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MEETING ABSTRACT

A3.27

Functional analysis of the docked vesicle pool in hippocampal mossy fiber terminals by electron microscopy

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Background: Understanding the relation between structure and function of synapses is a key question in neuroscience. Although structure and function of synapses have been studied for decades, several fundamental aspects of synaptic transmission remain unresolved. For example, the relation between functionally and structurally defined synaptic vesicle pools remains unclear. Some studies proposed that readily releasable pool (RRP) and docked vesicle pool are identical [1], whereas others suggested that they are only partially overlapping and that vesicles may be released from non-docked pools [2]. As central synapses are structurally and functionally diverse, addressing these questions requires correlated structural and functional analysis at rigorously identified synapses.
Methods: We implemented and further developed on the "flash and freeze" technique [3], to apply it to hippocampal acute slices and organotypic slice cultures and investigate the relationship between function and structural changes after synaptic stimulation at the hippocampal mossy fiber–CA3 synapse. We analyzed the docked vesicle pool immediately after brief and long stimulation trains.
Results: Our results reveal several unexpected aspects of structure and function at this synapse. First, our analysis shows that the docked vesicle pool at active zones at hippocampal mossy fiber synapses is substantially depleted by more than 80% after presynaptic stimulation. After depletion, we observed recovery of the depleted docked pool 20 s after the end of the stimulus. At this time point, the docked vesicle pool not only recovered, but both the number and the diameter of docked vesicles increased. Secondly, our results confirm that the vesicle diameter at the mossy fiber synapse is variable and skewed towards larger diameters, extending previous work on this synapse [4]. Intriguingly, we find that, initially, stimulation increases the mean diameter of docked vesicles, which then decreases after brief and long trains. Finally, moderate repetitive stimulation results in the formation of endocytic pits, presumably by a clathrin-independent mechanism, as previously suggested on the basis of capacitance measurements [5].
Discussion: Our main conclusions are: (a) RRP and docked vesicle pool are highly overlapping at this synapse. (b) The structural changes we observed with depletion and recovery of the RRP are consistent with our functional findings that after post-tetanic potentiation there is an increase in the RRP. (c) Smaller vesicles are released first, implying that they have a higher release probability. Consistent with this interpretation, vesicle fusion rate increases with membrane curvature [6, 7].

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Keywords: hippocampus – mossy fibers – readily releasable pool – electron microscopy

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