentylenetetrazol (PTZ-noncompetitive antagonism of the GABA(A) receptor complex)-induced kindling model.

NOTES:

1)) In Abstract: Paragraph «The study aimed to investigate how seizures contribute to abnormalities in generating new cells in the hippocampus and to explore the potential influence that these newly formed cells might have on subsequent seizure activity and the development of chronic epilepsy.»

replace with «The study aimed to investigate how seizure activity contributes to some signaling molecules' perturbation in generating new cells in the hippocampus and to explore the potential influence that these factors (GABAergic neurons, nitrergic neurons, BDNF, and NGF expression) and newly formed cells might have on subsequent seizure activity and the development of chronic epilepsy.»

2)) In Abstract:Paragraph «These results contribute to our understanding of the some factors involved in the onset of seizures and the development of chronic epilepsy. Additionally, they may aid in the development of strategies for preventing and treating epilepsy. However, further investigations are necessary to explore the potential role of newly generated cells in epilepsy development.»

Replace with «These results contribute to our understanding of some factors involved in the onset of seizures and the development of chronic epilepsy. Additionally, they may aid in the development of diagnostic and preventive strategies for

epilepsy. However, further investigations are necessary to explore i) a causative or contributing role of a wide range of factors in maintaining seizures, ii) modulatory mechanisms that control the interplay between neurogenesis and gliogenesis in these disorders.»

3)) In the INTRODUCTION, it is written «As neural stem cells give rise to both neuronal and glial progenitors, about fifty percent of newly born cells differentiate into neurons and fifteen percent into glial cells [7][10].» - These sources do not contain such information. I suggest that these and the following sentence be deleted: « Neurogenesis and gliosis following seizures have not been compared in investigations before.» and replaced with the following text – «The neural stem cells give rise to both neuronal and glial progenitors, and under certain circumstances, interaction occurs between neurogenesis and gliogenesis in the adult brain. Neurogenesis modifies neuronal connectivity in specific brain areas, whereas gliogenesis ensures that myelination occurs and produces new supporting cells by generating oligodendrocytes and astrocytes [Bergmann O, Frisén J. Neuroscience. Why adults need new brain cells. Science. 2013;340:695–696.]. Therefore, modulating the balance between neurogenesis and gliogenesis may present a perspective for neurorestoration of the adult brain. The study of the pathogenic significance of altered neurogenesis and gliogenesis in brain diseases continues to remain relevant. The aberrant neuronal activity, as occurs in disorders of recurrent seizures (epilepsy), could promote maladaptive myelination, contributing to pathogenesis [Knowles JK, Xu H, Soane C, Batra A, Saucedo T, Frost E, Tam LT, Fraga D, Ni L, Villar K, Talmi S, Huguenard JR, Monje M. Maladaptive myelination promotes generalized epilepsy progression. Nat Neurosci. 2022 May;25(5):596-606. doi: 10.1038/s41593-022-01052-2. Epub 2022 May 2.] . Actually, a type of brain plasticity, called activity-dependent myelination, contributes to a disease and, in particular, promotes worsening of epileptic seizures and contributes to epilepsy progression. The overall influence of altered neurogenesis on epileptogenesis is unclear. In particular, neural stem cells (NSCs) respond promptly to physiological and pathological stimuli, altering their neurogenic and gliogenic potential. In a mouse model of temporal lobe epilepsy, seizures triggered by the intrahippocampal injection of the glutamate receptor agonist kainic acid (KA) induce NSCs to convert into reactive NSCs, which stop producing new neurons and ultimately generate reactive astrocytes, thus contributing to the development of hippocampal sclerosis and abolishing neurogenesis [Muro-García T, Martín-Suárez S, Espinosa N, Valcárcel-Martín R, Marinas A, Zaldumbide L, Galbarriatu L, Sierra A, Fuentealba P, Encinas JM. Reactive Disruption of the Hippocampal Neurogenic Niche After Induction of Seizures by Injection of Kainic Acid in the Amygdala. Front Cell Dev Biol. 2019 Aug 20;7:158. doi: 10.3389/fcell.2019.00158.]. Reactive gliosis, which has been proposed to be key to the development of secondary recurrent seizures and associated morbidities [Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N. C., and Rogawski, M. A. (2013). Glia and epilepsy: excitability and inflammation. Trends Neurosci. 36, 174–184. doi: 10.1016/j.tins.2012.11.008], free radicals are generated in the brain, and their production is proportional to the brain activity in some animal models of seizures and epilepsy [Łukawski K, Czuczwar SJ. Oxidative Stress and Neurodegeneration in Animal Models of Seizures and Epilepsy. Antioxidants (Basel). 2023 May 5;12(5):1049. doi: 10.3390/antiox12051049.]. Reactive nitrogen species like nitric oxide (NO) play a variety of physiological and pathological roles in mammalian cells. NO behaves as a neuromodulator with dual action (proconvulsive or anticonvulsive) [Banach M, Piskorska B, Czuczwar SJ, Borowicz KK. Nitric oxide, epileptic seizures, and action of antiepileptic drugs. CNS Neurol Disord Drug Targets. 2011 Nov;10(7):808-19. doi: 10.2174/187152711798072347.] In the central nervous system, NO

may behave as a second messenger, neuromodulator, and neurotransmitter. NO promotes proliferation of NSCs in the hippocampus. It is shown that NO increased the proliferation of NSCs and the number of neuroblasts following seizures but was detrimental to the survival of newborn neurons [Carreira BP, Santos DF, Santos AI, Carvalho CM, Araújo IM. Nitric Oxide Regulates Neurogenesis in the Hippocampus following Seizures. Oxid Med Cell Longev. 2015;2015:451512. doi: 10.1155/2015/451512. Epub 2015 Oct 26.]. Moreover, the endothelial NO synthase acts as an anticonvulsant and the neuronal NO synthase as a proconvulsant, Different effects of NO on GABA transaminase activity and on GABA levels, depending on the isoform involved, may explain its contradictory role in seizures [Vega Rasgado LA, Reyes GC, Vega Díaz F. Role of nitric oxide synthase on brain GABA transaminase activity and GABA levels. Acta Pharm. 2018 Sep 1;68(3):349-359. doi: 10.2478/acph-2018-0022.]. A dysfunction in either GABA or glutamate availability will have important consequences regarding seizure genesis [Huguenard J. Neurotransmitter Supply and Demand in Epilepsy. Epilepsy Curr. 2003 Mar;3(2):61-63. doi: 10.1111/j.1535-7597.2003.03210.x..]. The idea that GABAergic neurotransmission is fueled by glutamate is provocative, and there are now more questions than answers. Epileptogenesis triggers molecular changes in the hippocampus, including enhanced expression of neurotrophic factors. There is a strong controversy concerning the potential role of BDNF in epilepsy [AlRuwaili R, Al-Kuraishy HM, Al-Gareeb AI, Ali NH, Alexiou A, Papadakis M, Saad HM, Batiha GE. The Possible Role of Brain-derived Neurotrophic Factor in Epilepsy. Neurochem Res. 2023 Nov 25. doi: 10.1007/s11064-023-04064-x. Epub ahead of print. Erratum in: Neurochem Res. 2024 Jan 6.] Moreover, stem/progenitor cells can secrete potent combinations of trophic factors that modulate the molecular composition of the environment (stem cell paracrine mechanisms for tissue regeneration) **[**Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. *Regen Med.* 2010;5:121–43.**]**»

4)) Next comes the sentence: «Understanding how proliferating cells affect the occurrence of seizures or the emergence of persistent epilepsy is particularly significant[11][12][13][14].» REFERENCES [11-14] do not support the content of the proposal. I quote from a source [14], where there is no mention of epilepsy at all – «Finally, we discuss some current challenges in the hippocampal neurogenesis field, and future research directions to address them, such as time course analysis across the lifespan, mechanisms regulating neurogenesis progression.» The article is devoted to «Features of Hippocampal Neurogenesis across the Lifespan. » The authors propose that hippocampal neurogenesis is one life-long developmental process with common features that remain consistent across the lifespan, as well as age-dependent properties. In particular, the number of NSCs decreases with age, and the NSC population becomes increasingly quiescent, contributing to the age-related decline in neurogenesis observed in adulthood (Berg, D. A., Su, Y., Jimenez-Cyrus, D., Patel, A., Huang, N., Morizet, D., et al. (2019). A Common Embryonic Origin of Stem Cells Drives Developmental and Adult Neurogenesis. Cell 177, 654.e–668.e.), (Harris, L., Rigo, P., Stiehl, T., Gaber, Z. B., Austin, S. H. L., Masdeu, M. D. M., et al. (2021). Coordinated Changes in Cellular Behavior Ensure the Lifelong Maintenance of the Hippocampal Stem Cell Population. Cell Stem Cell 28, 863.e–876.e.) THEREFORE, writing 50% differentiate into neurons and 50% fifteen percent into glial cells is incorrect (the specificity of age-dependence, pathological condition, etc., is lost). I suggest the authors enter these literary data about age-related changes, which will also be interesting to discuss for the 8-week group of animals in connection with possible aging).

5)) The following is the text «Considering this gap, the present work was designed to study the mechanism of onset of

seizures and how the seizures influence cell proliferation and differentiation. The role of various neurotransmitters and growth factors needs to be investigated in TLE. We thus used a kainic acid (KA)-induced status epilepticus model and a pentylenetetrazol (PTZ)-induced kindling model for the purpose of our study because these are thought to be characteristic models of the chronic epileptic system.» which I propose to present more reasonably in this form « In various epilepsy models, the hippocampus is one of the structures in which the **strongest neuronal loss is observed. However, the most damaged areas of the hippocampus may vary at different ages of the animals** [Velisek, L.; Kubova, H.; Pohl, M.; Stankova, L.; Mareš, P.; Schickerova, R. Pentylenetetrazol-Induced Seizures in Rats: An Ontogenetic Study. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **1992**, *346*, 588–591.] **In addition, in different models may recruit different cellular and molecular mechanisms, and different duration and severity of induced seizures depending on ontogenetic-specific characteristics. Available literature suggests the need for comparative studies impending role of hippocampal neurogenesis in different models and under identical age and species specific conditions.The present work was designed to study the factors (including neuronal nitric oxide synthase, growth factors, neurotransmitters) involved in the onset of seizures and the development of chronic epilepsy. We used a kainic acid (KA)-induced status epilepticus model and a pentylenetetrazol (PTZ)-induced kindling model for the purpose of our study because these are thought to be characteristic models of the chronic epileptic system** »

6)) On page 3/18 it says «three serial sections were examined at each -3.3, -4.3, and -5.8 coordinates[17] , as measured from bregma. » Need to add **AP**-3.3, -4.3, and -5.8 **mm** . The same « **NADPH-d positive cell count:** Every third coronal section passing through the hippocampus (bregma - 1.8 to -5.8) » add «mm»

On page 6/18 from the top, the third line says «Neurogenesis was thus reduced to 31% (P<0.001), whereas gliosis was reduced to 32.92% (P<0.001) in control rats.» replace with «……..compared to control rats»

7)) Quote on page 6/18 « When the magnitude of neurogenesis was compared between two time points, i.e., 48 hr and 8 weeks post SE, we observed that after 8 weeks it reduced to 49.64% (after 48 hr, P<0.01). Similarly, in kindled rats, neurogenesis decreased to 54.60% (P<0.001) after 48 hr. When the magnitude of gliosis was compared, we observed that after 8 weeks it reduced to 5.40% of the gliosis that had occurred after 48 hr (P<0.0001). In kindled rats, gliosis decreased to 54.38% (P < 0.05) after 48 hr.» in my opinion it needs some adjustments «When the magnitude of neurogenesis was compared between 48 hr and 8 weeks post SE, we observed that after 8 weeks it reduced to 49.64% (vs. 48 hr, P<0.01). Similarly, in kindled rats, neurogenesis decreased to 54.60% (vs. 48 hr, P<0.001). When the magnitude of gliosis was compared, we observed that after 8 weeks it reduced to 5.40% (P<0.0001) of the gliosis that had occurred after 48 hr. In kindled rats, gliosis decreased to 54.38% (vs. 48 hr, P < 0.05)»

8)) On page 8/18 from above «On the other hand, PTZ, which is a GABA antagonist, can directly promote cell proliferation by removing the inhibitory effects of GABA[19][20]. We hypothesize negative regulation of GABA on Neuronal Progenitor Cells (NPCs) proliferation, and a GABA antagonist like PTZ may promote NPCs proliferation . To further prove this hypothesis, changes in GABA levels were examined. Results showed that GABA immunoreactivity was decreased after 48 hr of kindling. This suggests that increased cell proliferation could be a result of the endogenous decrease of GABA

and the presence of PTZ. However, PTZ lasts for only two hours in the body. Still, we observed increased cell proliferation at 48 hr after the last PTZ injection. This observation suggests the role of other factors like seizures in cell proliferation.»

PEPLASE WITH «On the other hand, PTZ, which is a GABA antagonist, can directly promote cell proliferation by removing the inhibitory effects of GABA. Известно, что an increase in GABA concentration provides a signal to slow proliferation [Wang DD, Kriegstein AR, Ben-Ari Y. GABA regulates stem cell proliferation before nervous system formation. Epilepsy Curr. 2008 Sep-Oct;8(5):137-9. doi: 10.1111/j.1535-7511.2008.00270.x. PMID: 18852839; PMCID: PMC2566617.]. We hypothesize negative regulation of GABA on Neuronal Progenitor Cells (NPCs) proliferation, and a GABA antagonist like PTZ may promote NPCs proliferation . To further prove this hypothesis, changes in GABA levels were examined. Results showed that GABA immunoreactivity was decreased after 48 hr of kindling. This suggests that increased cell proliferation could be a result of the endogenous decrease of GABA and the presence of PTZ. However, PTZ lasts for only two hours in the body. Still, we observed increased cell proliferation at 48 hr after the last PTZ injection. This observation suggests the role of other factors like seizures in cell proliferation. So, the newborn neurons may sense neuronal network activity through tonic and phasic GABA activation, what suggests an unexpected mechanism for activity-dependent regulation of adult neurogenesis [19 inarticle Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H. GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature. 2006 Feb 2;439(7076):589-93. doi: 10.1038/nature04404. Epub 2005 Dec 11. PMID: 16341203; PMCID: PMC1420640]. The use-dependent decrease (run-down) of the currents (I_{GABA}) evoked by the repetitive activation of $GABA_A$ receptors (due to PTZ-induced sizure) is markedly enhanced in hippocampal and cortical neurons of temporal lobe epilepsy patients [20 in article Mazzuferi M, Palma E, Martinello K, Maiolino F, Roseti C, Fucile S, Fabene PF, Schio F, Pellitteri M, Sperk G, Miledi R, Eusebi F, Simonato M. Enhancement of GABA(A)-current run-down in the hippocampus occurs at the first spontaneous seizure in a model of temporal lobe epilepsy. Proc Natl Acad Sci U S A. 2010 Feb 16;107(7):3180-5. doi: 10.1073/pnas.0914710107. Epub 2010 Jan 26.]... The role of GABA signaling is becoming more intriguing considering that, paracrine GABA signaling of neuronal stem cells regulate the generation of excitatory neurons [LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron. 1995;15:1287.]. In the current literature are critical findings evidence about the role of neurotransmitters in epilepsy [Akyuz E, Polat AK, Eroglu E, Kullu I, Angelopoulou E, Paudel YN. Revisiting the role of neurotransmitters in epilepsy: An updated review. Life Sci. **2021** Jan 15;265:118826. doi: 10.1016/j.lfs.2020.118826. Epub 2020 Nov 28. PMID: 33259863.] [Perucca E, Bialer M, White HS. New GABA-Targeting Therapies for the Treatment of Seizures and Epilepsy: I. Role of GABA as a Modulator of Seizure Activity and Recently Approved Medications Acting on the GABA System. CNS Drugs. 2023 Sep;37(9):755-779. doi: 10.1007/s40263-023-01027-2. Epub 2023 Aug 21.].»»

9)) In the captions to Figures 2, 3, 4, add ***P<0.001 compared to the control group. For Figure 3, also add a*** KA vs. control, b*** PTZ vs. KA (I assume so). In Fig. 6, add a scaling bar for magnification.