Review of: "Synapse Weakening-Induced Caspase-3 Activity Confers Specificity to Microglia-Mediated Synapse Elimination"

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Potential competing interests: No potential competing interests to declare.

In this study, Yu and colleagues investigate the impacts of Caspase-3 activity on synapses in the developing retinogeniculate (RGC) pathway and in the dentate gyrus of APP/PS1 Alzheimer's disease mice. The authors find that Caspase-3 deficiency results in significantly less microglial, but not astrocytic, engulfment of presynaptic retinogeniculate (RGC) synapses during postnatal day 4 of development. Further, synapse loss in APP/PS1 mice is reduced in Caspase-3 deficient mice.

Overall, this is a well-written study with well-designed experiments that are a nice follow-up to their previous work. Enthusiasm for this work diminishes somewhat due to the lack of specificity of Caspase-3 ablation, as the authors only use a global knockout. This was mentioned as a limitation by the authors in the Discussion.

- To improve clarity further, it would help to add a clearer statement to the Results when describing that the synaptic engulfment was quantified in each individual microglial cell. In the confocal images of (Figure S9), it appears that the density of microglia is reduced in the Caspase-3 mutants. This could perhaps be linked to Caspase-3 activity associated with microglial apoptosis that mediates the turnover of the microglial population that others have described during both development and in the adult brain (e.g., Askew et al., 2017, DOI: 10.1016/j.celrep.2016.12.041).
- The rationale for investigating the AD mouse model in the Results is clear, but the data linking the findings in the RGC developmental model to the AD mice could be improved. For example, was Caspase-3 activity observed co-localized within microglia in their APP/PS1 mice (S17A) as it is in the Figure 1 RGC experiment?
- The authors' statements that in the APP/PS1 mice the synapse changes observed with Caspase-3 deficiency are "Aβ-induced" are unclear, particularly as the caspase-3 deficiency did not change the amyloid deposition. Could this instead be a secondary effect, mediated instead by e.g., complement pathways, as described by others to mediate synapse loss in Alzheimer's disease? I.e., (Wong et al., 2016, DOI: 10.1126/science.aad8373). The authors mention that expression of complement factors is not changed by caspase-3 knockout in their developmental model, but do not address this in the APP/PS1 mice.
- In Figure S17, it is unclear why only half the APP/PS1 mice (2 of 4) showed changes in Caspase-3 activity (but only at 4 months old). Are the authors looking at adding additional mice to this? If Caspase-3 activity is not upregulated in the dentate gyrus of these mice, what then is driving the synapse loss in the AD mice? Particularly in the 6-month-old females, where there is no obvious change in the caspase-3 activity, which was the age when the synapse loss was

quantified in Figure S15. It is unclear why the authors chose to quantify synapses at 6-month-old APP/PS1 mice, and not the 4-month-old mice, when caspase-3 activity was perhaps upregulated. Likewise, others, like the above-mentioned reference (Wong et al., 2016), observe hippocampal synapse loss in the APP/PS1 mice early, at 4 months old. Are the authors looking to characterize synapses for this age group? The minor differences in synapses in Figure S15 may be more obvious at the earlier age.

- To better support their statements, more robust statistical analyses would be preferable; e.g., for Figure 7, a two-way ANOVA with Bonferroni post-hoc is suggested to directly compare the caspase-3 wildtype to the caspase-3 deficient mice. Understanding that the magnitude of the differences is fairly minor, so the statistical difference may be lost with ANOVA.