Review of: "Disentangling the molecular mechanisms underlying the retrieval and extinction of morphine withdrawal-associated memories in the basolateral amygdala and dentate gyrus"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Franco-García et al. evaluated the expression of Arc, Homer 1a, GluN1, NMDAr, mTORC1 and Grin1, during the retrieval and the extinction of contextual aversive memories-induced by morphine withdrawal.
For this to be possible, they used a Conditioning Place Aversion (CPA) protocol to induce an aversive memory related to morphine withdrawal, after this, they used an extinction paradigm to reconsolidate the aversive memory. They evaluated protein levels of the above-mentioned molecules by means of immunofluorescence and immunoblotting, and their mRNAs by means of RT-PCR in the basolateral amygdala (BLA) and dentate gyrus (DG).

The amount of data of this research is impressive but the manuscript could be improved. I have three main concerns that I believe need to be addressed:
1) The way of how the manuscript is written gives the impression that the main goal of the authors is to find changes in mTOR; however, observed changes in mTOR are minimal (only a decrease of p-mTOR/mTOR ratio in DG). Introduction and discussion are grounded on mTOR literature, giving the appearance that Arc, Homer and GluN1 results are misleading, which, in fact are the clearest results they got in their research. I found the results of the correlation between Arc, Homer1a, GluN1 and the extinction index pretty interesting; especially in the context of explaining the molecular correlates of the retrieval and extinction of aversive memories. I think that discussing the possible role of Arc, Homer1a, GluN1 in the DG and BLA during retrieval and extinction of fear memories should be the central part of the research.
In this context, I recommend these papers:
Nakayama, D., Hashikawa-Yamasaki, Y., Ikegaya, Y., Matsuki, N., Nomura, H., (2016) Late Arc/Arg3.1 expression in the basolateral amygdala is essential for persistence of newly-acquired and reactivated contextual fear memories. Sci. Rep. 6: 21007

Guzowski JF, Timlin JA, Roysam B, McNaughton BL, Worley PF, Barnes CA. (2005) Mapping behaviorally relevant neural circuits with immediate-early gene expression. Curr Opin Neurobiol. 15(5):599-606.

Guzowski JF, Miyashita T, Chawla MK, Sanderson J, Maes LI, Houston FP, Lipa P, McNaughton BL, Worley PF, Barnes CA. (2006) Recent behavioral history modifies coupling between cell activity and Arc gene transcription in hippocampal CA1 neurons. Proc Natl Acad Sci U S A. 103(4):1077-82.

Montes-Rodríguez CJ, Rueda-Orozco PE, Prospéro-García O. (2019) Total sleep deprivation impairs fear memory retrieval by decreasing the basolateral amygdala activity. Brain Res. 1719:17-23

2) The way the results are written makes it difficult to follow the outcome of this paper, I suggest reorganizing the results in a more conceptual manner. For example, to write the result section making clearer the function of the DG and BLA in each memory process and, integrating all the evaluated molecules. Also, I recommend to create sub-sections in each result section.

Correlations. I suggest separating the correlations results, you can include a “correlation section” in the result section, centering on the negative correlation between Arc and Homer 1a with the extinction of a fear memory. And another section with the molecule’s correlation results.

After reorganizing the data, it would be easier to integrate the differential expression as Arc, Homer 1a, and GluN1, around the retrieval and extinction of fear memories.

3) Conditioned Place Aversion memories have a strong contextual (spatial) component which involves the hippocampus, I would think the CA1 and/or the CA3 region of the hippocampus are mainly involved. This has been suggested already by Ma Q. et al (2020) (see https://pubmed.ncbi.nlm.nih.gov/32760235/). In this context, please discuss why you did not include CA1 or CA3 in the hippocampus study.

Methodological issues

Signal in Fig. 2 b (GAD+ps6 in BLA) is not clear.

In general, signal/noise ratio is not optimal in your immunofluorescence pictures. It is hard for me to see a clear signal; it may be a resolution problem. I suggest including a marker to point out a positive cell (i.e., an arrow) and/or pictures at higher magnification.

Analyses

Table 2 is unnecessary because most of the results are reported in Fig. 4 and 5.

I am not sure that the last correlation analysis (Table 4) between the mRNA of Arc, Homer 1 and Grin1, is the best analysis. Maybe a clustering analysis would be more appropriate.

I believe you should use a repeated measures ANOVA in the extinction group for the behavioral analysis.

Other comments

I suggest strengthening the explanation of the extinction index measured as the difference of time spent in the naloxone-associated compartment during the extinction and CPA test.