Reviewer’s report

Title: Effects of Stress on Behavior and Resting-State fMRI in Rats and Evaluation of Telmisartan Therapy in a Stress-Induced Depression Model

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Reviewer: Na Cai

Reviewer's report:

General

This is an interesting paper that examines the effect of chronic mild stress on rat behavior, cognitive function and brain activities, and the possible alleviating effect of the telmisartan. I think it is a good study that is worth publishing, though both presentation and discussion of the results should be improved before publication. In particular, I am concerned that the authors did not correct for multiple tests when setting significance threshold for any analysis, and without a table showing all the statistical tests performed, and the effect size and p values in each of the tests, I find it hard to access if the trends the authors are seeing are significant and if the conclusions drawn are supported by the results.

Minor

1. The first paragraph for introduction can improved. "The most common consequence of prolonged depression is an impairment in learning and memory, which impairs social interactions in patients with depression" - Is it known that social impairment is due to learning and memory? I would consider rewriting this. "Stress is known to be a key factor in the development of depression and memory impairment. However, the etiology of depression and its effective therapeutic strategies have not been clearly identified." While both statements are true, one doesn't follow the other. Please consider rewriting.

2. "Analysis of the amplitude of the low-frequency fluctuations (ALFF) and the regional homogeneity (ReHo) are two methods that investigate the resting-state activity of the entire brain [13,14]." It seems both methods have regional resolution, is that right? If so, perhaps consider changing to "two methods that investigate the resting-state activity in regions across the brain".

3. "However, little is known about whether telmisartan can alleviate the brain alterations caused by chronic stress" Do the authors have a citation for chronic stress causing brain alterations?
4. Please write out all full names of abbreviated terms such as "ReHo" and "LFF"

Major

Methods section

1. "12h food deprivation, 12h water deprivation" for the CUMS procedure - could the changes the authors observe be due to periodical starvation?

2. Have the authors considered additional groups where the rats are administered the same doses of 0.5mg/kg or 1mg/kg telmisartan without CUMS? What does telmisartan do on its own? Does it alter behaviour, cognitive function or brain activity in the absence of stress? I think this is a very important control experiment to do, and while I appreciate this may not be possible for the scope of this study, I need to hear what the authors think about this, and unless there is evidence to suggest telmisartan has no such activities, this should be commented on in the discussion section.

3. Can the authors state in the "Animals" section how old the rats are? Are they all of the same age?

4. Are all the test procedures performed in the same order on all the rats?

5. The rats the authors used are outbred, so they are not genetically identical to each other, and this may be less ideal than using inbred strains in which we know variations are not due to genetic differences between rats. It would be good to write about this in the discussion.

Results section

6. For each procedure (eg OFT), the authors are performing at least 3 tests (C group vs each of the S and T groups), and if they are also comparing between the two T groups, even more tests. The significance threshold for each test should therefore be adjusted for number of tests performed to 0.05/(number of tests). I would suggest making both Tables 1 and 2 into figures (boxplots), and in place of them include a table with all the statistical tests performed on results from each procedure, their effect sizes and standard error of effect sizes, P values, and whether they are significant after multiple testing correction.
7. What are the T and Z values in Tables 3 and 4? Please state in either the text and the table legend, otherwise it's not possible to understand what they are saying. Tables 3 and 4 are both not easy for me to understand. As the information is also presented in Figure 2, I think it's better if the authors shift both tables to Supplemental Materials, and in their place include a table as described above - each statistical test the authors performed and the test statistics.

8. "We analyzed the correlations between selected behavioral test indices and the ALLF and Reho values in the altered brain regions. The only significant correlations were between the number of rearings in the OFT and the ReHo values in the thalamus (r = -0.446, p=0.029)." As I mentioned earlier, there is a need to correct for multiple testing. How many tests did the authors perform? It would be great to have a table in the supplemental materials on the outcome of each of the tests performed (correlation coefficient, p value). Also, please report r² instead of r.

9. Figure 2 - the colour bar that indicate the t value doesn't indicate whether yellow is higher or red is higher! Also, it may be good to label each of the brain regions in the figure.

Discussion section

10. In general, the authors can consider making the discussion section a lot shorter - information from literature are good for interpreting results from this study, but can be kept to those directly relevant to the results. It would be better to see more discussion of the results of the study (for example, what does increase ReHo in a brain region mean, is the increase expected for rats under chronic stress, what are the possible reasons for this increase, etc), the caveats for each analysis, and recommendation for future analyses that will take this research forward.

11. I think a few claims may need to be toned down. "The most notable effect of telmisartan in this depression model was its improvement of cognitive function. In the present study, we found that the novel object recognition in the ORT was significantly higher in the T-1mg/kg group compared to the S group, suggesting that telmisartan effectively improved cognitive function." I think it is premature to generalize novel object recognition is a good proxy for general cognitive function and conclude telmisartan improves it. It may be prudent to limit the claim to exactly the findings in this study, improving novel object recognition, and recommending other experiments and further analyses to be performed to access cognitive function in this rat model of depression.

12. Along the same veins: "Our study also demonstrated a possible antidepressant effect with telmisartan. In the OFT, the level of locomotor activity was much higher in the T-1mg/kg
groups compared to the S group, although no significant differences were found. Besides, there were no significant difference between the T-1mg/kg and C groups in the level of locomotor activity." Do the authors mean there is no significant difference between any of the three groups (C, S and T) in locomotor activity, but there seems to be a trend? I don't think results that are not significant can on their own be sufficient for drawing the conclusion telmisartan can be a "complementary antidepressant therapy for individuals with depression, particularly those with cognitive impairments". I would feel more comfortable if the authors suggest further studies to look into the potential of telmisartan in alleviation of symptoms of depression.

13. I think the discussion should comment on the two dosages of telmisartan used in the study.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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I recommend additional statistical review

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