High resolution microscopy of synaptic vesicle recycling

A readily retrievable pool

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Abstract:
Fusion of synaptic vesicles (SVs) during fast synaptic transmission is mediated by SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex assembly formed by coil-coiling of three members of the protein family: synaptobrevin 2 (syb2) and the presynaptic membrane SNAREs syntaxin-1A and SNAP-25. In order to maintain neurotransmission exocytosed SV components need to be retrieved from the surface by compensatory endocytosis. Clathrin-mediated endocytosis (CME) is thought to be the predominant mechanism of SV recycling. However, it might be too slow for fast SV recycling. Therefore, it was suggested that a pre-sorted and pre-assembled pool of SV proteins on the presynaptic membrane might support a first wave of fast CME. We monitored the temporal dynamics of such a 'readily retrievable pool' of SV proteins in hippocampal neurons using a novel probe, CypHer 5, a new cyanine dye-based pH-sensitive exogenous marker, coupled to antibodies against luminal domains of SV proteins. This way we could for the first time demonstrate the preferential recruitment of a surface pool of SV proteins upon stimulated endocytosis. Using fluorescence nanoscopy (isoSTED and FPALM) of labeled SV proteins we could resolve the spatial distribution of the surface pool at the periactive zone. Recently, we identified dimerisation of vesicular SNARE syb2 as a first important step in self-assembly of surface nanodomains. It has been shown before that syb2 can dimerize, and a glycine residue in the transmembrane domain of syb2 was found to be important for dimerization. Fluorescence Photo-Activation Localization Microscopy (FPALM) of membranes of secretory (PC12) cells reveals re-assembly of exocytosed syb2 into nanoclusters harbouring a few ten molecules, which is abolished when the glycine residue is mutated. Likewise, in cultured hippocampal neurons, using a combination of two pH-sensitive dyes (pHluorin and cypHher) we could show that dimerization of syb2 is necessary for efficient sorting into newly endocytosing SVs.