# Synaptotagmin I Functions as a Calcium Sensor to Synchronize Neurotransmitter Release

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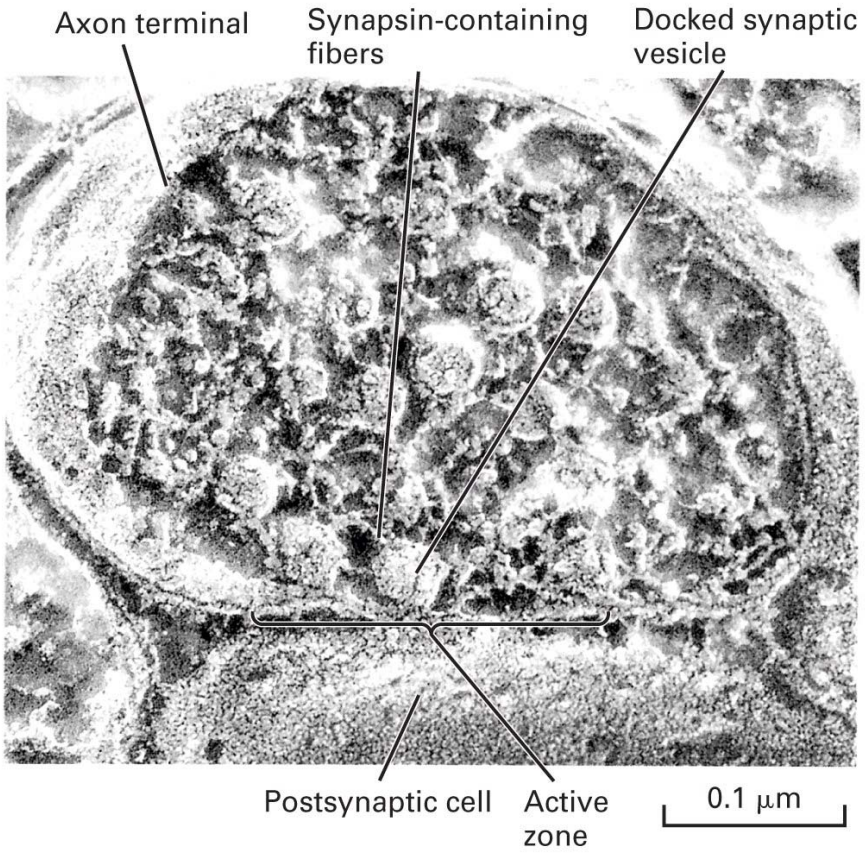
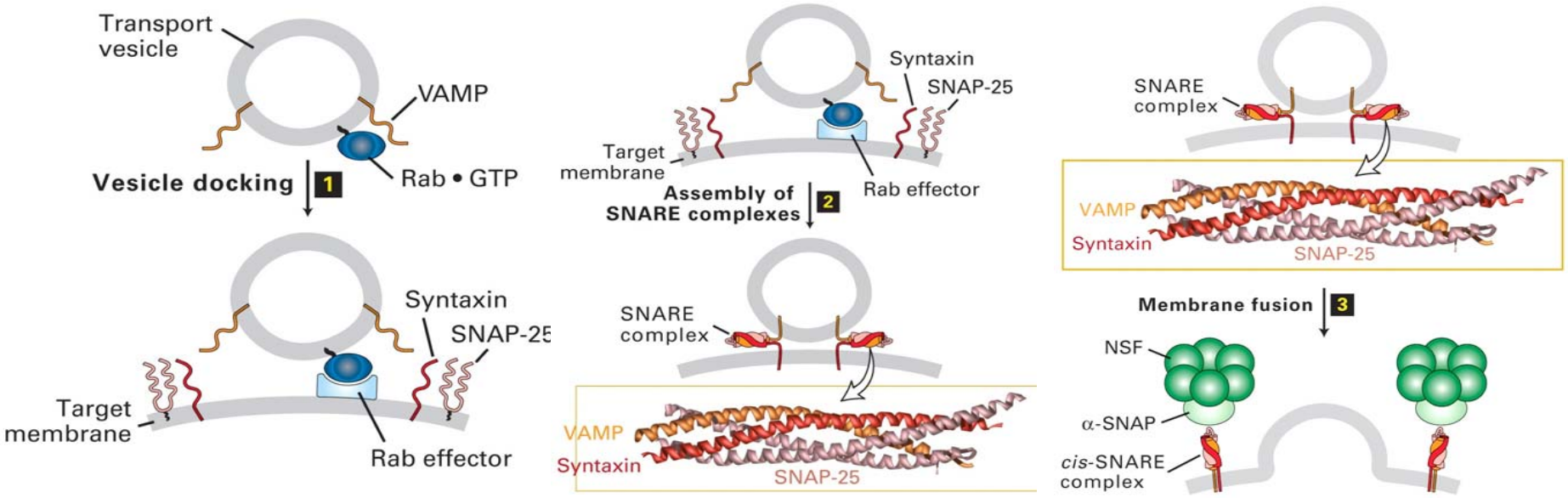


# Finding the mechanism of late neurotransmitter release

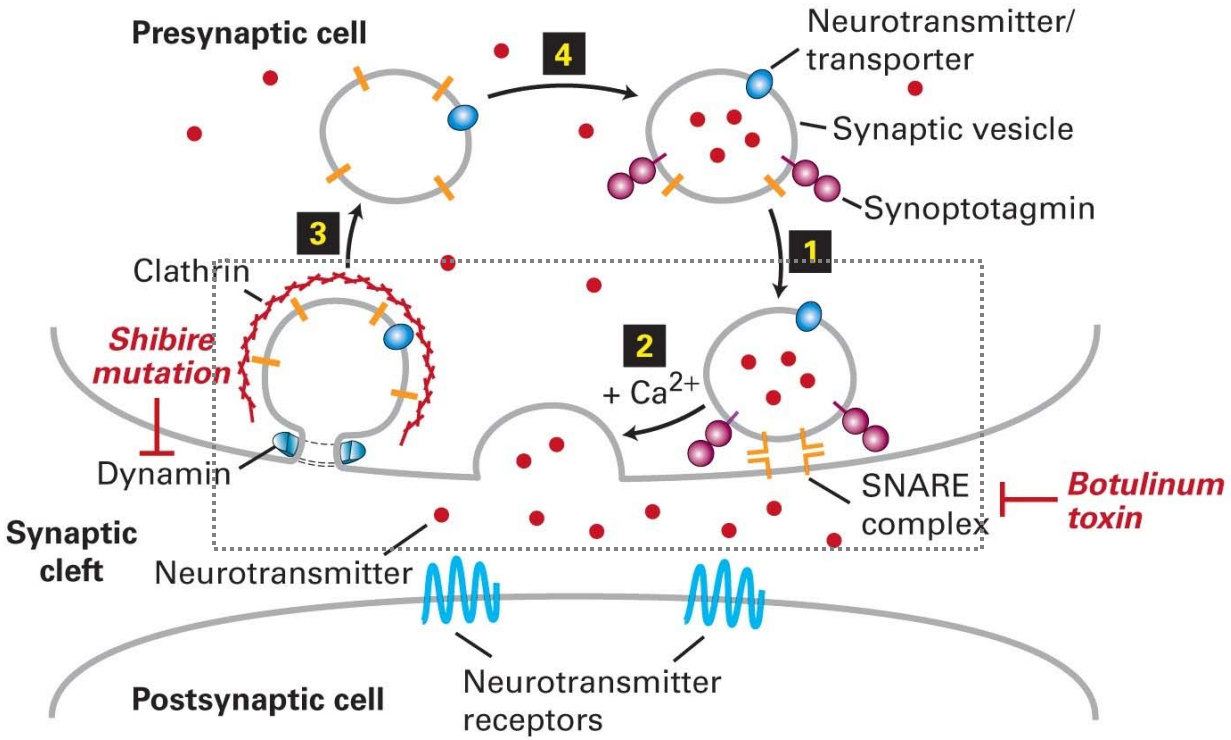
- Hypothesis: Ca++ influx into the presynaptic nerve triggers neurotransmitter release (1967)
- Release happens in milliseconds even though vesicle must go through translocation, docking and priming
- Reconstitution of the SNARE components is slow and Ca++ independent (1998)
- Few neuronal preparations allow the control of Ca++ stimulus sufficiently for quantitative analysis
- The advancement of caged signaling compounds (1997) and genetic studies drove most analysis of process

# What properties might this molecule possess?



# Synaptotagmins

- Synaptotagmin have well characterized Ca++ binding motifs (1990)
- Synaptotagmin binds the SNARE complex and phospholipids in a Ca++ dept. manner (mid 90's)
- Ca++ dept. interaction with t-SNARE and SNAP-25



# *Drosophila Syt* Mutants

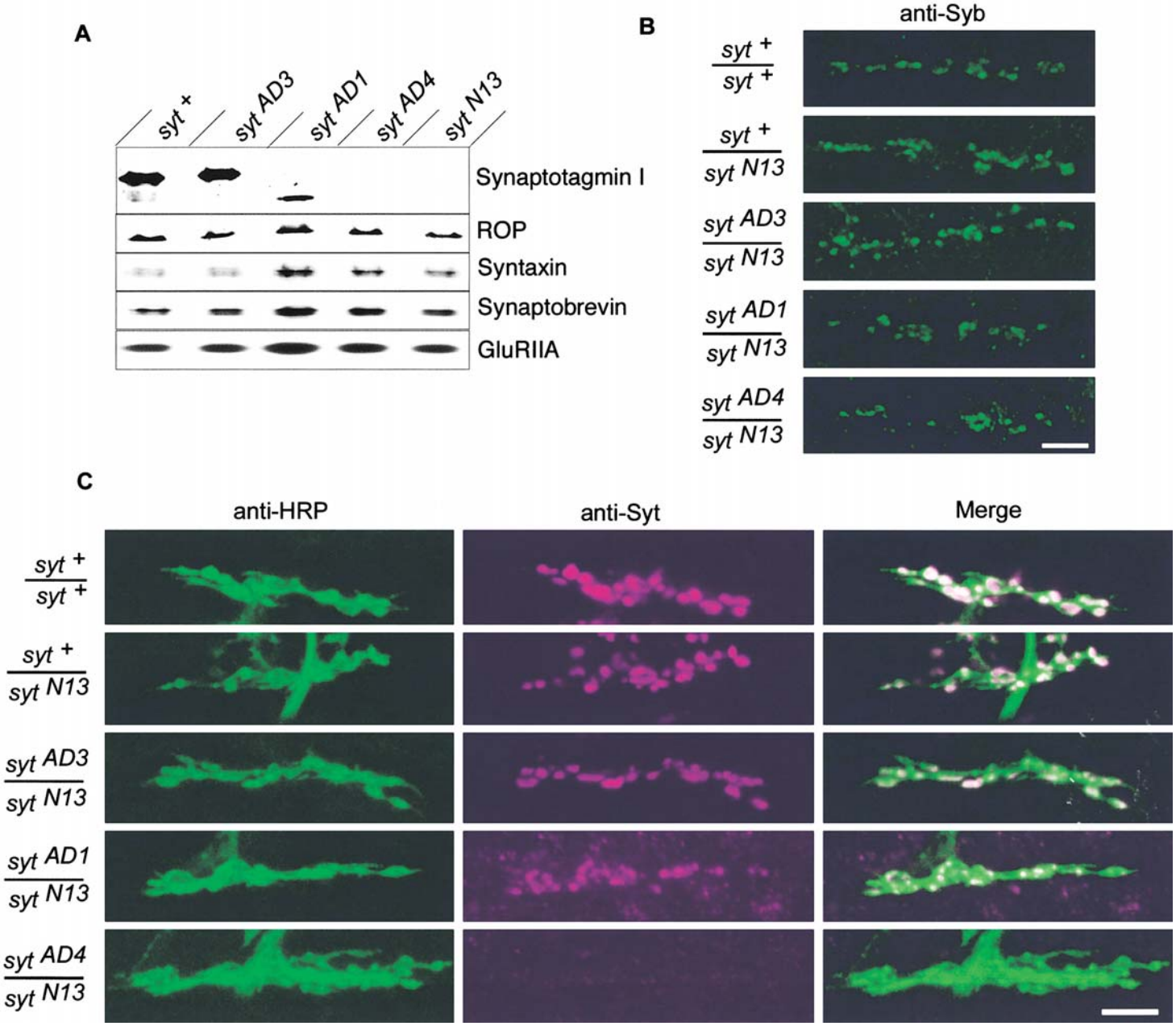
- **AD4=NULL=no interactions** ; deletion of transmembrane and cytoplasmic domains

- **AD1=NO C2B= little assc. with SNAREs**; deletion of C2B domain reducing Ca++ dept. assc with SNAREs and oligomerization, phospholipid binding preserved

- **AD3= NO C2B Ca++ binding=no oligomerization;**Y364N in C2B does not abolish SNARE or phospholipid binding

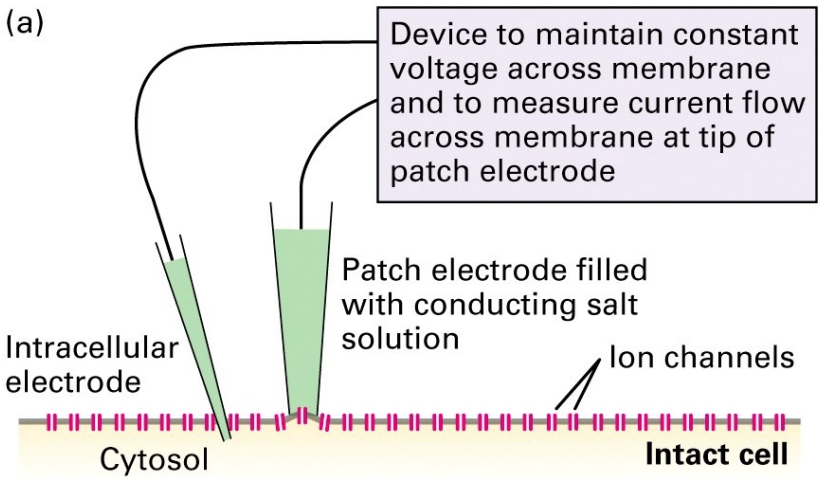
- **N13=NULL=no interactions**; Deleted at 5' of gene so *no protein made*

# Figure 1: Characterization of Mutants

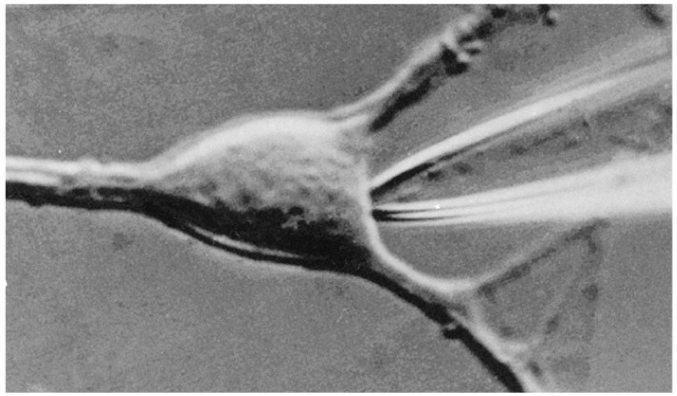


# Electrophysiological Analysis

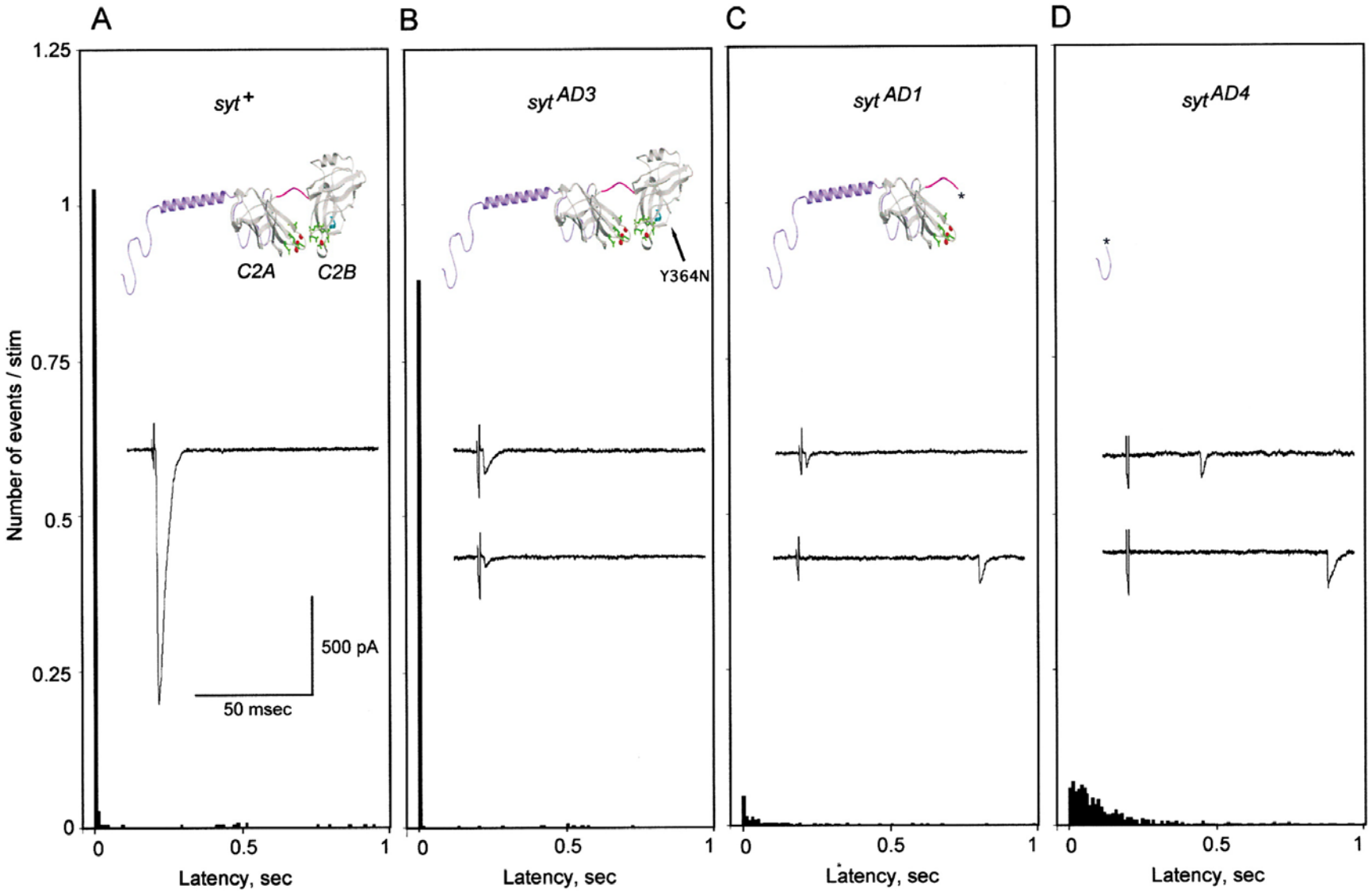
- Whole cell patch clamp to embryonic muscle fibers
- Motor nerves positioned at a suction electrode at the site of their emergence from the CNS
- Quantal content=ln(number of stimuli/number of failures of synapic current w/I 6ms)
- Mhc null mutant backgrounds to inhibit contractions



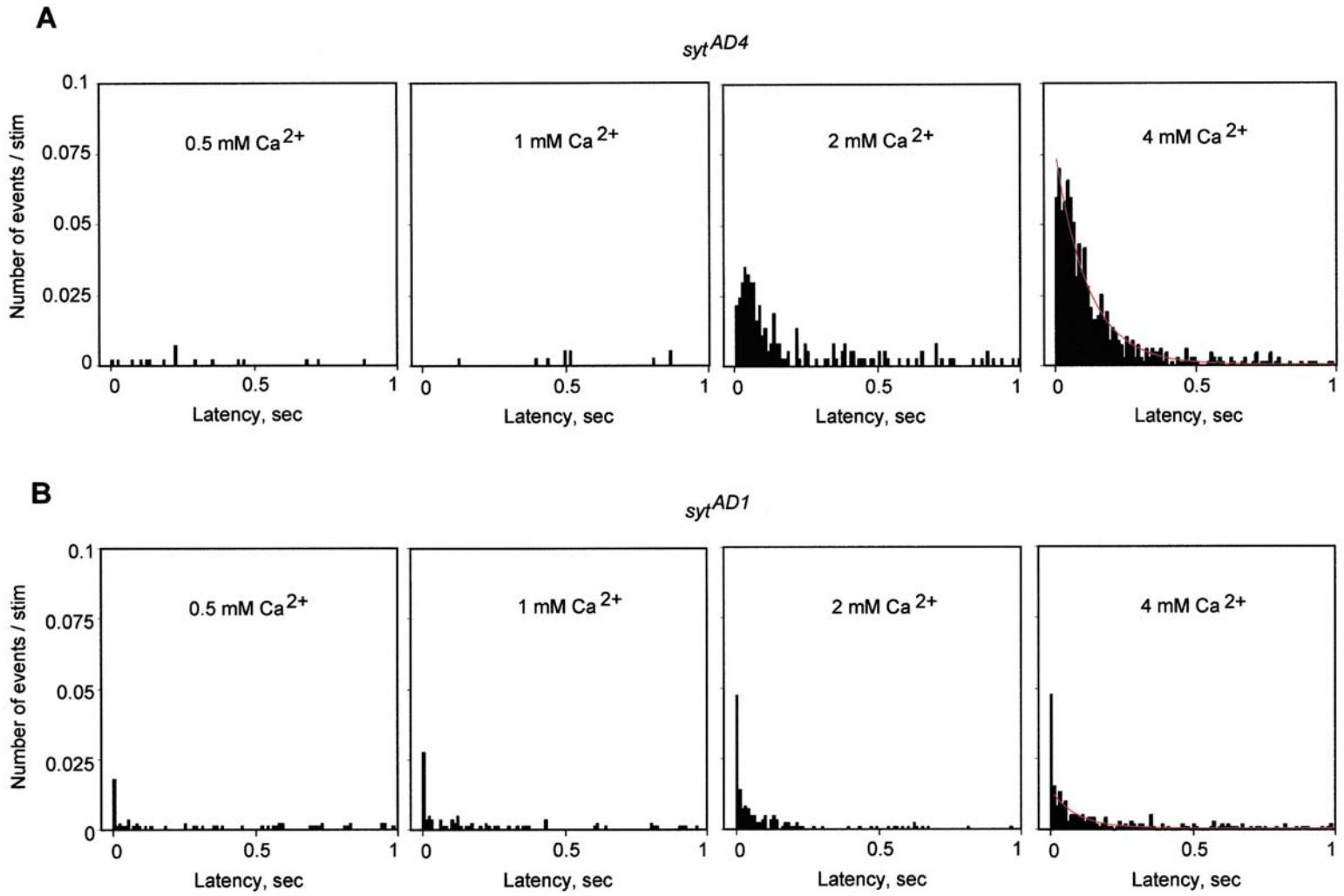

(b)



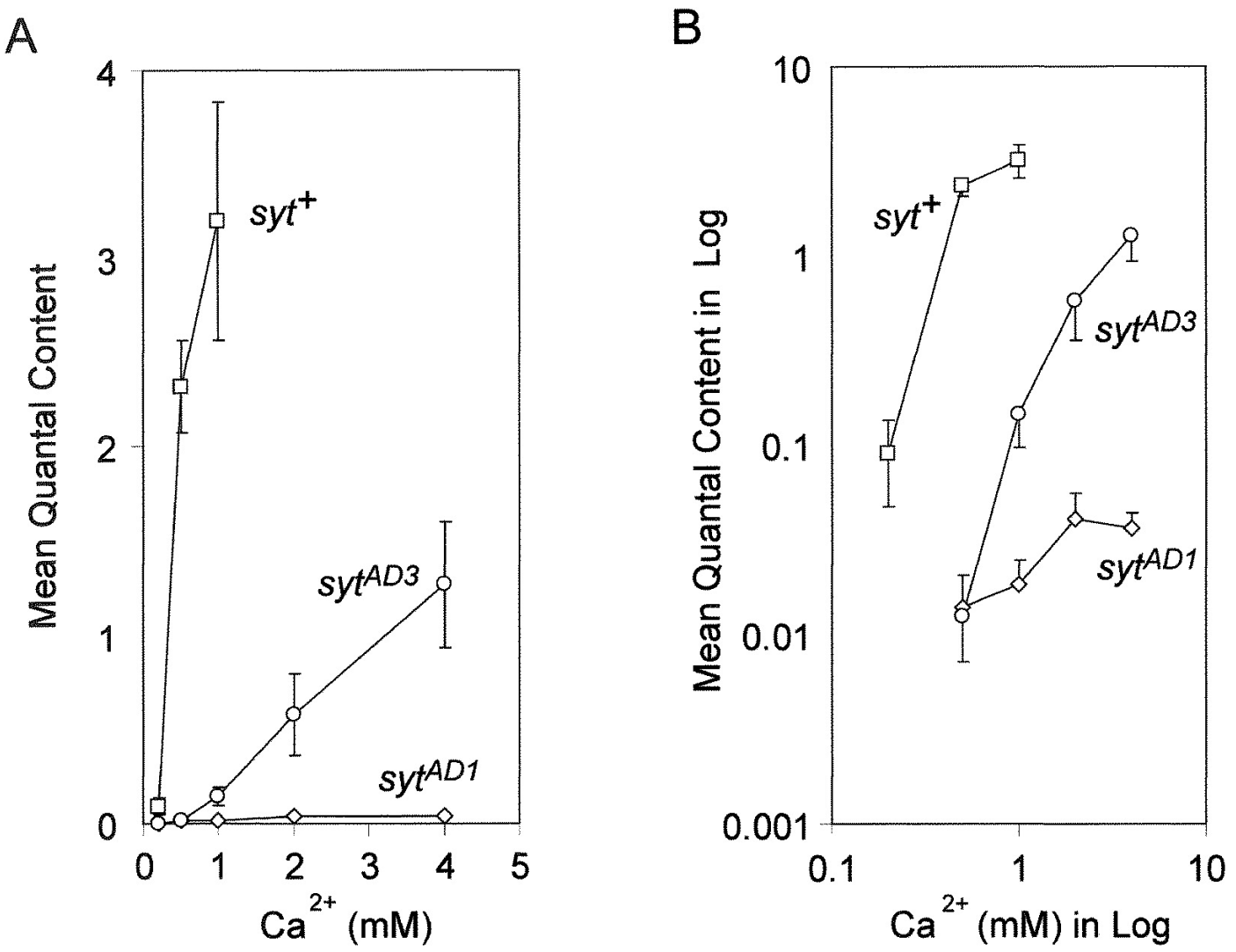
# Figure 2: Mutants Disrupt Distinct Functions of Synaptotagmin



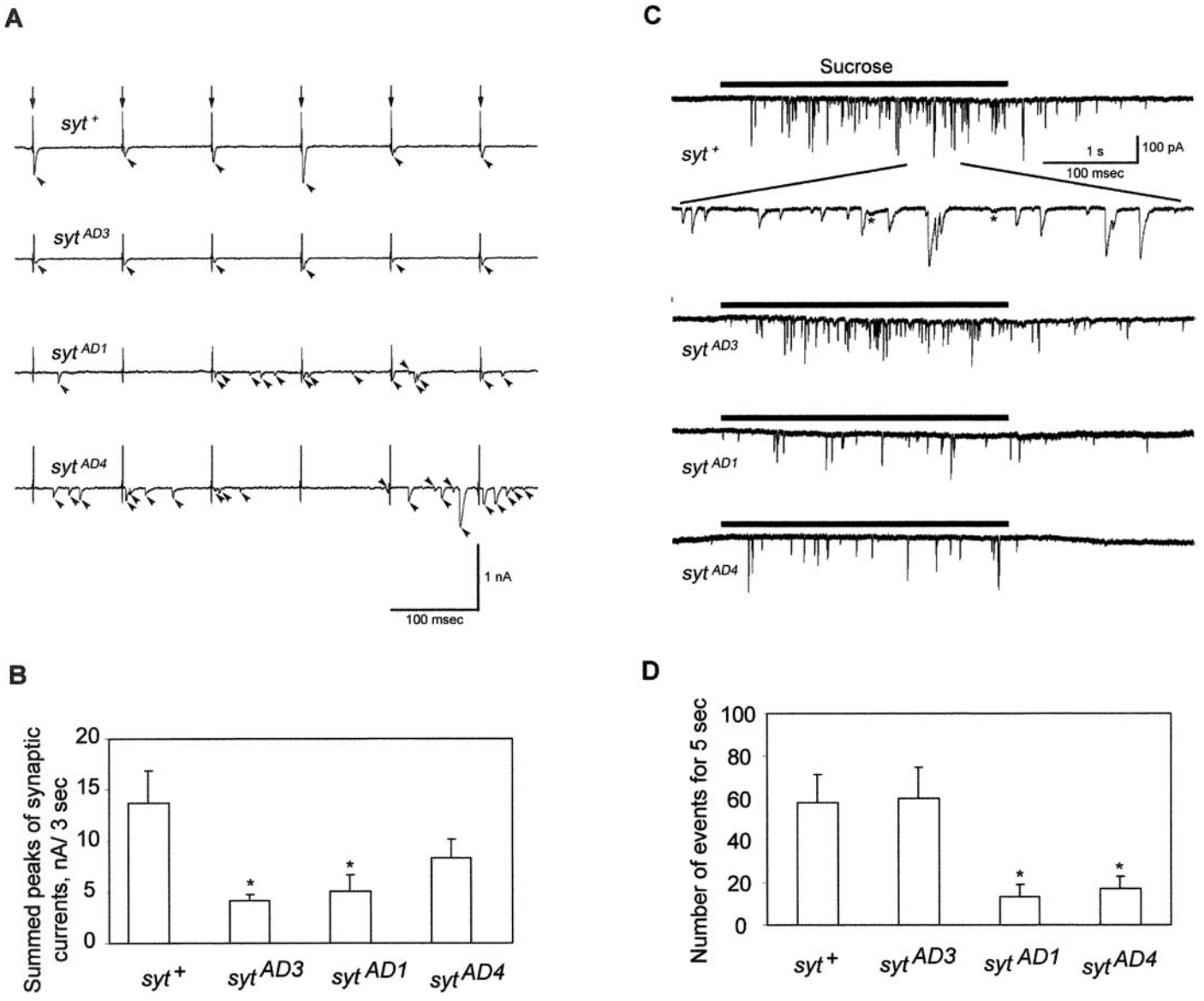
# Figure 3: Molecular Features Required for Suppression of Asynchronous release



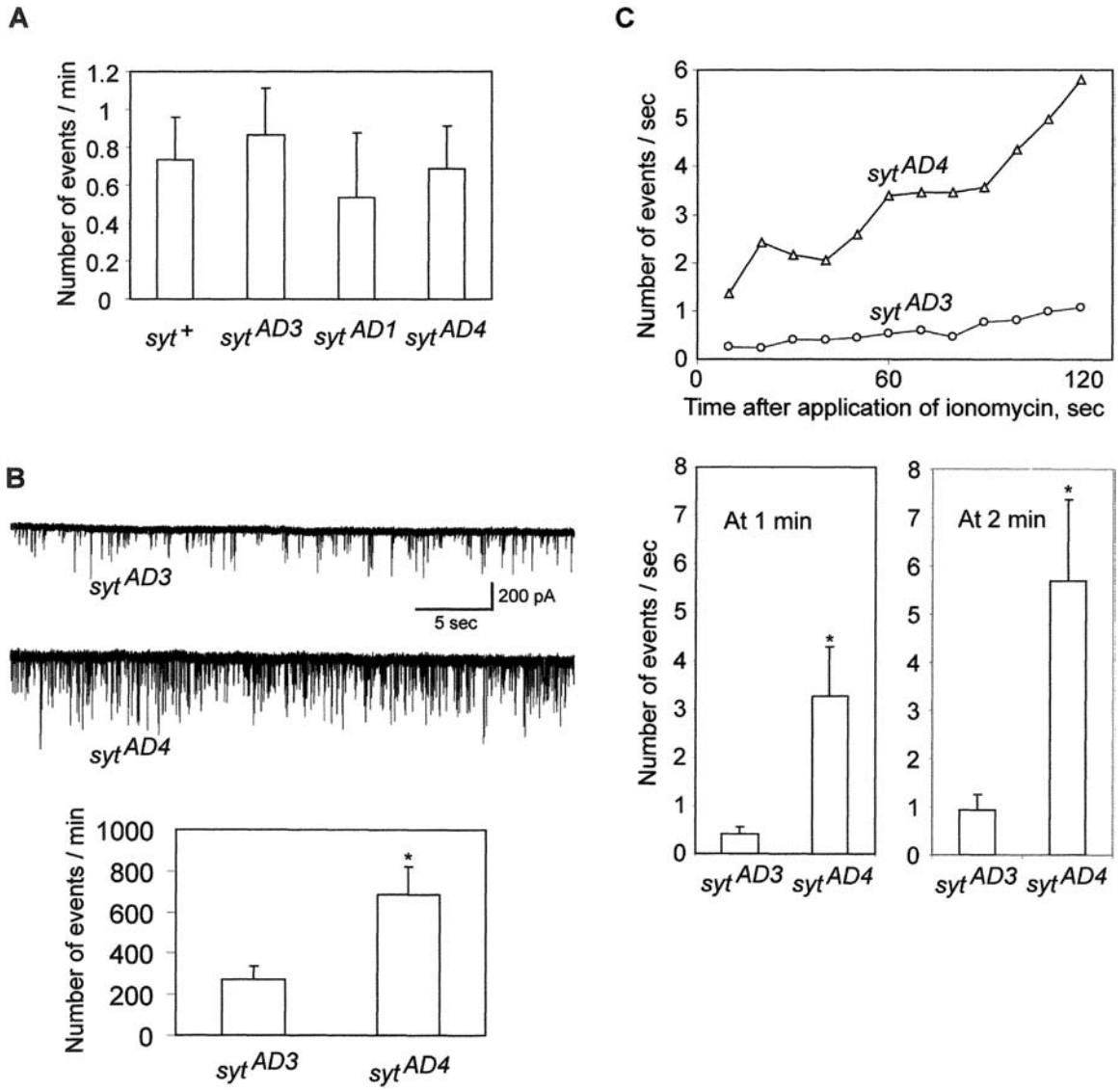
# Fig 4. Comparison of Synchronous Release Cooperatively



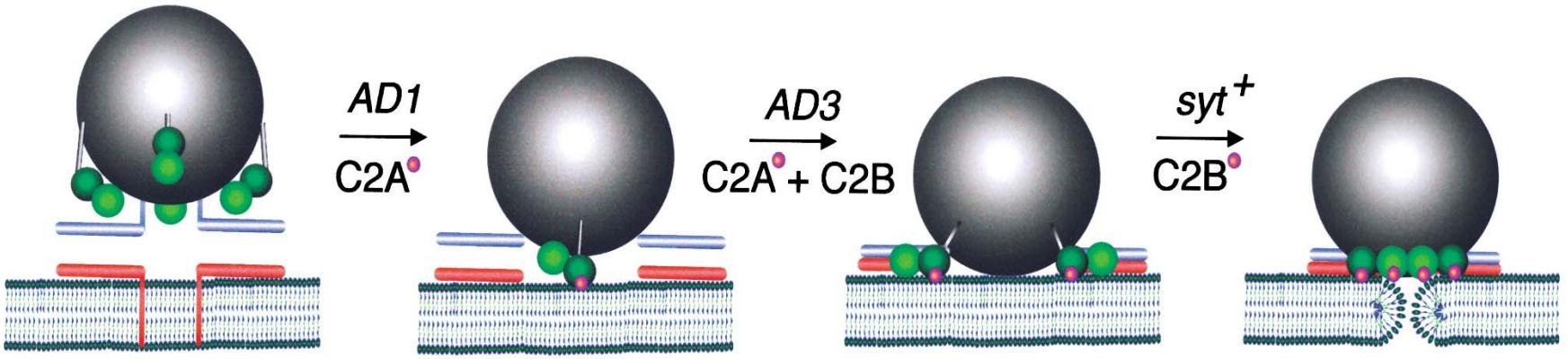
# Figure 5: Synaptotagmin role in Vesicle Recycling



# Fig 6: Synaptotagmin function as a Fusion Clamp



# Conclusions




Cooperativity 1, needed for Ca++ dept binding of phospholipids

Cooperativity 4, needed for Ca++ dept binding of phospholipids and SNARE complex

- AD4, no synchronous release is observed
- AD1, can trigger synchronous release of vesicles, but in the absence of interaction with the SNARE complex there is a low release probability
- AD3, when this interaction with the SNARE complex is made, Ca++ dept cooperativity of release is seen and there is a higher probability of release
- Syt+, Ca++ dept oligomerization of C2B domain maximizes release probability
- Drawn stepwise but actual sequence of events in vivo unknown , likely to be simultaneously

# Conclusions

- There are two kinetically and mechanistically distinct phases of release: a fast component (5-10ms) by a low affinity Ca++ sensor and a second distinct asynchronous component by a high affinity Ca++ sensor (100-200ms)
- In the AD1 mutant these synchronous and asynchronous phases coexist so the protein has the properties necessary to trigger the fast phase but cannot fully suppress the slow phase
- BEST EVIDENCE: cooperativity of neurotransmitter release is abolished in AD1, indicating this is the key Ca++ sensor
- Propose that syt protein rapidly triggers opening and stabilization of the fusion pore while preventing early opening at low Ca++ concentrations by the another high affinity sensor
- Syt does not have a role in docking and endocytosis
- Syt is a suppressor of delayed release during sustained Ca++ elevation, this is possibly mediated through the high affinity Ca++ sensor