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.

The manuscript entitled "*Synapse Weakening-Induced Caspase-3 Activity Confers Specificity to Microglia-Mediated Synapse Elimination*" by Yu *et al.* provides an exciting investigation into activity-dependent synapse pruning driven by post-synaptic caspase-3 activity.

The study is divided into three parts: (1) the early development of the retinogeniculate pathway, (2) the refinement of the retinogeniculate circuit, and (3) amyloid-β-induced synapse loss in an Alzheimer's disease (AD) model.

The experiments in each part are well-conceived, and the interpretations are largely sound. However, I believe that the overall organization of the manuscript could be improved to enhance the impact of the work. Specifically, there seems to be a notable disconnect between part (3) and the earlier sections. While I understand the authors' attempt to generalize the role of caspase-3 in synapse elimination and maintenance, focusing solely on the first two parts might strengthen the coherence of the narrative. Removing part (3) could help achieve this focus.

Additionally, the flow of the manuscript is somewhat disrupted by the splitting of part (1) around part (2). For example, the data for part (1) (Fig. 1-3 and S1-S6) is presented first, followed by part (2) (Fig. 4 and S7-S8), and then part (1) is revisited (Fig. 5-6 and S9-S12). I suggest reorganizing the manuscript to present a more seamless progression of ideas.

There are a few critical aspects that need further attention:

1. While the use of conventional caspase-3 knockout mice is appropriate, conditional knockout strains should be considered, given the important role of caspase-3 in microglial function. Additionally, demonstrating the result by post-synaptic-specific elimination of caspase-3 activity, at least *in vitro*, is crucial to firmly establish the role of caspase-3 in the current work.
2. In Fig. S11, the authors concluded that "*astrocyte-mediated synapse elimination does not appear to depend on caspase-3*" because of its small effect size. However, they also mentioned that "*Astrocytes were present in the P5 dLGN at a much higher density than microglia and possess very fine processes*". This observation may indicate that a higher number of astrocytes contribute to synapse pruning even though each effect is small. It would be beneficial to include experiments similar to those in Fig. 6 to more rigorously assess astrocyte involvement.

Lastly, I have not addressed part (3) in detail since I believe its removal might enhance the focus and clarity of the manuscript.