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

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Article by Zhou Yu, Andrian Gutu, Namsoo Kim, Erin K. O'Shea: "Synapse weakening-induced caspase-3 activity confers specificity to microglia-mediated synapse elimination".

This outstanding and technically very advanced paper was clearly intended for a top-tier journal, but for obvious reasons, it was not accepted. I read this paper with great interest, and I want to express this to the authors before I move on to criticism. Nothing I write below will change the importance of the presented study. Below, I will briefly go over the points that seemed questionable or even strange to me.

1. First, the paper seems too complicated and overloaded. It could easily have been, without any loss of quality, or perhaps for the sake of quality, divided into two or three separate studies. One or two would have dealt with the visual input to the lateral geniculate nuclei, and the other with the Alzheimer's model in the hippocampus. I did not find any compelling arguments to combine these topics.

2. Figure 1 contains a very important message regarding the concentration of activated caspase-3 specifically in the postsynaptic zone. That is, caspase-3 is concentrated not in axons, not in glial cells of one nature or another, but in the postsynaptic areas of dendrites. But where are these post-synapses, what is their shape and size? What types of cells are talked about? We know that the lateral geniculate nucleus is by no means homogeneous. It consists of six cellular layers that receive input from the optic tract fibers and optic radiations, divided into magnocellular and parvocellular layers. The nucleus contains neurons of various morphologies (please see: https://pmc.ncbi.nlm.nih.gov/articles/PMC6623799/). In addition, spiny stellate cells distribute input signals from the lateral geniculate nucleus to the cerebral cortex. So, do postsynaptic cells have spines? The article ignores these obvious questions. The single unclear image of 1G raises more questions than it answers. Figure S4, which shows MAP2 labeling (i, ii, iii), can be considered a failure. This is not the quality of visualization that one expects from a section that is fundamental to the entire article.

3. The data in Figure 2 are also difficult to accept without a great deal of effort. I could not understand why the average case (single injection) suggests synaptic competition, while the control and dual injection do not. The cell as a whole and a specific dendrite in particular, «do not know» whether the inputs from two retinas overlap or not. To make such a bold statement, it would be necessary to show co-localization of these inputs or their specific distribution in specific zones of

the dendritic tree (proximal, distal, etc.). The authors do not show anything like this. I will put it modestly: synaptic competition exists only in the given scheme 2A, and not in reality. Many alternative explanations can be thought up for the statistically significant differences shown in Figure 2C, but I do not see the point in giving them here. I deliberately dwell on the first figures in more detail because they lay the foundation for the entire subsequent work. If these cornerstones are removed, the entire edifice of the article will shake.

4. The chapter "Caspase-3 is required for segregation of eye-specific territories" presents us with a Casp3–/– model, but in essence, it is of no importance for the main narrative.

5. The electrophysiological data also seem to me to be irrelevant to the essence of the problem. Yes, caspase-3 taken out of context (upstream, downstream elements) is important for the development and action of neurocirculatory systems, but this is not the topic of this article. Perhaps the data are important for describing the outstanding role of caspase-3, but not in the given context. In addition, the question of direct or indirect participation of caspase-3 is not (and could not be) resolved here.

6. The question of the participation of microglia in the pruning of labeled synapses seems to me to be the weakest side of the presented article. I don't question the fact that caspase-3 tags were found in microglia. But how did they get there? Are we supposed to assume that glial cells crawl up to the tagged areas of dendrites and simply rip out the postsynaptic membranes along with all the local proteins? But the dendrites somehow magically remain intact. This scenario is extremely unlikely. It's safe to say that this question is beyond the scope of this article, but it's far more important than anything else. At least, I would have expected this question to be addressed in more detail than it is in the discussion.