OPEN PEER REVIEW REPORT 2

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Title: Time-course pattern of neuronal death and gliosis in gerbil hippocampi induced by mild, severe and lethal transient global cerebral ischemia
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COMMENTS TO AUTHORS

In this study the authors analysed the impact of different durations of bilateral carotid artery occlusion on: regional neuronal death, astrocytosis and inflammation markers, while evaluating these responses at different time points after ischemic onset.
The aim of analysing hippocampal cell damage depending on the duration of the ischemic event as well as of evaluating cell death and damage markers at different time points is a topic of interest. However, there are many concerns regarding this study.
In my opinion, a clear rationale for the study and a proper framework for understanding the contribution of the work is missing. Therefore, even when the results are interesting, they lose strength as the study seems merely descriptive in the form it is currently written. Also, there seem to be important concepts to be revised: The authors refer to be using a transient ischemia model. Given that they analyse different durations of ischemia with further recovery of the animals, I then understand that they are using a model of ischemia/reperfusion. However, the authors do not address reperfusion along the manuscript, which is a hallmark in their model.
An example of this conceptual misunderstanding can be found in several sentences:

Introduction: "Transient global brain ischemia happens by particular ischemic conditions such as return of spontaneous circulation after cardiac arrest".

Materials and methods: the authors do not mention that carotid ligation was liberated, which is the procedure that gives raise to reperfusion.

Overall, methods should be described in more detail.

-A proper framework of the study with previous results is missing. The authors claim that: "…chronological alteration of ischemic injury in the hippocampus, which consists of hippocampus proper (CA1-3 fields) and dentate gyrus, induced by mild, severe and lethal transient ischemia have not been fully reported". However they fail to state the major previous findings in the filed, which are many. Therefore, the rationale for performing the study is not evident to the reader.

The introduction would also need a round up.

Results:
- There is no reference to behavioral studies nor are behavioral aims exposed in the introduction. Therefore the analysis of spontaneous motor recovery does not seem to have a ground.

-The images are neatly presented and convincing. The graphs also seem convincing and are

representative of the images. However, it is not completely clear how data were analysed: Experimenters analyzed subfields in 7 serial sections. Did they average the data from these 7 seven subfields? Was this considered n=1? Detailed information should be provided.

Authors should be specific in regard to their analysis. Statistical comparisions along the text should clearly state if measurements were significantly different or not (e.g):

"In the 15 min BCCAO operated group, SMA was more increased than that in the 5 min BCCAO operated group"

It seems that there is no gial infiltration in the granular layer of the dentate gyrus even when neurons show to be picnotic. Are there known reasons for this lack of gliosis?

In my understanding, microglia does not behave as astrocytes after damage and one should better refer to "microgllial activation states" more than to a hypertrophic state. Activation states should in turn be defined according to the observations.

Discussion: In my view, the Discussion section does not discuss relevant information, such as the vulnerability of cells in the infragranular layer of the DG to die and discussion is very superficial.

Some examples of ideas that I would ask to be discussed:

The density of GFAP-IR is mainly evident in the subgranular zone of the DG, where Type1-GFAP+ neural precursor cells activate (and proliferate) after damage. Can the authors distinguish between gliosis and proliferation of Type-1 cells in this region and in this context of damage?

What does a "paler"signal mean for NeuN-IR?

Hypothermia has been extensively shown to protect against damage.
The authors are basing their discussion on hyperthermia, which was not introduced, nor mentioned before in the manuscript.

Disconnected ideas are found along the manuscript and should be rewritten:

"Granule cells of the dentate gyrus project mossy fibers to CA3 field and are very resistant to transient ischemia (Schmidt-Kastner and Freund, 1991; McAuliffe et al., 2011). It has been studied that the subgranular zone of the dentate gyrus offers neurogenesis for granule cells in lifespan (Kempermann et al., 2015).

"This being so, astrocyte activity is linked to blood flow in the brain, and that this is what is actually being measured in fMRI (Swaminathan, 2008; Figley and Stroman, 2011)".

-There are many redundant phrases. Some examples:

"Bilateral common carotid artery occlusion (BCCAO) for just 5 min in gerbils evokes transient forebrain ischemia and causes the death/loss of pyramidal neurons in the CA1 field of the hippocampus 4-5 days after 5 min of BCCAO…"

-"…. and transcardially perfused with 0.1 phosphate-buffered saline" Should say: 0.1M

-Rephrase: "Each immunoreactivity was measured by…"

-Table 1 does not provide substantial information. Data can be mentioned in the text.

-Cells cannot be positive for crescyl violet. The term "positive for" is used in immunohistochemistry, not in this type of staining procedure. Instead, cells cannot be "stained" with NeuN. They are immunoreactive or immunopositive to the marker.

-Numerical data, "n" and significance should be provided along the text in the "Results" section.

-Change "hypertrophied" for "hypertrophic" and please define the term the first time it is being used.

Circular sentence: "Based on this finding, gerbils subjected to 15-min BCCAO might be used for study on neurogenesis in the dentate gyrus, because 15-min BCCAO may present a tool of neurogenesis of granule cells in gerbils subjected to 15-min BCCAO."