

Peer Review File

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Review Comments:

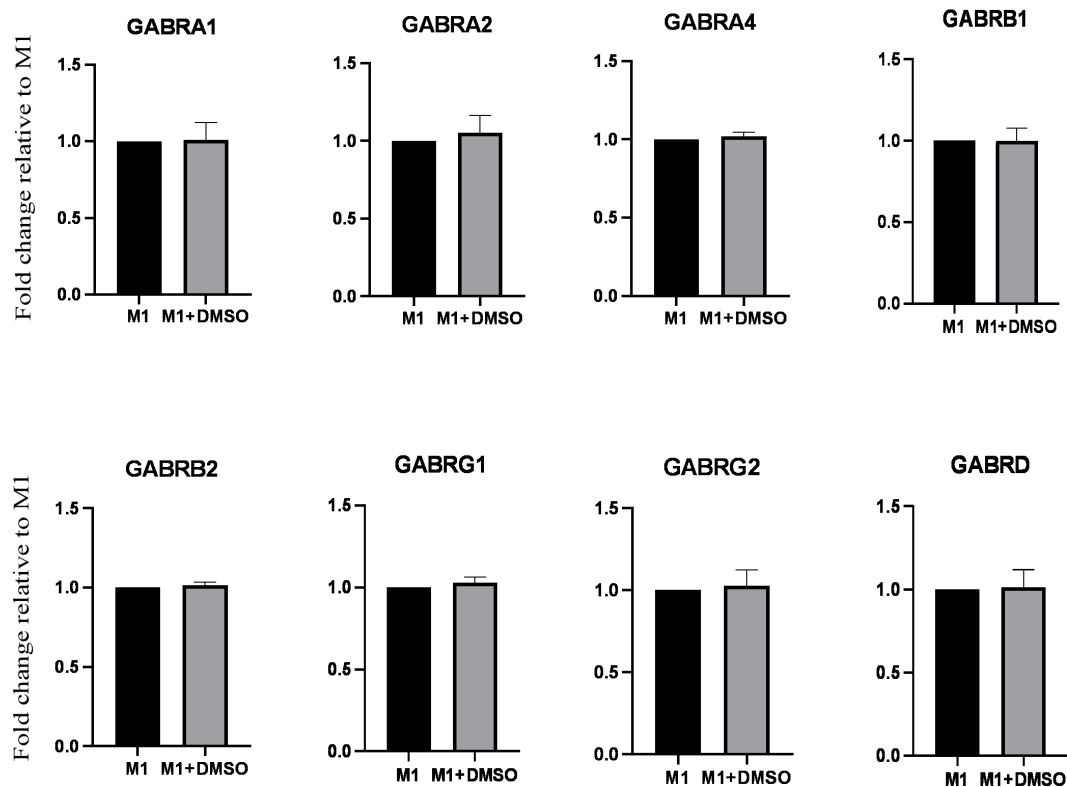
Reviewer A

The manuscript from Kochiyama et al describes the important finding that THP-1 cells increased the expression of GABA(A)R subunits after differentiation. Other groups have reported similar observations for other immune cells. In addition, administration of propofol further changed the expression of $\alpha 1$, $\alpha 4$, and $\beta 3$ GABA(A)R subunits. GABA(A)Rs do not directly change gene transcription thus, signal transduction, most likely through changes of calcium homeostasis might be involved. The authors should comment on a possible signaling cascade. Secondly, the delta-delta method was used for the presentation of fold changes of gene expression. I'm wondering if for Figure 4 the authors should use the cycle numbers for the M1-THP-1 from Figure 3 and relate them to DMSO (vehicle) and to propofol (25, 50, 100 μ M) cycle numbers. This will allow them to then compare a true vehicle with a SD to the treatment groups.

Reply: Thank you for your valuable suggestions. It has been reported that intravenous anesthetics, including propofol alters cellular calcium homeostasis (European Journal of Pharmacology 884: 2020: 173303, Journal of Anesthesia 2006: 20: 17-25), so there may be a possibility that propofol can alter THP-1 calcium homeostasis.

We previously reported that propofol suppresses inflammatory cytokine production during M1 macrophage differentiation via activation of nuclear factor-E2-related factor 2 (Nrf2) (Mediators Inflamm. 2019;1919538). It is speculated that in the present study activation of Nrf2 may also be involved in the genetic alterations of GABA_A receptor subunits. In the next study, we will analyze whether the Nrf2 signaling cascade is involved in the change of GABA_A receptor subunits expression.

We analyzed gene expression changes in each GABA_A receptor subunit in samples of M1-THP-1 and M1-THP-1 with the solvent control DMSO. There were no gene expression changes between M1-THP-1 and M1-THP-1 with DMSO, and then we performed propofol experiments. The results of the experiment are described below. This figure is original one and made by us.



Reviewer B

The manuscript details an *in vitro* study utilizing macrophage differentiation and QPCR as its main techniques. The experiments are straight-forward and well designed.

Future studies should seek to replicate these findings in an *in vivo* model if possible, and establish a causal connection between propofol-mediated differentiation of M1 macrophages and the described changes in GABA receptor subunit expression.

The following minor concerns should be addressed prior to publication:

- 1.) In the results section 3.1, several subunits' expression levels are stated to be tested but undetected following M0 differentiation in THP-1 cells. These results are not shown in Figure 3. However, undetectable subunit expression changes in the propofol experiment are shown in figure 4. For the sake of transparency and consistency, the aforementioned undetected expression level changes in 3.1 should be reported in figure 3, or in a similar supplementary figure.

Reply 1.): In the present study, we used RT-PCR to analyze gene expression changes in 13 GABA_A receptor subunits: α 1-6, β 1-3, γ 1-3, and δ . Of the 13 GABA_A receptor subunits, gene expression of five subunits (α 3, α 5, α 6, β 3, and γ 3) could not be detected in THP-1 cells. Therefore, changes in gene expression of the eight detected subunits (α 1, α 2, α 4, β 1, β 2, γ 1, γ 2, δ) by M0 and M1 macrophage differentiation and by propofol administration have been shown

in figures 3 and 4, respectively.

2.) The manuscript requires extensive proofreading for clarity and grammar throughout. For example, the intro refers to "GABA expression" when it should say "GABA receptor subunit expression" to avoid potential confusion with expression of the neurotransmitter itself.

Reply 2.): Thank you for your valuable comment. We rewrote the document after careful proofreading of the text and grammar, including the points you pointed out.

Changes in the text: The function of GABA has been well studied in the central nervous system; however, few studies are available on GABA_A receptor subunit expression in relation to physiological functions in other tissues. (page5, line 6)