**Reviewer’s report**

**Title:** Differential role of dose and environment in initiating and intensifying neurotoxicity caused by MDMA in rats

**Version:** 1 **Date:** 09 Jan 2019

**Reviewer:** Patrizia Romualdi

**Reviewer's report:**

The authors seek to determine the cause that can generate serotonin syndrome instead of serotonergic injury based on the hypothesis that this two events take different course during MDMA toxicity. The purpose of the research is interesting but the manuscript requires a profound revision in the form, as it is difficult to understand what the authors want to say in some parts of the text, and also in the experimental details.

- In the introduction, the author should deepen the explanation of what happens at the central level during hypothermia or hyperthermia conditions. In this way the background of the study would be much more complete.

- In the methods section is unclear how many animals were used to perform the experiment (and also the abbreviations HD and LD, or EGG and many others should be explained at first time).

In addition, the doses and timing should be explained more clearly by the authors; for the readers it is totally confusing and hard to understand time intervals at which MDMA was administered.

Moreover from the results arise that there are different groups of animals (how many analyzed 7 days after MDMA treatment?) , even though it is not clear which one (under normal condition or modified condition).

Why do the authors decide to wait 7 days? the reason is not explained in the manuscript and it should be added to the text.
- Can the language of the sentence "the chamber temperature was set at 26 °C (± 1 °C) for testing the HD" be clarified?

- How long after the last administration the tests are carried out? This aspect should be clarified.

- The paragraphs organization in the methods section should be carefully reviewed by the authors, in order to clarify which vehicle was used, the dose and timing of administrations.

The use of the terms "experiments" and "approaches" does not allow to understand the analyses performed.

Basically, the information present from line 23 up to line 48 on page 5 should be appropriately inserted and divided in the subsequent paragraphs.

- Can the description of the paragraph "approach 4" be clarified?

- In the figures the dose should be reported as 10mg/kgx3 and 2mg/kgx3, because 10mg/kg or 2mg/kg are incorrect.

What should represent the number 0, 2 and 4 on the X axis in the figure 1A and also in the figure 1B? the author described that the samples were collected at 15 minute intervals to measure the 5-HT extracellular levels, and that the Tcor results from the mean of 4 consecutive measurement. Could the authors clarify this aspect?

In the legend of this figure they say that 2h represent the intervals between administrations. It would be more appropriate to explain this aspect before, for example in a paragraph entitled "drugs and treatments" in the method section
The meaning of the symbol # is not mentioned in the legend of Fig 1. The Y axis for the Fig 1B reports number as 0, -1, -2, what do they mean? The author wrote in the results that the Tcor at baseline is around 38.57°C.

In the results the author reported the statistic values but don't the T° values, never, neither under the unmodified nor under modified environmental conditions.

- The value expressed by the Y axis in the figure 2B, 3B and 5B should be written in the same way.

- Line first of the paragraph "Experiment 2, Identify determinants for the syndrome initiation and intensification under the modified environment" page 9 the sentence "Rats with a similar level of hyperthermia (H+) were used to compare the effects of the HD and LD on the syndrome".

Can the authors clarified the meaning? It seems like rats have the same level of hyperthermia before to start MDMA treatment after both HD and LD.

- In the fig 5 data refer only to the animals that were hypothermia, evaluated after the previous test shown in fig 4, it is correct? in this case it should be clarified in the methods that only a subset of animals underwent further analysis.

- Data reported in the fig 6 were obtained in the animals under the unmodified or modified environment conditions?

Unfortunately the lack of clarity in the results makes very difficult the understanding of the results and the speculations present in the discussion. The experimental design is very confusing and is not clearly described to allow the reader to understand how many experimental groups have been analyzed and for what purpose.

The English form and the construction of the sentences is very bad, making impossible the flowing reading of the manuscript.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

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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