Reviewer's report

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

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Reviewer: BRIAN PIPER

Reviewer's report:

The manuscript "Identify determinants for toxic initiation and intensification: Different roles of drug doses and environments in the two distinct aspects of MDMA toxicity" contains much mechanistic information.

General Concerns

A) The authors seem to be unacquainted with some of the key issues in this field. Carefully reading the Green review (Pharmacological Reviews 2003; 55 (3): 463-508), or something more recent, and citing repeatedly would be extremely beneficial.

B) Direct administration of MDMA into the brain did not cause serotonergic depletions (Ricaurte study). MDMA is extensively metabolized and some metabolite(s) are key players in that process (and more important than just MDMA). At the very least, the choice to only study MDMA and not also measure metabolite(s) should be noted in a limitations paragraph. This rationale should be provided in the methods too. The absence of measurement of the serotonin syndrome behavior is also very odd and should be noted here (unless it is to be reported elsewhere?).

C) As noted in the Green review, changes in serotonin reflect multiple processes. There's acute release, but then inactivation of tryptophan hydroxylase which depletes 5-HT (see also many papers by Schmidt CJ), then long-term depletions. Not all of these are reflective of serotonin "toxicity". Similarly, although the term "neurotoxicity" is widely used to refer to long-term reductions in the serotonin transporter, there is also evidence for neuroadaptation (Beizonski et al. J Neurochemistry 112(4):951-62. and Current Neuropharmacology 2011; 9:84-90). The abstract, intro, and discussion should be revised to be more attentive to these issues as the current coverage of "toxicity" and "injury" is pretty simplistic.
Minor:

Minor issues including with language are noted below.

1) The title could be refined. Perhaps "Differential role of dose and environment in the effects of MDMA"

2) Abstract, Background: Not sure if "toxicity" can undergo anything. Perhaps "MDMA toxicity includes ..". Clear definitions of toxicity and injury in the intro would be beneficial.

3) Abstract, B: 2nd sentence: indicating that they differ in the course of initiation and intensification.

4) Abstract, Results: … only the high dose could cause serotonergic injury.

5) Intro, p. 3, line 45: but likely suggest … differ from those

6) Intro: Regarding "but what molecules behind the drug doses are there for initiating or intensifying serotonin syndrome and serotonergic injury" please see the Green review. A dopamine adduct was believed to be important. A bit more depth on the current status on this topic is needed.

7) Just an fyi that interspecies scaling is less useful for extensively metabolized drugs like MDMA. Response scaling may be more appropriate.

8) Intro, p. 4, line 13: toxic development.

9) Methods: p. 5, line 26: humidity controlled environment

10) p. 6, line 10: avoid starting a sentence with a # (5).

11) p. 6, line 50: Just to double-check, the guide cannula were previously (pre-drug) implanted under anesthesia, correct?

12) p. 5, line 53: Under measures of "syndrome initiation", the authors appear to have neglected to report the actual serotonin syndrome (e.g. videotaped rating of forepaw treading, head weaving, and low body posture). How can you report on the syndrome without actually measuring the syndrome? That section heading needs reconsideration. Perhaps, "Neurophysiological and neurochemical measures for syndrome initiation and intensification"?

13) p. 7, line 28: and the brain rapidly removed

14) p. 7, line 48: 5,000
15) p. 8, line 21-24: 1,000 … 2,000

16) The terms "Approach 1… 4" are not informative. Delete.

17) p. 8, line 39: if a significant

18) Results, p. 8, line 57: to the two doses were compared

19) Fig 1: Double-check that this is high resolution (> 300 DPI). Fig 5, 7C, 8C too. In Fig 6, if space, just spell-out front cortex. Why is serotonin in all-caps? A & B labels should be lined up vertically.

20) Discussion, p. 11, line 48: "5-HTiA"?

21) p. 12, line 12: consider "psychobiological" instead of "psychological"

22) p. 11, line 52: Perhaps, "The activity balance … in adaptation to the drug environment."

23) p. 12, line 41: hallucinations

24) p. 13, line 28: consider "Our plasma results are consistent with previous reports [13,41,43] which revealed"

25) p. 13, line 49: Perhaps "are unlikely to contribute to serotonergic injury."

26) volume or pages for ref 15?

27) The statement "The authors declare that data supporting the findings of this study are available within the article." Is inadequate. Are the authors willing to make their raw data available or not?

28) Were any of the same animals used for both EEG and other measures or were they separate? The N may have been too low here. For future studies, the authors should consider trying to conduct some exploratory correlations across different levels of measurement (e.g. AUC of homogenous temperature changes or EEG and axonal function).

29) In Sup Fig 1A, are the yellow and red lines supposed to reflect warm and hot temperatures? If available, an image of a rat in a running position would be more informative.
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.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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