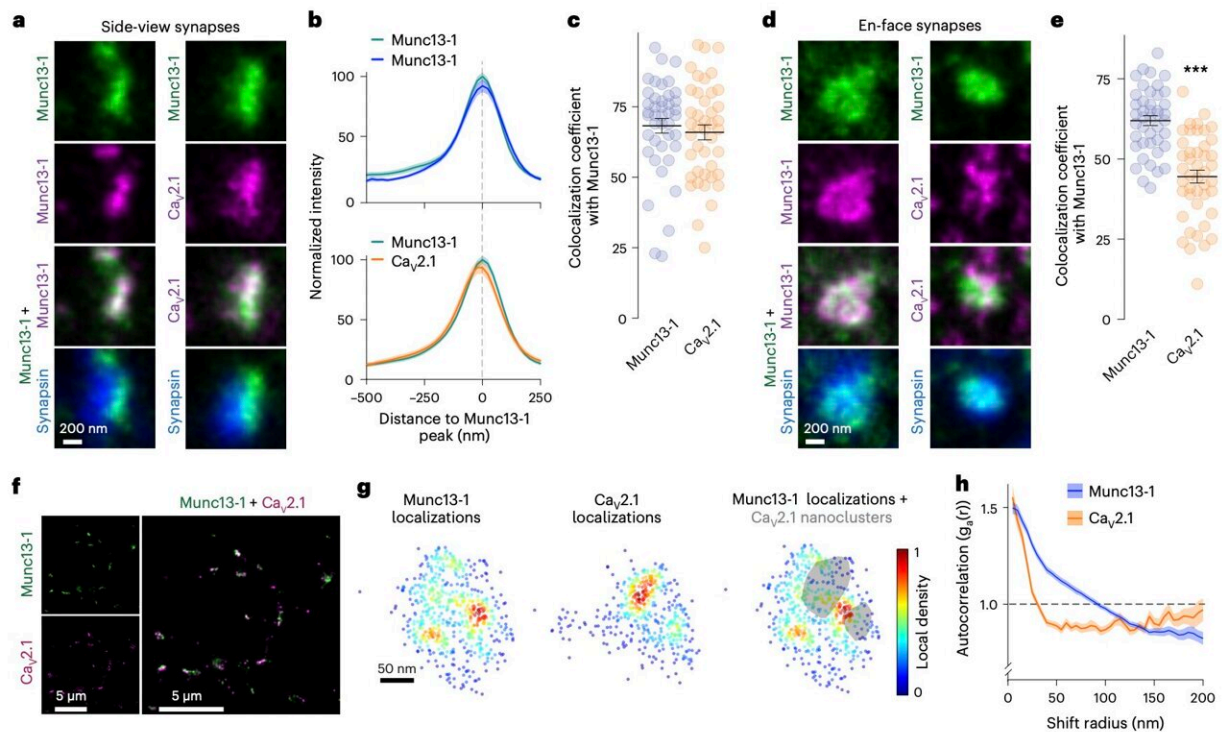


Hippocampal study unveils distinct protein machineries for calcium channel clustering and vesicle priming

September 11 2024, by Ingrid Fadelli



Munc13-1 and CaV2.1 nanoclustering. a–c, Example STED images of sideview synapses (a), quantification of line profiles (b) and Pearson's colocalization coefficients (c). Cultured hippocampal neurons stained with antibodies against Synapsin and Munc13-1, and a second Munc13-1 antibody or a CaV2.1 antibody. Side-view synapses were identified by the presence of an elongated Munc13-1 object (shared Munc13-1 antibody) at the edge of the Synapsin-positive vesicle cloud. A line profile (750 nm \times 250 nm) was positioned perpendicular to the Munc13-1 center, and signals were aligned to the Munc13-1

peak in the averaged line profiles (b); Munc13-1, 42 synapses/3 cultures; CaV2.1, 42/3. d,e, Same as a–c, but for en-face synapses identified by the presence of a Munc13-1 area (shared Munc13-1 antibody) surrounded by Synapsin and without line profiles. Munc13-1, 43/3; CaV2.1, 44/3. f, Zoomed-out, rendered DNA Exchange-PAINT images after Munc13-1 and CaV2.1 staining. g, Zoomed-in view of the density of protein localizations in an example en-face synapse imaged with DNA ExchangePAINT showing Munc13-1, CaV2.1 and Munc13-1 with shaded CaV2.1 nanoclusters identified with a density-based clustering algorithm. h, Autocorrelation of Munc13-1 and CaV2.1 synaptic densities measured as the average probability of detecting localizations for the same protein at increasing distances from any given localization. Munc13-1, 147 synapses/3 cultures; CaV2.1, 147/3. Credit: Emperador-Melero et al. (*Nature Neuroscience*, Springer, 2024).

In neuroscience, the term 'active zone' is used to refer to a specialized area in the presynaptic membrane of synapses (i.e., the connections via which neurons transmit electric nerve signals to each other or to other cells). The active zone is the site where neurotransmitters are released at synapses, thus it plays a central role in neuron-neuron communication.

During an [action potential](#) (i.e., an [electrical signal](#) that travels within the membrane of neuron to transmit information across the [nervous system](#)), [synaptic vesicles](#) fuse with the pre-synaptic membrane in response to an influx of Ca^{2+} ions. This process, which occurs within the active zone, ultimately leads to the release of neurotransmitters into the small gap between the neuron sending an electric signal and the neuron receiving it, known as the synaptic cleft.

Researchers at Harvard Medical School and University of Maryland School of Medicine recently carried out a study investigating [protein machinery](#) in the active zone that mediates synaptic vesicle priming and clustering of Ca^{2+} channels. Their findings, [published](#) in *Nature*

Neuroscience, unveil two separate active zone protein machineries at hippocampal synapses that independently execute these functions.

"Action potentials trigger neurotransmitter release at the presynaptic active zone with spatiotemporal precision," Emperador-Melero, Jonathan W. Andersen and their colleagues wrote in their paper.

"This is supported by protein machinery that mediates synaptic vesicle priming and clustering of Ca_v2 Ca^{2+} channels nearby. One model posits that scaffolding proteins directly tether vesicles to Ca_v2 s; however, here we find that at mouse hippocampal synapses, Ca_v2 clustering and vesicle priming are executed by separate machineries."

As part of their studies, the researchers examined floxed mice and previously prepared hippocampal cultures extracted from mice pups using various microscopic, imaging and processing techniques. Their analyses showed that two proteins, namely Ca_v2 and Munc13, each form nanoclusters positioned at variable distances from each other at hippocampal synapses.

" Ca_v2 nanoclusters are positioned at variable distances from those of the priming protein Munc13," wrote Emperador-Melero, Andersen and their colleagues.

"The active zone organizer RIM anchors both proteins but distinct interaction motifs independently execute these functions. In transfected cells, Liprin- α and RIM form co-assemblies that are separate from Ca_v2 -organizing complexes. At synapses, Liprin- $\alpha1$ –Liprin- $\alpha4$ knockout impairs vesicle priming but not Ca_v2 clustering. The cell adhesion protein PTP σ recruits Liprin- α , RIM and Munc13 into priming complexes without co-clustering Ca_v2 s."

Based on their observations, the researchers conclude that Ca_v2 clusters

and vesicle priming sites are organized independently and are supported by distinct machineries. They also concluded that Liprin- α and PTP σ proteins specifically support priming site assembly.

Overall, these recent findings suggest that there are at least two distinct active zone machineries marked by independent mechanisms that mediate Ca^{2+} channel clustering and vesicle priming. The researchers hypothesize that the sub-structuring of the active zone they observed contributes to the resilience of synapse and active zone assembly reported in various previous neuroscience studies.

The results gathered as part of this research effort could pave the way for new studies aimed at further investigating the distinct active zone protein machineries identified. This could lead to more interesting observations about the sub-structuring of the active zone, potentially identifying other protein machineries mediating Ca^{2+} channel clustering and vesicle priming.

More information: Javier Emperador-Melero et al, Distinct active zone protein machineries mediate Ca^{2+} channel clustering and vesicle priming at hippocampal synapses, *Nature Neuroscience* (2024). [DOI: 10.1038/s41593-024-01720-5](https://doi.org/10.1038/s41593-024-01720-5)

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