

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

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This work demonstrates the need for caspase-3 activity to eliminate weaker synapses in the well-known visual system where most of the pruning work has been done. It presents several experiments aimed at demonstrating an interesting mechanism of synapse pruning in the retinogeniculate visual pathway. The paper includes many figures and really interesting results, but it makes reading difficult due to the mix of results, interpretations, and hypotheses along the results section of the work. In addition, several methods of analysis were designed by the authors (i.e., the definition of "synapses" on confocal images without further TEM confirmation, the analysis of "activity" of caspase 3 by ML-assisted confocal images, etc.). Are these methods validated before being used here? The result showing caspase 3 KO mice having no alterations in pruning is intriguing, and the explanations provided by the authors are not very convincing. Please improve this, show images, etc. The images provided for microglia and astrocytes synaptic material engulfment are nice but not very convincing. Please provide higher magnification or larger details, or 3D moving representations, etc. The caspase 3 blockage may affect typical neuronal loss in AD models; how do the authors prevent that effect that might confound the implications of the findings?

In addition, I have a few suggestions to make the reading easier:

- During the description of the results, it would be better to avoid including descriptions of methods that are already detailed thoroughly in the Material and Methods section. Also, do not include discussion of the implications of these results in this section.
- Supplementary figures should be moved to the end of the article, as placing them between the main text makes reading more complex.
- It is probably unnecessary to clarify phrases like "contralateral [opposite side ...]," as seen in the legend of figure S1. I believe readers can understand the meaning, and this would make the paragraph shorter.
- In figures S9, 5, S11, and 6, it is necessary to add insets with a magnification of microglia and astrocytes so that the morphology and localization of Cd68 and axon terminals, for example, can be better appreciated.

