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Roles of VAMP7 in Drosophila Synaptic Transmission
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Soluble N-ethylmaleimide-sensitive factor Attachment Receptor (SNARE) proteins control the fusion of intracellular membrane compartments. The vesicle-associated membrane proteins (VAMPs) are a family of SNAREs essential to establish the interaction with target SNAREs like Syntaxin and SNAP. Tetanus-toxin Insensitive VAMP (TI-VAMP) is different from the Synaptobrevin VAMP family which majorly controls synaptic vesicle (SVs) fusion. Ti-VAMP or VAMP7 has a longing domain necessary to sort proteins to internal compartments. In neurons, VAMP7 is thought to regulate SV trafficking and spontaneous fusion. In addition, VAMP7 participates in neurite extension and SV cycling through a reserve pool, however, its function is still under investigation. Here, we characterized a VAMP7 loss of function Drosophila animal (VAMP-$7^{-/-}$) generated by imprecise p-element excision. Null animals survive until third instar larval stage in low density condition and display sluggish phenotype. Basal synaptic transmission analyses by two-electrode voltage-clamp indicate unaltered spontaneous and evoked SV fusion. Additionally, exo/endocytosis imaging with FM 1-43 revealed normal exo/endo-cycling pool but altered reserve pool formation. Neuronal driven expression of Drosophila VAMP7 tagged with GFP display punctate morphology at nerve terminals.

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Synapsin Null Increases Calcium Sensitivity of Vesicle Fusion and Alter Short-Term Synaptic Memory at Drosophila NMJ
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The availability of synaptic vesicles (SVs) and their timing for fusion are critical for neurotransmitter release and neural communication. Synapsins (Syn) are abundant phosphoproteins associated reversibly with SVs and cytoskeleton. Syn is highly conserved in animal kingdom and is thought to regulate SVs trafficking and short-term plasticity. Syn function compromises learning and memory, however, its role in synaptic transmission is still under investigation. Here we analyzed Syn null by voltage-clamp recording of synaptic transmission at Drosophila glutamatergic model. We found that Syn null increases the probability of SV fusion by decreasing the sensitivity to calcium. In addition, Syn null increases asynchronous release, alters short-term plasticity and synaptic memory. A use-dependent model for neurotransmission induced by high-frequency nerve activity and a masked plasticity under depression is discussed.

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Mathematical Modeling Supports the Hypothesis that Synaptotagmin Rings Clamp Fusion
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Synaptotagmin (Syt) is a component of the cell's machinery that mediates membrane fusion during synaptic release and other fundamental processes. Syt is an integral membrane protein in synaptic vesicles with calcium binding domains C2A and C2B and binds SNARE proteins whose assembly drives fusion. SYT is the calcium sensor during evoked release, but the mechanisms are unclear. Recently, electron microscopy (EM) showed Syt C2AB domains spontaneously forming rings on negatively charged phospholipid monolayers. Ca^{2+}disassembled the rings, suggesting Syt rings could serve as membrane spacers that clamp fusion by preventing SNARE assembly until the Ca^{2+} pulse, when their disassembly would trigger fusion (Wang et al, 2015).
Here we used mathematical modeling to test the hypothesis that SYT rings form on target membranes and clamp fusion. We developed a coarse-grained model that incorporates features from the Syt crystal structure and treats the membranes in a dynamic triangulation scheme.
The model showed that Syt monomers self-assemble into rings on monolayers, with poly-lysine SYT patches on the inner edge of the ring attracting and buckling the charged membrane. The model reproduced experimental ring size distributions and structures seen in EM: smaller rings produced dome-shaped membrane deformations, larger rings produced volcanoes, reflecting competition between membrane bending energy and unbinding energy from the substrate. Further, we find that docking of a SYT-containing vesicle will generate SYT rings on the target membrane (trans-binding) due to the charged PS and PIP2 components, consistent with the ring spacer clamping hypothesis. Interestingly, rings buckled the membrane towards the synaptic vesicle, a deformation that might be expected to promote fusion. Overall, we find that charged membranes promote formation of Syt rings that may clamp fusion, but the rings simultaneously deform membranes into potentially fusion-promoting shapes.

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Molecular Dynamics Simulations of Synaptotagmin Binding to Lipid Bilayers
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Synaptotagmin 1 (Syt1) is a protein associated with synaptic vesicles that serves as a neuronal calcium sensor. In response to an action potential, calcium ions inflow into the nerve terminal, bind Syt1, and trigger fusion of vesicles with the plasma membrane. Although the structure, dynamics, and calcium binding properties of Sy1 have been extensively studied, it is still debated how specifically Syt1 triggers fusion. Syt1 includes a transmembrane region and two cytosolic domains, C2A and C2B, connected by a flexible linker. Both domains have calcium binding loops, and calcium binding is thought to promote the insertion of domain tips into lipid bilayers. To elucidate this mechanism, we performed molecular dynamics (MD) simulations of calcium free and calcium bound forms of Syt1 in water/ion environment and took advantage of periodic boundary conditions to model the dynamics of Syt between bilayer bilayers. We found that calcium binding loops of C2A domain have strong affinity to lipids even in the absence of calcium, and calcium biding strongly promotes their penetration into bilayers. In contrast, C2B binding loops did not interact with lipids in the absence of calcium, and even upon calcium binding only a week interaction of C2B calcium binding loops with lipids was observed. Apparently, this interaction may depend upon lipid composition and may require specific lipids which were not included in our simulations. Calcium binding pockets of C2 domains had a strong preference for facing opposing membranes during the entire length of the simulation. These results suggest a scenario whereby the tip of Syt1 C2A domain preferentially interacts with the vesicle membrane and immerges into lipids upon calcium binding, while C2B domain interacts with the plasma membrane, possibly being anchored by membrane-specific lipids, such as PIP2.

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Synaptotagmin-1 Binds to PIP2-Containing Membrane but not to SNAREs at Physiological Ionic Strength
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The Ca^{2+} sensor synaptotagmin-1 is thought to trigger membrane fusion by binding to acidic membrane lipids and SNARE proteins. Previous work has shown that binding is mediated by electrostatic interactions that are sensitive to the ionic environment. However, the influence of divalent or polyvalent ions, at physiological concentrations, on synaptotagmin's binding to membranes or SNAREs has not been explored. Here we show that binding of rat synaptotagmin-1 to membranes containing phosphatidylinositol 4,5-bisphosphate (PIP2) is regulated by charge shielding caused by the presence of divalent cations. Surprisingly, polyvalent ions such as ATP and Mg^{2+} completely abrogate synaptotagmin-1 binding to SNAREs regardless of the presence of Ca^{2+}. Altogether, our data indicate that at physiological ion concentrations Ca^{2+}-dependent synaptotagmin-1 binding is confined to PIP2-containing membrane patches in the plasma membrane, suggesting that membrane interaction of synaptotagmin-1 rather than SNARE binding triggers exocytosis of vesicles.

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Inversion of Ligand Binding Preferences in Re-Engineered Dysferlin C2Av1
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Dysferlin is a 237 kDa skeletal muscle protein that plays a key role in Ca^{2+}-mediated membrane repair of muscle cells. The full length protein possesses seven tandem C2 domains and a single C-terminal transmembrane helix. Mutations in the dysferlin protein have been linked to Limb-Girdle Muscular Dystrophy in humans. The canonical C2A domain of dysferlin binds Ca^{2+} and phospholipid similar to other Ca^{2+}-dependent phospholipid binding C2 domains; however, another co-expressed isoform of dysferlin C2A, C2A variant 1 (C2Av1), binds negatively changed phospholipid, but not Ca^{2+}.