Reviewer’s report

Title: Maternal Sciatic Nerve Administered Bupivacaine Induces Hippocampal Cell Apoptosis in Offspring

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Please explain the rationale for looking for hippocampal apoptosis at 30 days postnatal in rats exposed to bupivacaine on day 15 of gestation. Peak expression of activated caspase 3 occurs hours after an insult and is quite low days after the insult. I would not expect activated caspase 3 expression at 30 days of age to result from an insult more than 5 weeks earlier, and I don't know what this single time point means. To attribute the increased activated caspase 3 to the maternal exposure during pregnancy would require study at multiple other time points.

The references cited for accumulation and retention of bupivacaine in fetal tissues after maternal administration also show short term (hours) results, with declining levels in fetal tissues at the end of their study time points. These studies do not provide evidence that bupivacaine persists in postnatal animals weeks after intrauterine exposure.

If caspase 3 has been activated in neurons for this prolonged time period since exposure, there should be evidence of considerable hippocampal neuron loss. This should be easily demonstrated by histological examination.

If this delayed or prolonged caspase 3 activation is hypothesized to be in other non-neuron cell types, this could be better demonstrated by immunohistochemistry than by Western blots.

The use of ketamine to anesthetize the pregnant rats complicates interpretation of any results as being due solely to exposure to bupivacaine. Ketamine is highly neurotoxic to the developing brain, and while all groups were exposed to ketamine, the neurotoxic effects of ketamine have been shown to be worse when ketamine is combined with a number of other drugs.

Why was only one pup from each litter used in this study? The other pups could have been used for other time points or other types of assessments.

No data is provided on the pups used in this experiment. Were they similar in birth weight, growth and behavior to their littermates?

Increased activated caspase 3 five weeks after a single maternal local anesthetic exposure would require the presence of an on-going or delayed insult. Was there any evidence of seizures or other neurologic abnormalities that might explain delayed apoptosis?

Are the methods appropriate and well described? If not, please specify what is required in your comments to the authors.
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

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?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
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Needs some language corrections before being published

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