Reviewer’s report

Title: Nitrosamine exposure exacerbates high fat diet-mediated neurodegeneration

Version: 2 Date: 27 October 2009

Reviewer: angel cedazo-minguez

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I read with attention the new version of this manuscript. The authors have now included some of the suggestions raised earlier.

My main concern was that the brain area selected for this study is of minor relevance for AD. The manuscript is oriented and discussed in a way that is AD related.

The reasons by which authors choose cerebellum instead of ie. hippocampus were: that 1) this area is affected also in late AD, 2) shows high insulin response, and 3) by focusing in only one area they were able to make a digestible manuscript.

These justifications are included in two areas of the paper.

I am not at all convinced that cerebellum is a right choice, mainly because, as I said before, the results obtained are discussed from an AD pathology perspective (cholinergic lose, Abeta, tau, p-tau). The existing neurodegeneration in cerebella of AD brains is not following those patterns. As a consequence some aspects of the discussion remain very speculative.

Following the suggestions by other referees, the new version includes supplementary behavioral studies. Rats under HFD and NDEA showed cognitive deficits as seen by MWM. Since this test reflects memory impairment, it stress the fact that to explore the effects of the treatments on hippocampi would have been of great interest. There is not information on the effects on behavior controlled by the cerebelum (ie. balance deficits or ataxia).

I have also some differences in interpreting the results.

- It is reported a decrease in p-GSK3. In the previous version we did not known if it was Ser9-GSK3 (inactive GSK3) or Tyr216-GSK (active GSK3). Now, we now that it was the inactive one. The obtained results were badly interpreted in the discussion. In page 19 it can be read: “although GSK3-b signaling is increase in AD, in the present model both GSK3b and p-GSK3b were reduced in the HFD+NDEA group. We suspect that this finding may represent a compensatory response…. “ In fact what the authors found is an apparent INCREASE of GSK3b activity, since a reduction in the inactive form of GSK3 was reported. This would be consistent with a reduction in p-akt (in materials, an anti p-akt is listed, but not results can be seen in the manus), but not with the decrease seen in
p-tau.

- Page 18 “Reductions in total tau, ..., could result in synaptic cytoskeletal collapse and synaptic disconnections” It could be also possible that reductions in tau, in total GSK3b, in ChAT ... would simply reflect neuronal death caused by the treatments by unknown mechanisms. This possibility is not discussed.

-Page 18 “ The reduced levels of ChAT reflects deficits in Acetylcholine homeostasis that contribute to cognitive impairment in AD”. I do not understand how cholinergic deficits in the cerebellum could contribute to the cognitive impairment seen in AD.

The study has a tremendous among of work, and I found some of the results interesting, but in my opinion, it should be re-written from a different perspective, much more conservative regarding comparisons with the neurodegenerative mechanisms seen in AD.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests