Review of: "Properties and proximity proteomics of synaptopodin provide insight into the molecular organization of the spine apparatus of dendritic spines"

Bence Racz

Potential competing interests: The author(s) declared that no potential competing interests exist.

Authors aimed to reveal new insight into the molecular properties of the mysterious spine apparatus. In order to do so, they mostly used model systems to find fundamental mechanisms contributing to the possibble formation of the SA, using synaptopodin as a proxy.

Initially, they used TEM and FIB-SEM to study the overall organization of the SA in brain tissue of mice. Next, hippocampal neurons *in vitro* were analysed for SA.
Next, they went on, and studied the role of synaptopodin in model systems (RPE1 and COS-7 cells), that do not normally express this protein. To further investigate the interactions of synaptopodin, it was directly targeted to the ER by fusing it to the N-terminus of Sec61ß, an ER resident protein anchored to this organelle by a C-terminal transmembrane region.

The study is elegant and well designed, using multiple, state-of-the art approaches to broaden our understanding of the role of synaptopodin in the formation of the SA.

Some minor issues:
With EM, they describe a significant morphological variance in the number of stacks, and shapes of different spine SAs. Yet, when they study the dynamic properties of EGFP-synaptopodin puncta in neurons by FRAP, they only study ~2 spines /neurons, overall 11 sppines. As the SA has siginificant morphological heterogeneity, how can the data gained by observing so few spines applied to describe fundamental mechanisms?

Most EM analysis of brain tissue was from adult mice (3 months old). However, the overwhelming majority of the experiments either used cultured cells or cells from pups. It is well known, that neurons at so early developmental stages have siginificantly different internal organization, and *in vitro* hippocampal neurons share very little morphological characteristic once compared to in vivo pyramidal neurons in the brain's neuropil - including the SA. This issue needs to be address in text, as readers may have the misleading impression, that results obtained from highly simplified model systems or from very young animall's barin cells may be readily applicable to adult brain.

While I really appreciate their results pointing to a role of synaptopodin in enriching Pdlim7 in spines and thus to a special relation between synaptopodin and Pdlim7, expressing these proteins with a fluorescent tag in neurons and detecting co-

localization may not be the best way to support such conclusions.

Taken together, this is a very well designed study wich provides novel insight into the function of synaptopodin in the formation of the SA. I'd definitely strengthen the manuscript by using higher sample sizes, and also providing a stronger link between neurons in brain vs. ther very interesting findings in model system.