Reviewer’s report

Title: Influence of isoflurane exposure in pregnant rats on the learning and memory of offsprings

Version: 0 Date: 03 Sep 2017

Reviewer: Misha Perouansky

Reviewer’s report:

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This study attempts to answer the question of whether in-utero exposure to isoflurane can have long-lasting effects on cognition. There are a number of stylistic issues e.g. the uncritical choice of references and the lack of references to fundamental issues underpinning the actual experiments (MWM, CREB) and problematic interpretation of existing literature which require some thoughtful editing. The methods lack detail, results are reported very sparsely. Figure legends should be rewritten to be helpful and utilize accepted terminology. There are also methodological flaws which, in the current version limit my enthusiasm. This is unfortunate as the reported results are intriguing and potentially important. Moreover, instead of repeating the need for 'guidelines' the discussion should aim at interpreting the MWM results in a neuropsychological context and embed the biochemical results into the learning&memory framework.

Overall, the authors draw appropriately modest conclusions

Introduction:

1. Tighten references, please quote only the most relevant one for the statement you would like to support e.g. #2 seems sufficient, #1 unnecessary. Please select the most appropriate and highest quality publication in the best journal e.g. refs 9/10 - both necessary? be especially critical when quoting papers written by the authors of this study e.g.

2. Most lab studies in rodent are done 1 week postnatal - place this date into the human pregnancy timeframe with respect to brain development. Ref 11 is not a good source to confirm but ref 12 is. This should be in the introduction when you explain.

Methods

1. What does 'predesigned' gas mean?
2. What is the source for the claim that most women undergo surgery at 12-16 weeks?

3. Rats used in the 'further study' cannot be also part of a pilot study. The whole point of the pilot is to find out what works and perhaps to estimate the number needed for statistics.

4. How did the randomization work?

5. What was the litter size and sex distribution in the three experimental groups? What were the numbers of healthy / unhealthy male rats?

6. When you say 'resuscitate' you actually mean 'recover'.

7. If you followed a published protocol for interpreting the different aspects of the MWM interpretation, please indicate what the statement e.g. 'The swimming distance in the platform quadrant reflects spatial localization and the times of crossing the platform reflects the accuracy of spatial memory' is based on.

8. How many rats were excluded 2/2 'visual problems' (how do you know they were visual?) in the different groups?

9. How were the rats housed during development? How many to a cage? Standard or enriched environment?

10. How can you exclude rats after randomization? Figure 1 indicates that pregnant rats were randomized. The description refers however to exclusions of animals 2/2 poor performance. Specifically, would inclusion of the 'poorly performing' rat in the control group have abolished any significant differences in behavioral experiments?

Results

1. What is Mirros water maze?

2. Fig 2 legend 'what's the percentage of platform quadrant'

3. Fig 2. There is no difference between control and ISO 1 and a striking difference between ISO1 and ISO2 for one parameter (platform crossing) at both time points (2b and 3b). It is notable that at the same time the complementary measure i.e. 2c/3c (I do not exactly what % target quadrant means) shows no difference between any measures. Is this expected based on the theory underlying these assays? Please provide a convincing neuropsychologically / learning theory-based interpretation for why these two measures diverge and how the two tests are related from the point of view of neural substrate? How can one yield such a different result than the other and be so consistent at P28 and P90?

4. The same 'outlier' is found in the pCREB assay. How does one interpret the lack of a 'dose response.'
5. Could you provide the individual values for these experiments? With other words, does individual performance in the water maze correlate with pCREB levels across experimental groups? Does a poorly performing rat in the control group have lower or higher pCREB expression than a well-performing rat in ISO2? Please cite literature showing that poor performance in a learning/memory task correlates with CREB/pCREB ratios.

Discussion:

1. The Clancy study does NOT claim that whole brain development is equivalent at certain age between rodents and humans. Clancy et al. are very careful to state that different parts of the brain reach certain milestones at different ages in different species i.e. brain development does not proceed at a uniform, easily comparable pace.

2. In all animals, a substantial proportion of neurons undergo apoptosis during normal development. Please be more accurate.

3. You repeat the 'lack of guideline' issue - what kind of studies would you envisage that could provide 'guidelines' for humans? Is this feasible? Shouldn't this be based on evidence of a potentially detrimental effect in humans? If you insist (and I think these repeated inferences do not strengthen this MS) on this analogy - how relevant is a multi-hour exposure to these doses of isoflurane for the type of procedures commonly performed in pregnant women?

4. Roughly speaking, your experiments compare 3 MAC hours of isoflurane to 4.5 MAC hours occurring weeks prior to your CREB-assay. The similarity of the behavioral results with the biochemical one (i.e. a quantitative qualitative difference between the two doses of isoflurane) while not impossible, are not easy to understand. Are you postulating a 'threshold' effect captured by fortuitous choice of these two doses which affects only one of the two learning assays?

5. pCREB/CREB are certainly important biochemical correlates of learning and memory. However, a single isolated assay does not provide a waterproof link especially considering the lack of a dose-response relationship. How does, as you say, can an effect on CREB phosphorylation in utero be still visible and affect behavior weeks later after all the experiences the animals had after birth? Phosphorylation is a dynamic process with high turnover times. A deeper explanation of CREB physiology would be helpful.

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

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