OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-19-00353
Title: Modified Constraint Induced Movement Therapy Altered Synaptic Plasticity of Contralateral Hippocampus in MCAO Rats
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Reviewer’s country: USA
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COMMENTS TO AUTHORS
The current study is an effort to provide an insight for a stroke therapy induced molecular mechanisms in the animal model. The study, in general, is of particular interest as it relates a system-level description with neurobehavioral presentation and reflections upon downstream expressions. There are a few general advice:
- The authors are highly encouraged to improve the paper writing.
- The sample size is considered low with the number of unknowns given in the study. One possible strategy, to gain more power, may be to use larger size for control group.
- Consistency is encouraged in statistical interpretation (<0.005 and <0.05) and mathematical conventions (p vs. P).

Here are more detailed suggestions:
Abstract
The goal is presented as to be understanding molecular mechanisms of mCIMT in treating neurologic impairments after MCAO procedure. The research question is interesting and relevant. Further detail must be included to evaluate whether the provided results, in the abstract, support the claims ("the study demonstrated mCIMT as the effective rehabilitation therapy in MCAO rats" and "mCIMT facilitating synaptic plasticity through AMPAR expressions of contralateral hippocampus") made. For example, lack of statistical evidence for the MCAO effect on motor recovery statement; As a reader, one expects reporting neurotransmitter receptor-related genes, PSD-95, Synapsin I result.
The authors are also encouraged to provide a statement on the rational or linkage of selected genes, proteins, and ratio (cycles) within the context of synaptic plasticity through AMPAR expressions. The hemispheric sites of investigation may be a routine for such models and experts in the field, but It may also help to bring readers' attention onto possible hippocampus-thalamic networks communication. Please note that two different statistical thresholding is given. Conjunction of reduction in Glutamate/GABA ratio with reported alterations in AMPA receptors related protein markers is an interesting finding for the therapy, yet generalization of finding into "facilitating synaptic plasticity through AMPAR expressions" requires more evidence than It's given. Can there be a role for alterations in excitatory neurotransmitters, or the precursor feature of glutamate? The final claim (supported by the current data) may be better to be limited to "effects of rehabilitative training by the changes in post-synaptic membrane AMPAR expression", although direction of the effect must be investigated.
Literature review
Provided literature are recent, but not comprehensive. The lack of relevancy and continuity is apparent. The authors are encouraged to pay further attention to linking their review sections together. For your benefit, I summarized major parts of the lit review as a reader: 1) The existing gap of knowledge for understanding mechanisms of improvement following mCIMT, and lack of comprehensive behavior assessments in the animal. 2) fMRI measured increase in hippocampal activity in adults and pediatric CIMT studies, 3) The hypothesis of remote contributions 4) motor-sensorimotor-hippocampus-thalamic networks 4) plasticity and activity-dependent plasticity in hippocampus. However, there is less of proper linkage among these parts convincing a general reader to bridge the gap into the works reported.

There are long connected sentences with poor message transfer, e.g. "Although most studies on motor control have focused on the primary motor cortex, it is also likely to relate to the hippocampus, which has a unique contribution to memory, also plays a role in motor control"

Statements such as plasticity as a sufficient known for information storage in the hippocampus may be better supported by providing reference.

Study design and analysis
Type of the study design seems to be observational and learning experiment, though not clearly stated. Please pay a closer attention into the written language, e.g. "The rats were divided into three groups randomly into …". IACUC related information is provided. Standards are explained for the data collection.
Although the choices of statistical hypothesis testing are complex; however, having no hypothesis set invalidates their use. The sample size is relatively small. This sample size may not allow for it, but a stronger design for such studies could be to include the interaction effects, rather than just main effects to study behavioral effects and molecular markers. Providing a statement for ANOVA assumptions of independence and normality improves quality of the report.

Results and discussions:
An interesting observation is made about the neurobehavioral improvement (when assessed by CatWalk and Adhesive Removal tests) on day 21. An interesting linkage here could be the effect of time on learning. One critique that seems to be disregarded here is the effect of multiple behavioral tests and trainings together with the current small sample size. The choices for markers are not appropriate to distinguish groups from each other. Error bars are inappropriately used. Please consider replacing it with box-plots or transparent chart overlaid by actual data points (especially with this sample size).
Unfortunately, the presentations are of poor resolution (Figure 2 and 6), making an interpretation difficult. The use of stock-bar is discouraged in all figures. The authors are encouraged to utilize large font size and when possible color-coding in higher-resolution setting. For all of these results, a better choice of presentations would be standard error or confidence interval, however with this small sample size, data points are required for inferential and validation purposes.