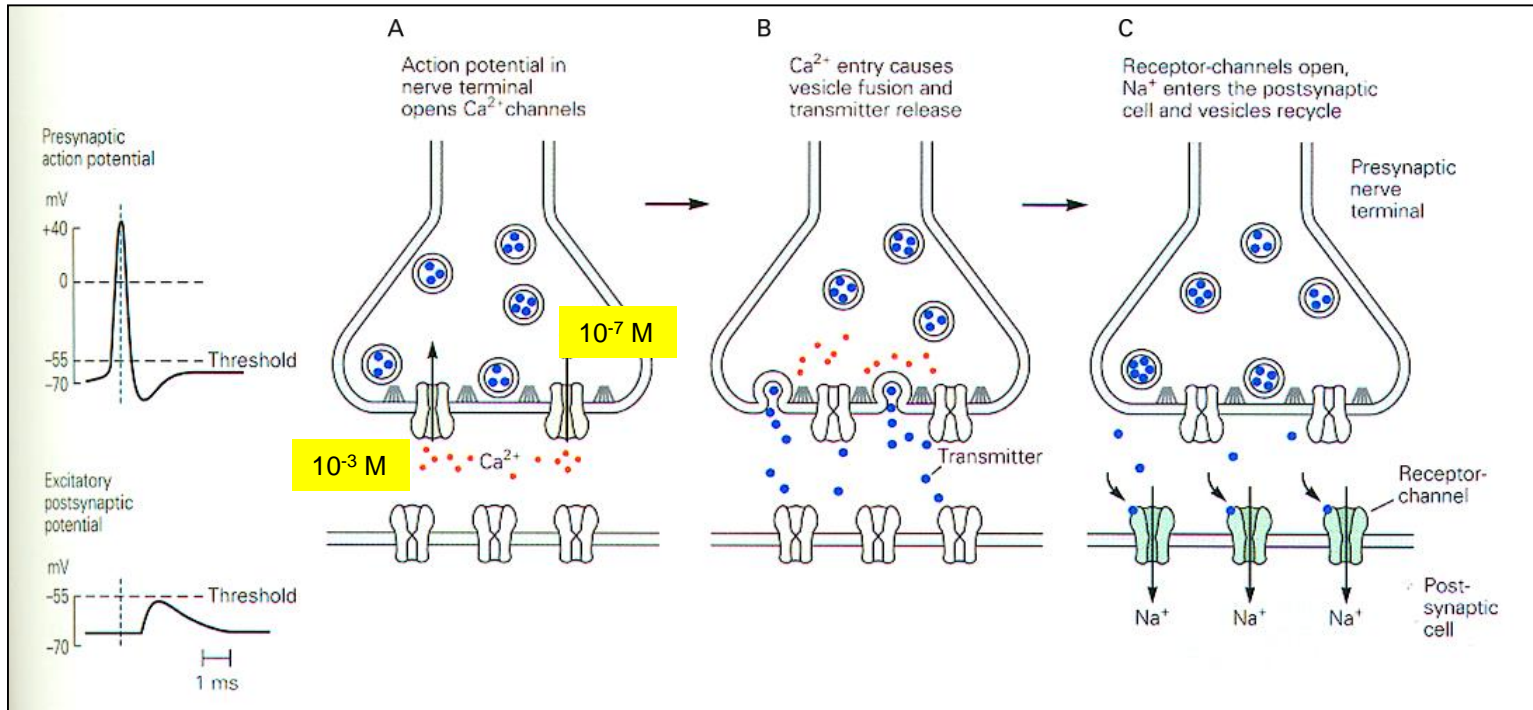


# Molecular mechanism of neurotransmission at chemical synapses

Neurotransmitter secretion is very fast (~100 times faster than insulin secretion by pancreatic  $\beta$ -cells) and is induced by  $\text{Ca}^{++}$  increase in the presynaptic terminal



**Figure 8-8** Synaptic transmission at chemical synapses involves several steps. The complex process of chemical synaptic transmission accounts for the delay between an action potential in the presynaptic cell and the synaptic potential in the postsynaptic cell compared with the virtually instantaneous transmission of signals at electrical synapses (see Figure 8-2B).

**A.** An action potential arriving at the terminal of a presynaptic axon causes voltage-gated  $\text{Ca}^{2+}$  channels at the active zone to open. The **gray filaments** represent the docking and release sites of the active zone.

**B.** The  $\text{Ca}^{2+}$  channel opening produces a high concentration of intracellular  $\text{Ca}^{2+}$  near the active zone, causing vesicles containing neurotransmitter to fuse with the presynaptic cell membrane and release their contents into the synaptic cleft (a process termed *exocytosis*).

**C.** The released neurotransmitter molecules then diffuse across the synaptic cleft and bind specific receptors on the postsynaptic membrane. These receptors cause ion channels to open (or close), thereby changing the membrane conductance and membrane potential of the postsynaptic cell.

**Release of neurotransmitter is quantal**

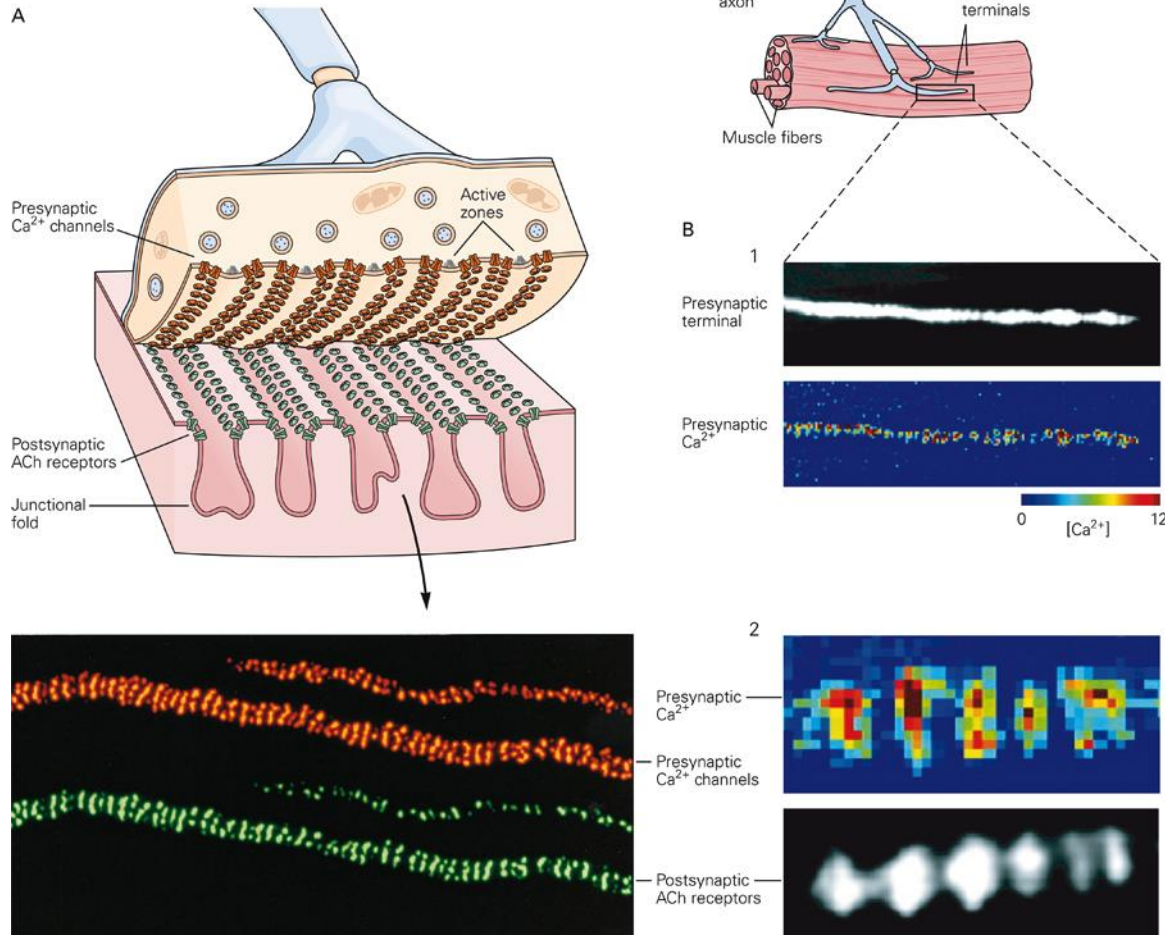


Bernard Katz

The anatomical specializations of the synapse and the properties of the pre-synaptic ion channels and postsynaptic receptors all contribute to achieve **FAST, QUANTAL TRANSMISSION**

For additional information on this subject, see Chapter 15 of the textbook 'From Molecules to Networks' by Byrne et al.

# Calcium channels are concentrated at the active zone



**Figure 12-4** Calcium flowing into the presynaptic nerve terminal during synaptic transmission at the neuromuscular junction is concentrated at the active zone. Calcium channels in presynaptic terminals at the end-plate are concentrated opposite clusters of nicotinic acetylcholine (ACh) receptors on the postsynaptic muscle membrane. Two drawings show the frog neuromuscular junction.

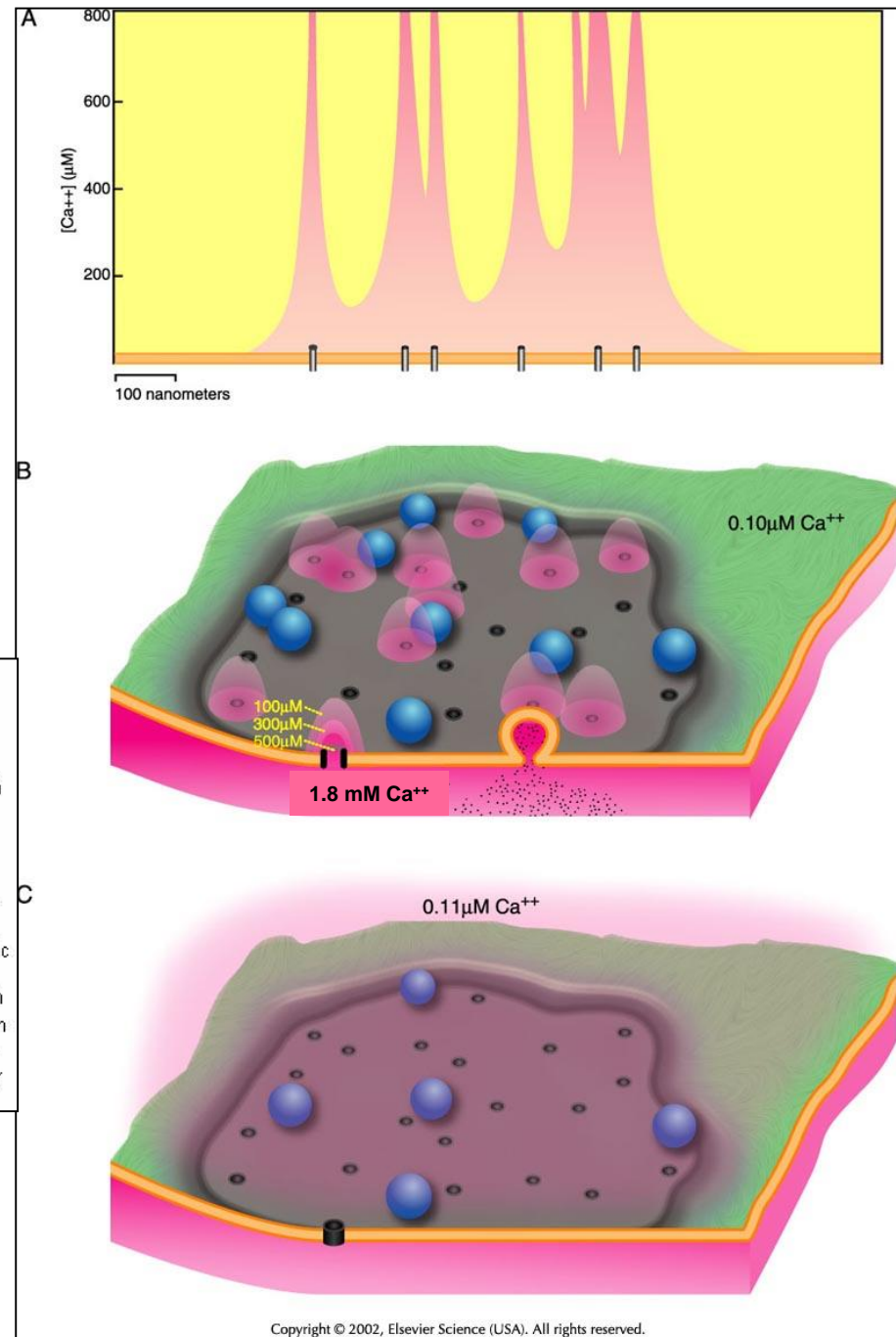
**A.** The enlarged view shows the microanatomy of the neuromuscular junction with the presynaptic terminal peeled back. A fluorescent image shows the presynaptic  $\text{Ca}^{2+}$  channels (labeled with a Texas red-coupled marine snail toxin that binds to  $\text{Ca}^{2+}$  channels), and postsynaptic ACh receptors (labeled with fluorescently tagged  $\alpha$ -bungarotoxin, which binds selectively to ACh receptors). The two images are normally superimposed but have been separated for clarity. The patterns of labeling with both probes are in almost precise register, indicating that the active zone of the presynaptic neuron is in almost perfect alignment with the postsynaptic membrane containing the high concentration of ACh receptors. (Reproduced, with permission, from Robitaille, Adler, and Charlton 1990.)

**B.** Calcium influx in presynaptic terminals is localized at active zones. Calcium can be visualized using calcium-sensitive fluorescent dyes. **1.** A presynaptic terminal at a neuromuscular junction filled with the dye fura-2 under resting conditions is shown in the black and white image. The fluorescence intensity of the dye changes as it binds  $\text{Ca}^{2+}$ . In the color image, color-coded fluorescence intensity changes show local hot-spots of intracellular  $\text{Ca}^{2+}$  in response to a single presynaptic action potential. **Red** indicates regions with a large increase in  $\text{Ca}^{2+}$ ; **blue** indicates regions with little increase in  $\text{Ca}^{2+}$ . Regular peaks of  $\text{Ca}^{2+}$  are seen along the terminal, corresponding to the localization of  $\text{Ca}^{2+}$  channels at the active zones. **2.** The color image shows a high-magnification view of the peak increase in terminal  $\text{Ca}^{2+}$  levels. The corresponding black-and-white image shows fluorescence labeling of nicotinic ACh receptors in the postsynaptic membrane, illustrating the close spatial correspondence between areas of presynaptic  $\text{Ca}^{2+}$  influx and areas of postsynaptic receptors. The scale bar represents 2  $\mu\text{m}$ . (Reproduced, with permission, from Wachman et al. 2004.)



# Microdomains with high $\text{Ca}^{2+}$ concentrations form near open $\text{Ca}^{2+}$ channels and trigger the exocytosis of synaptic vesicles

**FIGURE 3** Microdomains with high  $\text{Ca}^{2+}$  concentrations form in the cytosol near open  $\text{Ca}^{2+}$  channels and trigger the exocytosis of synaptic vesicles. (A) In this adaptation of a model of  $\text{Ca}^{2+}$  dynamics in the terminal, a set of  $\text{Ca}^{2+}$  channels is spaced along the x axis, as if in a cross section of a terminal. The channels have opened and, while they are open, the cytosolic  $\text{Ca}^{2+}$  concentration (y axis) is spatially inhomogeneous. Near the mouth of the channel, the influx of  $\text{Ca}^{2+}$  drives the local concentration to as high as  $800 \mu\text{M}$ , but within just  $50 \text{ nm}$  of the channel, the concentration drops off to  $100 \mu\text{M}$ . Channels are spaced irregularly but are often sufficiently close to one another that their clouds of  $\text{Ca}^{2+}$  can overlap and sum. (B) In the active zone (gray), an action potential has opened a fraction of the  $\text{Ca}^{2+}$  channels and microdomains of high cytosolic  $\text{Ca}^{2+}$  (pink) arise around these open channels as  $\text{Ca}^{2+}$  flows into the cell. In the rest of the cytoplasm, the  $\text{Ca}^{2+}$  concentration is at resting levels ( $0.10 \mu\text{M}$ ), but within these microdomains, particularly near the channel mouth,  $\text{Ca}^{2+}$  concentrations are much higher, as in A. Synaptic vesicles docked and primed at the active zone may come under the influence of one or more of these microdomains and thereby be triggered to fuse with the membrane. (C) A few milliseconds after the action potential, the channels have closed and the microdomains have dispersed. The overall  $\text{Ca}^{2+}$  concentration in the terminal is now slightly higher ( $0.11 \mu\text{M}$ ) than before the action potential. If no other action potentials occur, the cell will pump extra  $\text{Ca}^{2+}$  out across the plasma membrane and restore the initial condition after several  $100 \text{ ms}$ .



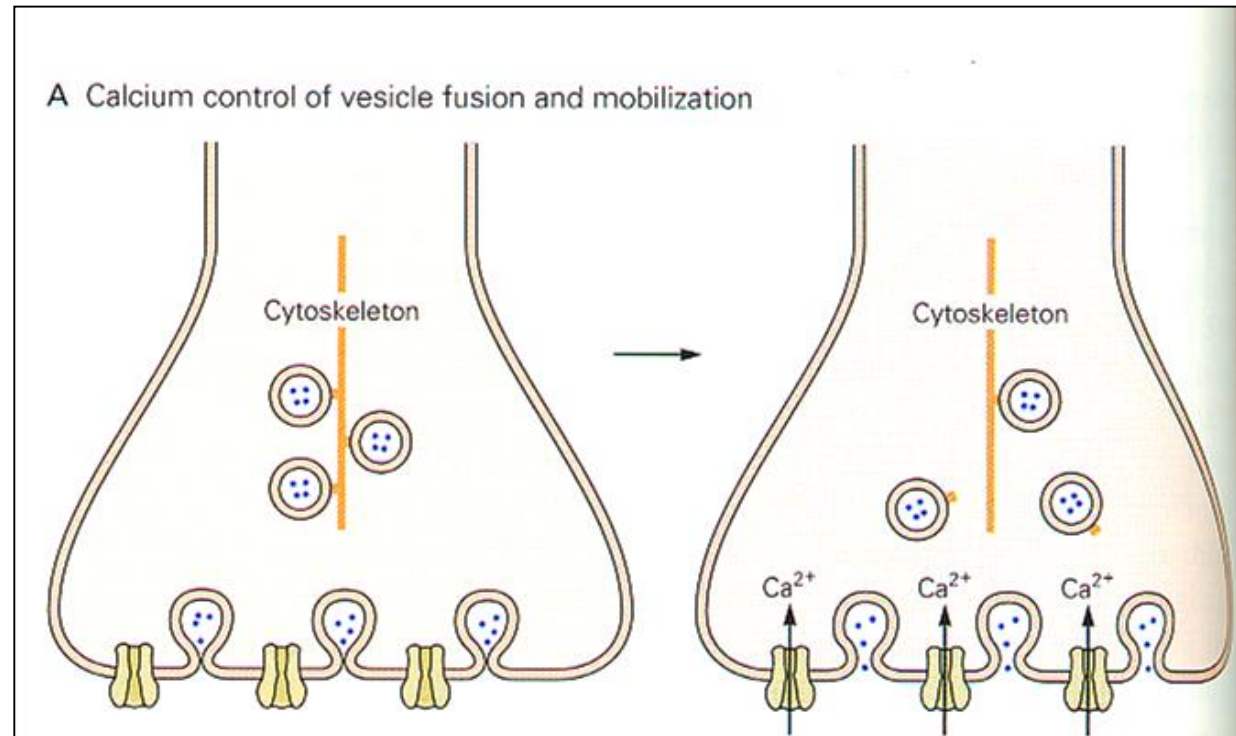
## Calcium controls:

1) exocytosis

2) mobilization of synaptic vesicles

**Figure 14-14** The mobilization, docking, and function of synaptic vesicles are controlled by  $\text{Ca}^{2+}$  and low-molecular-weight GTP-binding proteins.

A. Synaptic vesicles in nerve terminals are sequestered in a *storage compartment* where they are tethered to the cytoskeleton, as well as in a *releasable compartment* where they are docked to the pre-synaptic membrane. Entry of  $\text{Ca}^{2+}$  into the nerve terminal leads to the opening of the fusion pore complex and neurotransmitter release. Calcium entry also frees vesicles from the storage compartment through phosphorylation of synapsins, thus increasing the availability of vesicles for docking at the presynaptic plasma membrane.



**Dephosphorilated Synapsin 1** (Syn 1) links vesicles to the actin cytoskeleton.

**Phosphorilation of Syn 1** induces vesicle mobilization, increasing the number of vesicles available for docking/fusion with the plasma membrane

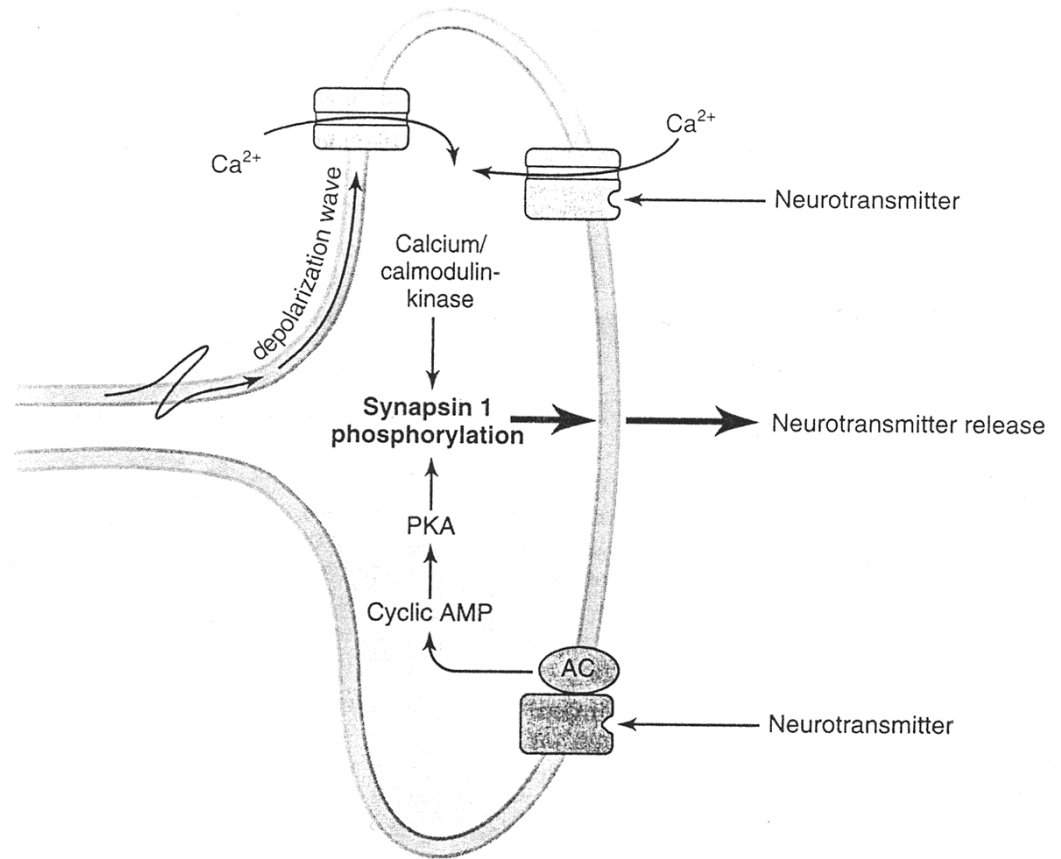
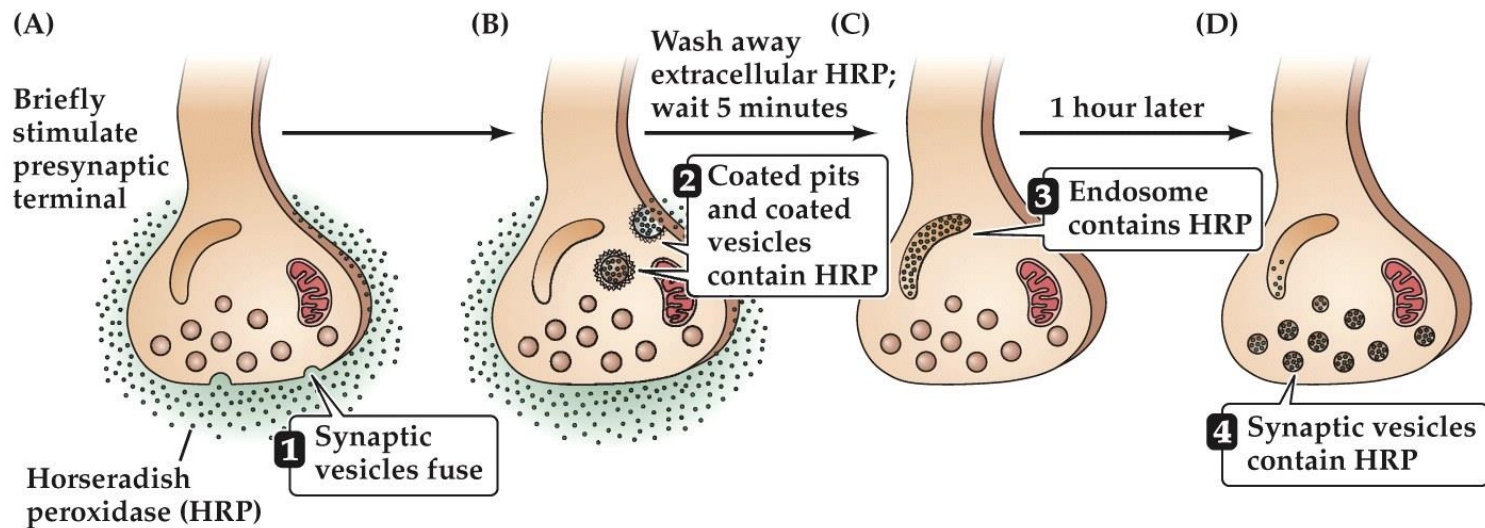


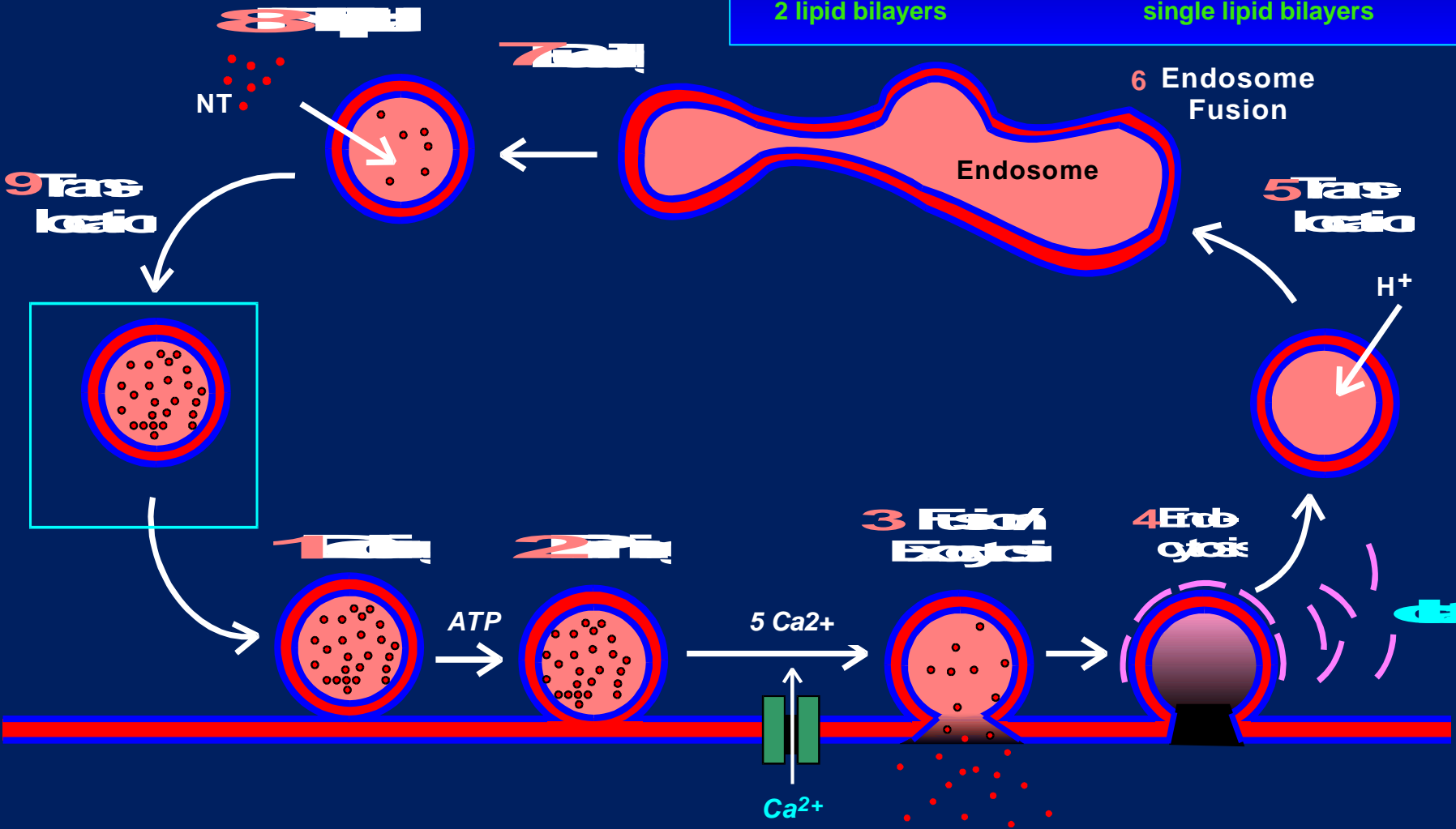
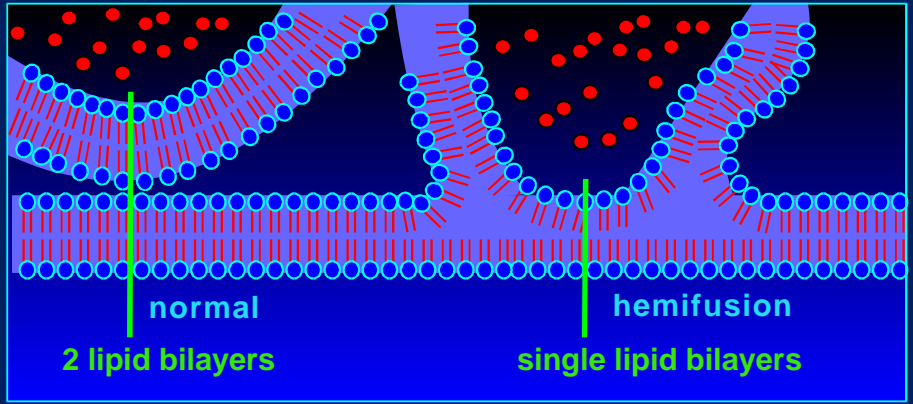
FIG. 6. Schematic diagram of the regulation of synapsin I phosphorylation in nerve terminals. Nerve impulses stimulate synapsin I phosphorylation through depolarization of the nerve terminal plasma membrane, an increase in free  $Ca^{2+}$  levels, and the activation of  $Ca^{2+}$ /calmodulin-dependent protein kinases. Phosphorylation of synapsin I then modulates neurotransmitter release. Phosphorylation of synapsin I appears to be involved in various  $Ca^{2+}$ -dependent mechanisms of regulation of neurotransmitter release, including the phenomenon of posttetanic potentiation. Some neurotransmitters stimulate (or inhibit) synapsin I phosphorylation by binding to presynaptic receptors and thereby altering  $Ca^{2+}$  levels and  $Ca^{2+}$ /calmodulin-dependent protein kinase activity. Such phosphorylation (or dephosphorylation) of synapsin I may be involved in  $Ca^{2+}$ -dependent mechanisms through which certain neurotransmitters acting on presynaptic receptors of axon terminals regulate neurotransmitter release. Other neurotransmitters stimulate (or inhibit) synapsin I phosphorylation by binding to other presynaptic receptors and thereby altering adenylyl cyclase (AC) activity, leading to changes in cAMP levels and cAMP-dependent protein kinase (PKA) activity. Such phosphorylation (or dephosphorylation) of synapsin I may be involved in cAMP-dependent mechanisms through which neurotransmitters acting on receptors of axon terminals regulate neurotransmitter release. Nerve impulse conduction would be expected to stimulate synapsin I phosphorylation in all nerve terminals throughout the nervous system. In contrast, most neurotransmitters would be expected to stimulate synapsin I phosphorylation only in certain nerve terminals. (Modified from Nestler, E. J., and Greengard, P. *Prog. Brain Res.* 60:323-340, 1986.)

# Demonstration of synaptic vesicle recycling in the presynaptic terminal

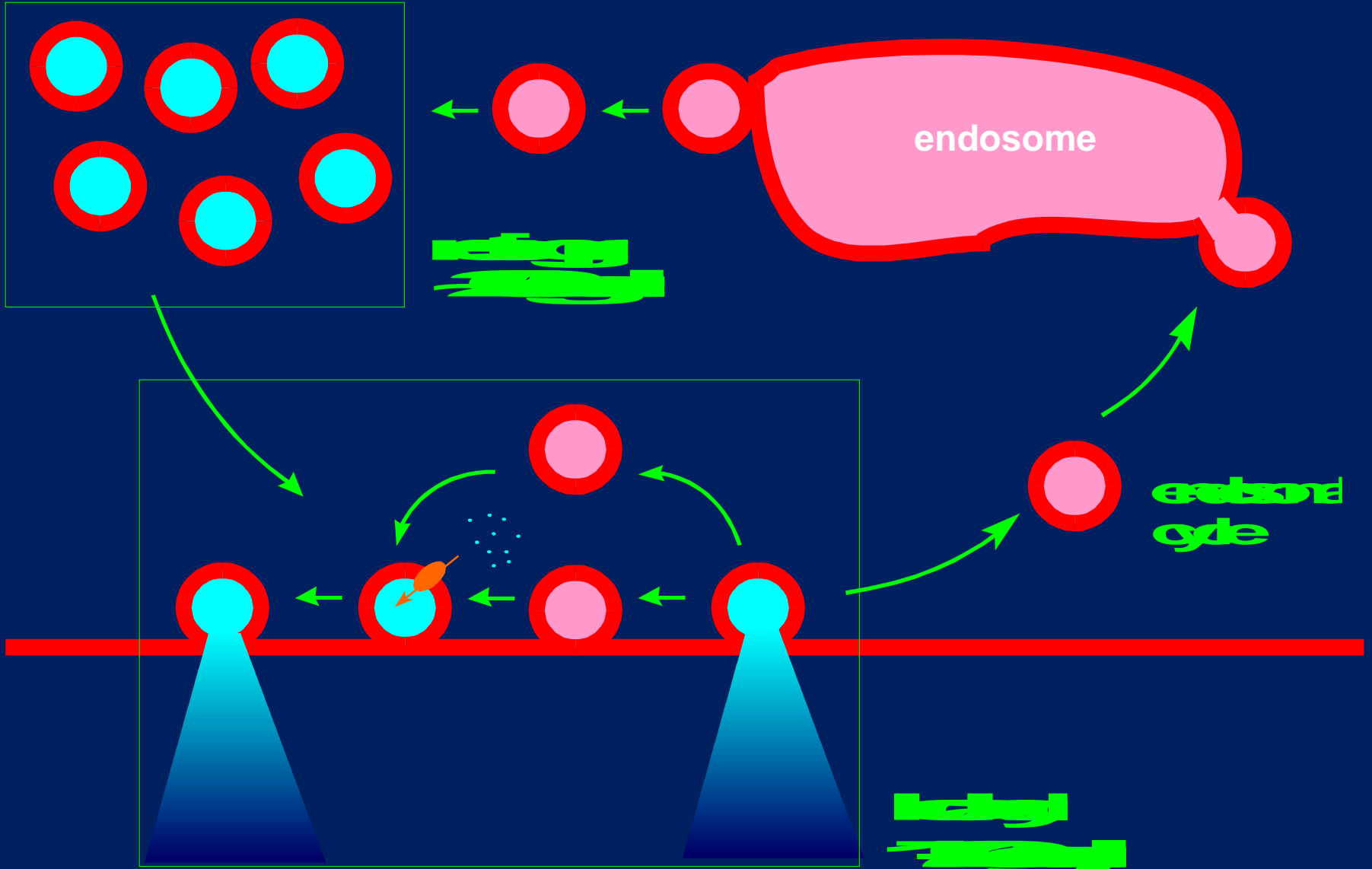




# The Nine Steps of the Synaptic Vesicle Cycle







The synaptic vesicle cycle revisited

# Schematic representation of the three vesicle pools found in the presynaptic terminal

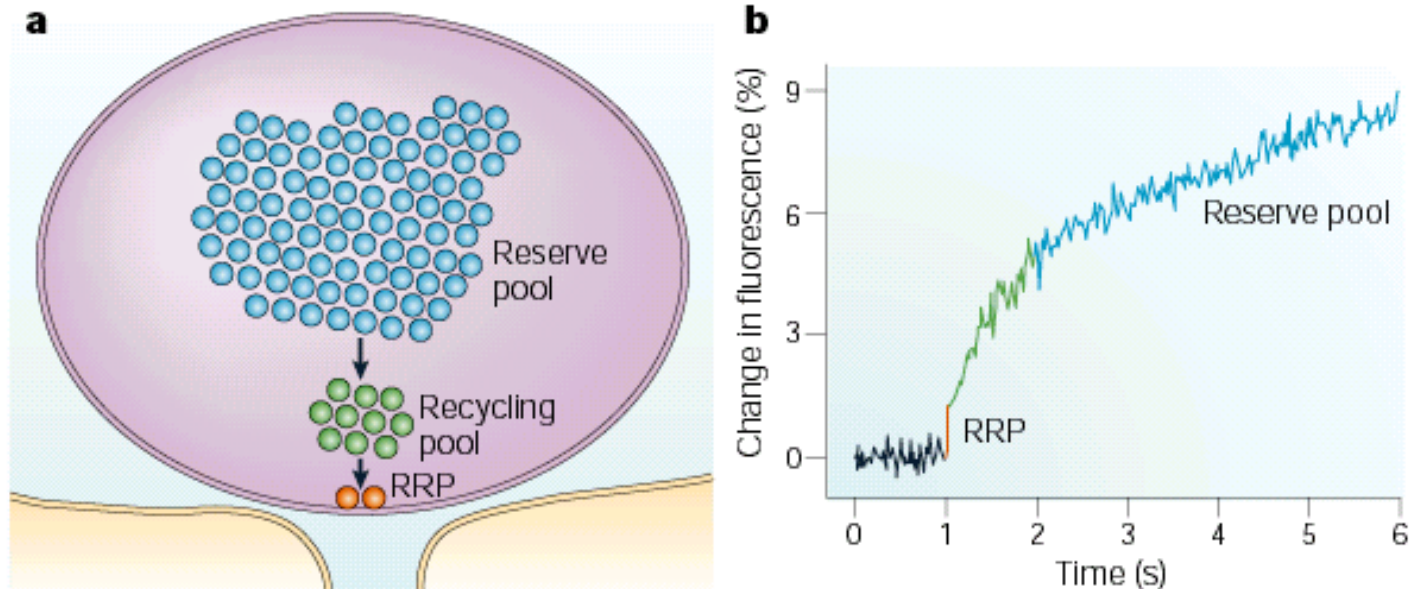


Figure 1 | **Three vesicle pools. a** | The classic three-pool model. The reserve pool makes up ~80–90% of the total pool, and the recycling pool is significantly smaller (~10–15%). The readily releasable pool (RRP) consists of a few vesicles (~1%) that seem to be docked and primed for release. **b** | Three kinetic components of release (indicating release of three vesicle pools) on depolarization of goldfish bipolar cells. The cell was stimulated in the presence of the styryl dye FM 1-43, and the increase in fluorescence gives a direct measure of exocytosis. Panel **b** modified, with permission, from REF. 12 © (1999) Blackwell Scientific Publishing.

# Presynaptic proteins and their roles in synaptic vesicle cycling

(B)

## Transmitter loading

Transmitter transporters  
Proton pump

## Mobilization

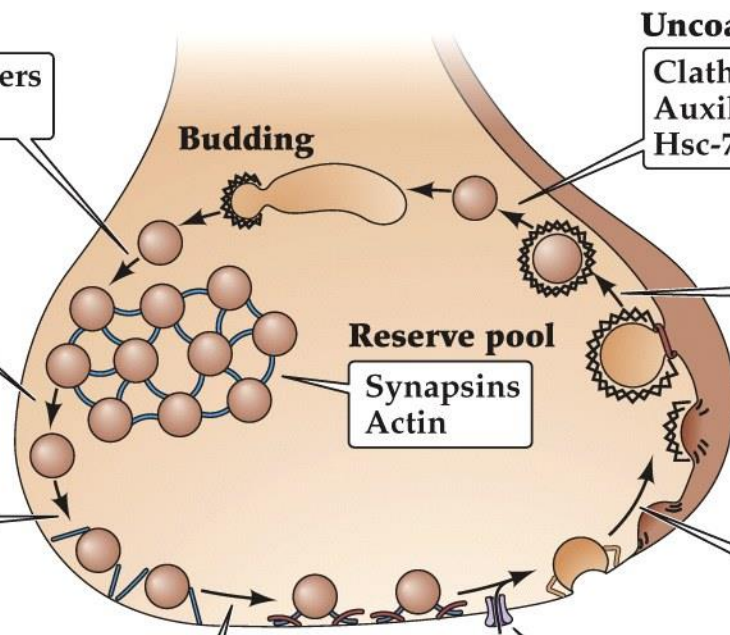
Synapsins  
Actin

## Docking

GTP-binding protein  
SNAREs?

## Priming

SNAREs	Complexin	Syntaphilin
(m)unc13	Tomosyn	Rab3a
(m)unc18/nSec1	CAPS	RIM
NSF	SV2	Doc2
SNAPs	Snapin	



## Uncoating

Clathrin Endophilin  
Auxilin Synaptojanin  
Hsc-70

## Budding

Dynamin Clathrin  
Amphiphysin Actin  
Endophilin Syndapin  
Synaptojanin WASP

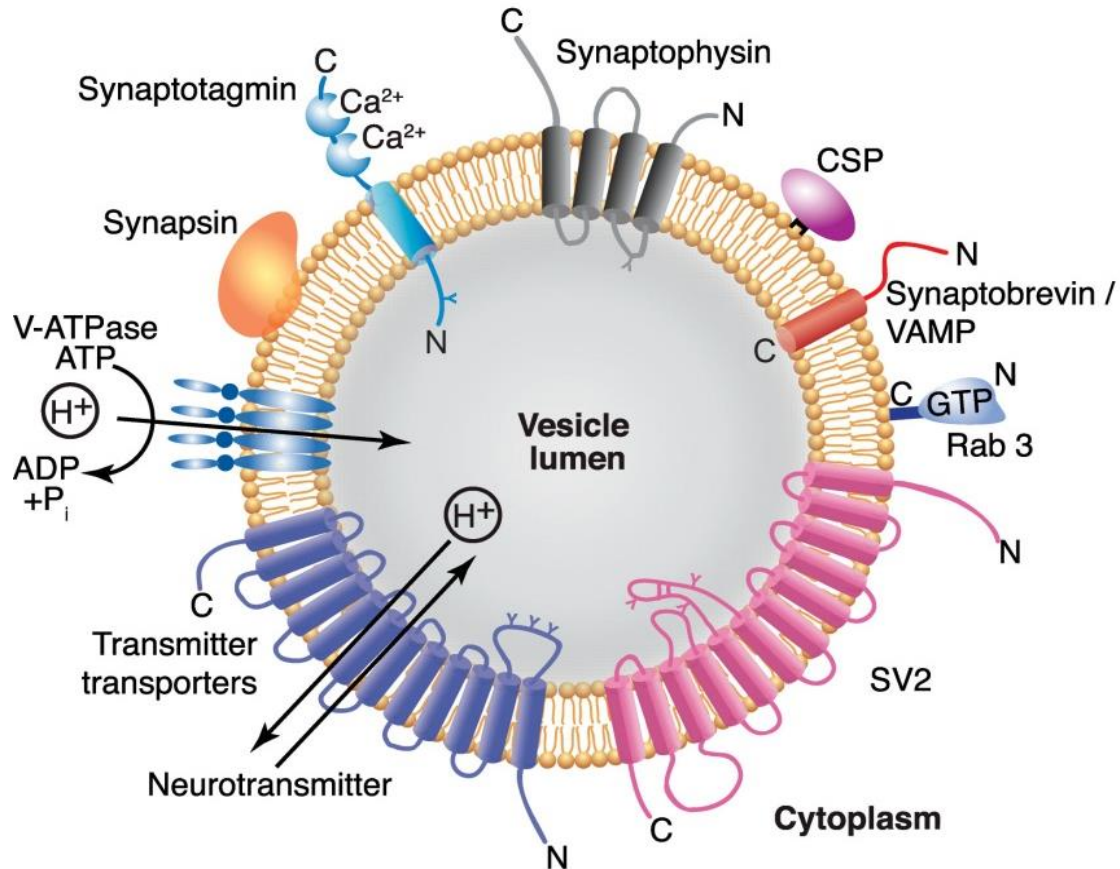
## Coating

Clathrin	Epsin	NSF
AP-2	Eps-15	SNAP
AP-180	Endophilin	Syntaphilin
Synaptotagmins	Stoned	SNIP
Synaptobrevin		

## Fusion

Ca<sup>2+</sup>  
Synaptotagmin 1  
SNAREs

# Synaptic vesicle proteins



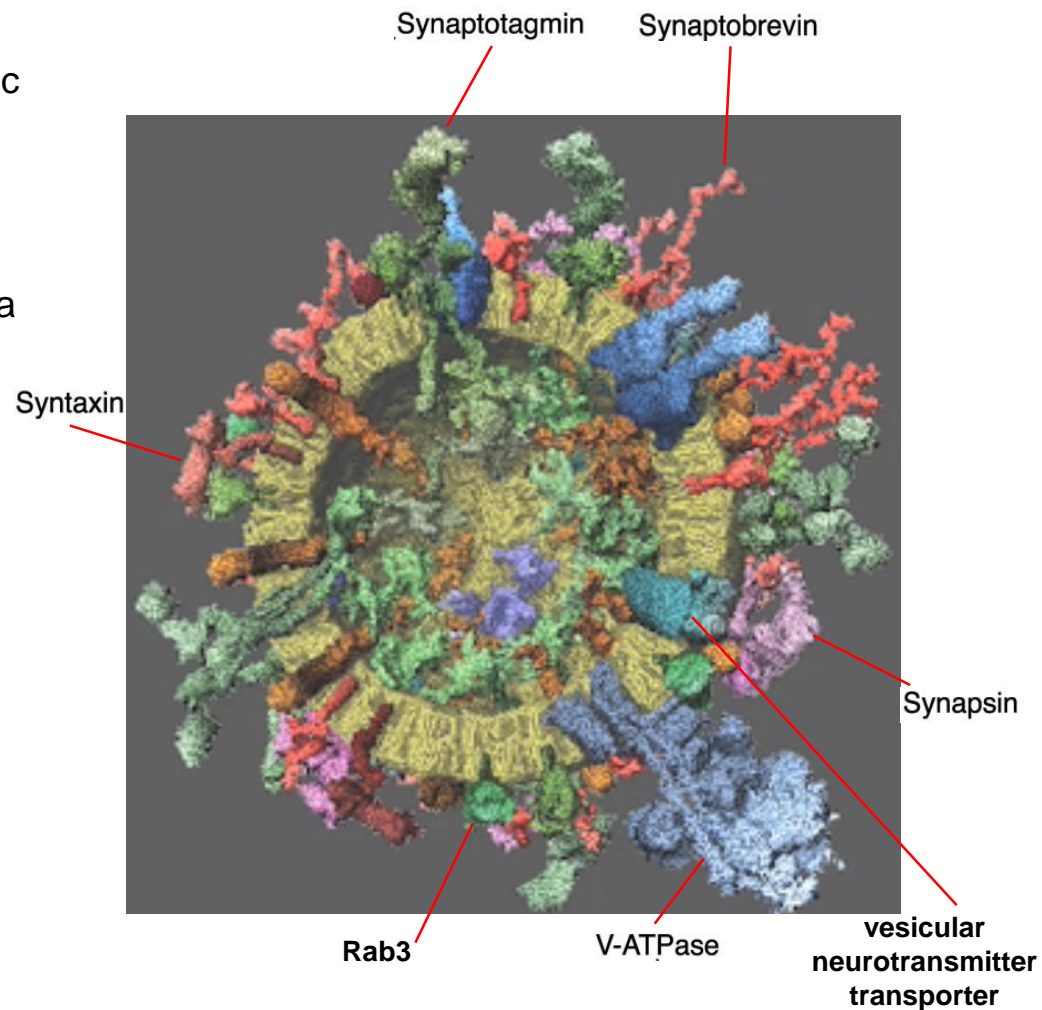
Schematic representation of the structure and topology of the major synaptic vesicle membrane proteins . N, amino terminal; C, carboxy terminal.



# The molecular anatomy of a synaptic vesicle

This model represents a cutaway view of a synaptic vesicle, with the membrane lipids and proteins drawn to scale. Each synaptic vesicle membrane contains close to 50 different integral membrane protein molecules, which vary widely in their relative abundance. The most abundant protein is a SNARE protein, called v-SNARE **synaptobrevin**. This molecule participates in membrane fusion at the synaptic terminal, and there are about 70 copies of this protein per vesicle. By contrast, the vesicle contains only one to two copies of **V-ATPase**. V-ATPase uses energy from ATP hydrolysis to pump  $H^+$  into the vesicle lumen. The resulting electrochemical gradient provides the energy to import neurotransmitter molecules, such as glutamate, into the vesicle.

This model was created, in part, from an electron tomogram of a real synaptic vesicle. Other data, including structural data of the synaptic vesicle's proteins, have been combined with the data from the tomogram to create the three-dimensional model. Note, only 70% of the membrane proteins estimated to be present in the membrane are depicted in the model.



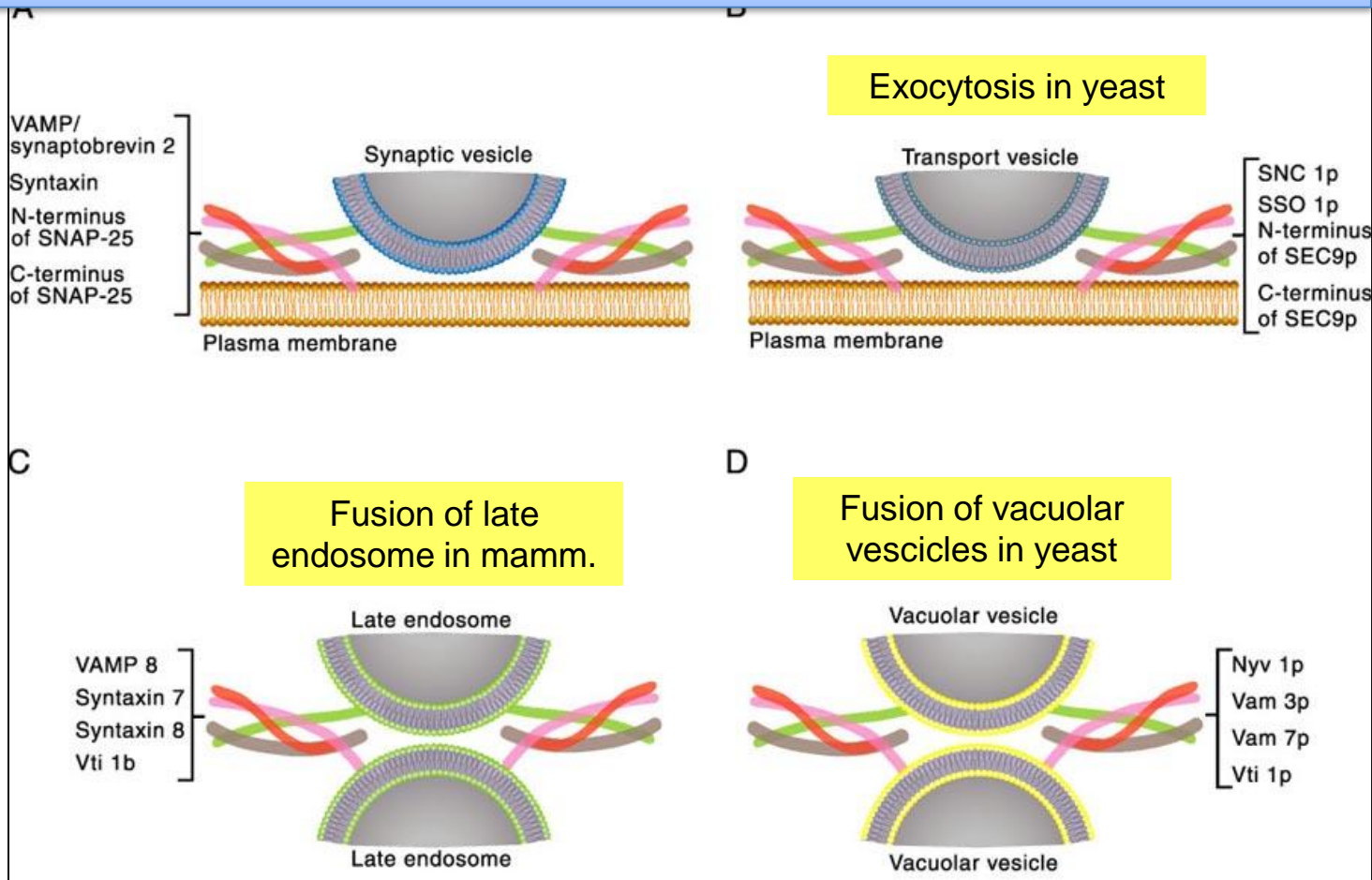
## Critical questions in neurotransmitter release:

- How do the synaptic vesicle and the plasma membrane fuse during transmitter release?
- How does calcium trigger synaptic vesicle fusion?
- How is calcium influx localized to release sites in order to enable the fast coupling of an action potential to transmitter release?

# The mechanism of synaptic vesicle exocytosis was co-opted from general vesicle trafficking common to all eukariotes



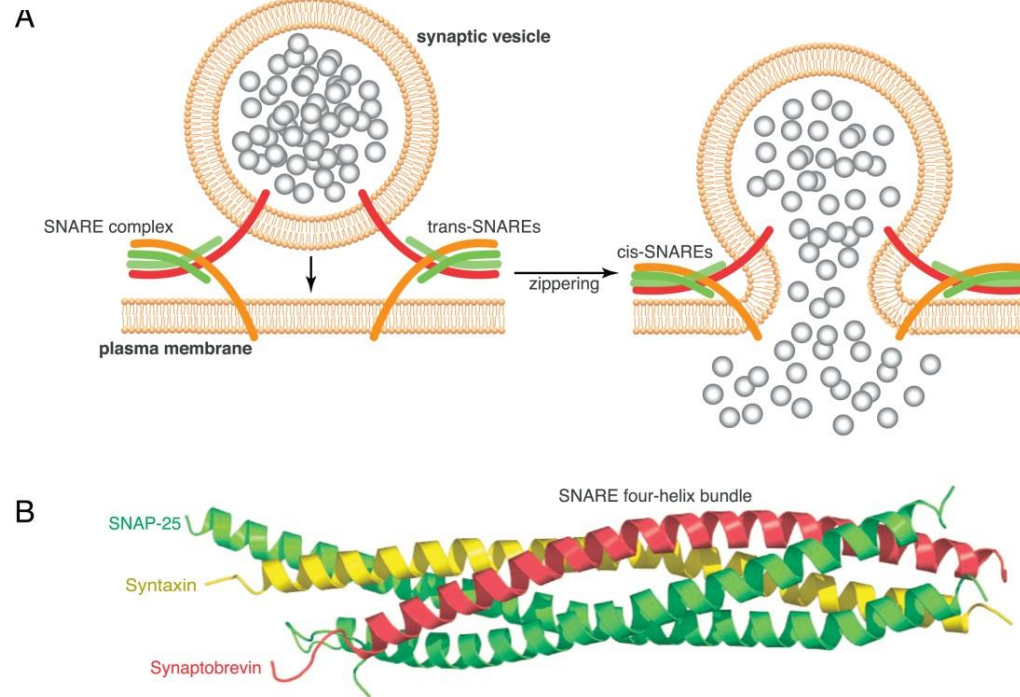
Discovery of SNARE proteins in membrane fusion



**FIGURE 7** Neurotransmitter release shares a core mechanism with many membrane fusion events within eukaryotic cells. The fusion of synaptic vesicles (A) is driven by a particular complex of four coiled-coil domains contributed by three different proteins. Exocytosis in yeast (B), the fusion of late endosomes in mammalian cells (C), and the fusion of vacuolar vesicles in yeast (D) exemplify the closely related four-stranded coiled-coil complexes required to drive fusion in other membrane-trafficking steps.

# Structure of the SNARE complex

**SNARE** = SNAP receptors



**Figure 15.6** The mechanism of membrane fusion.

(A) Fusion of synaptic vesicles is driven by a *trans*-SNARE complex of four coiled-coil domains contributed by three different proteins located on the vesicular and the target membrane. Zippering of this complex from the N- to the C-terminus forces the two membranes together for fusion. Once fused, the SNARE complex is in a *cis* configuration. Neurotransmitter release shares this core mechanism with membrane fusion events within eukaryotic cells. (B) Structural ribbon rendition of a tightly assembled SNARE complex. The four-helix bundle consists of one SNARE motif from synaptobrevin and syntaxin, while SNAP-25 contributes two SNARE motifs.

Adapted from Rizo and Südhof (2012); structure originally published in Sutton et al. (1998).

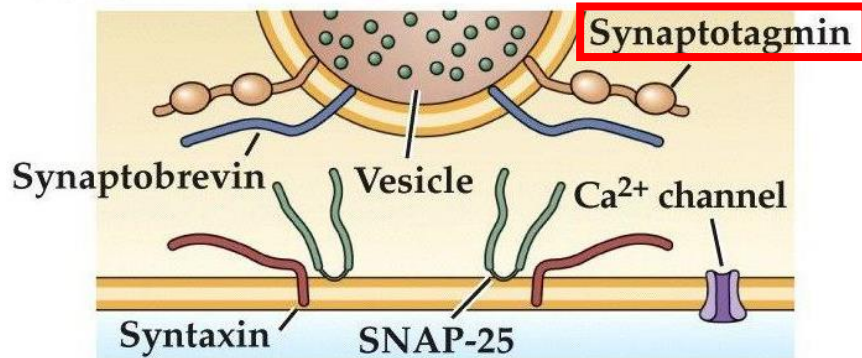


# Critical questions in neurotransmitter release

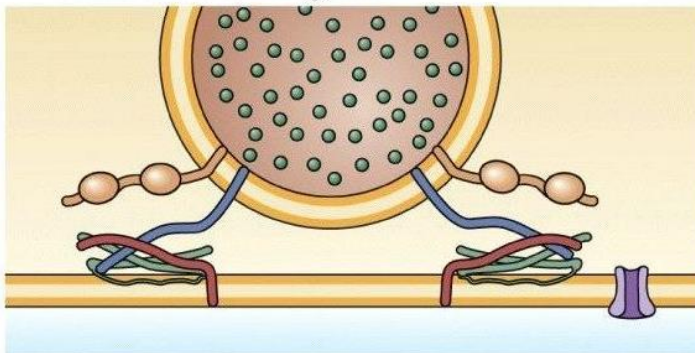
- How do the synaptic vesicle and the plasma membrane fuse during transmitter release?
- How does calcium trigger synaptic vesicle fusion?
- How is calcium influx localized to release sites in order to enable the fast coupling of an action potential to transmitter release?

# Synaptotagmin is the $\text{Ca}^{++}$ sensor that triggers vesicle fusion

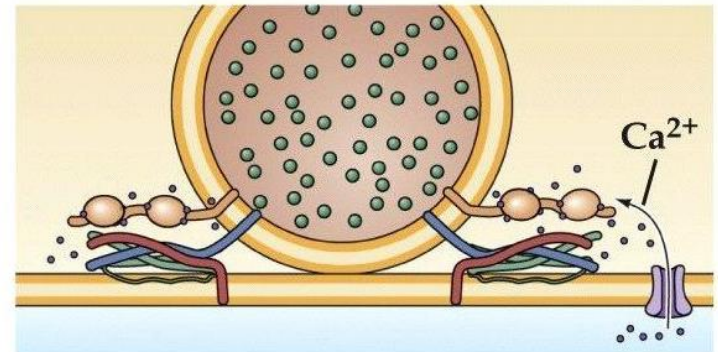
(B) (1) Vesicle docks



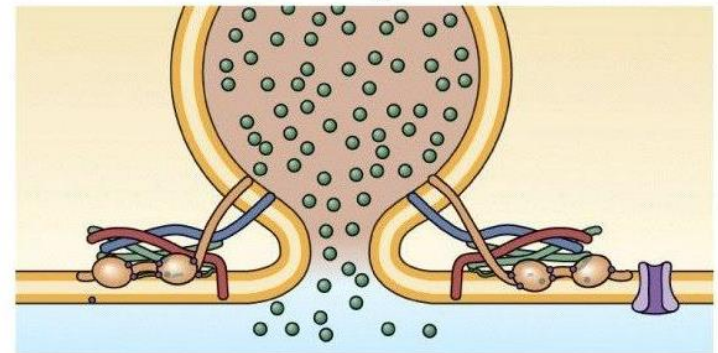
(2) SNARE complexes form to pull membranes together



(3) Entering  $\text{Ca}^{2+}$  binds to synaptotagmin



(4)  $\text{Ca}^{2+}$ -bound synaptotagmin catalyzes membrane fusion by binding to SNAREs and the plasma membrane

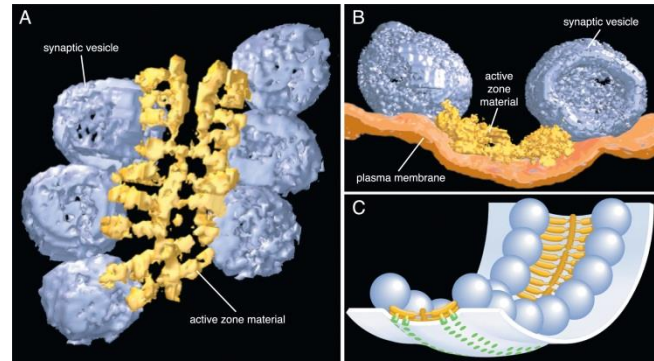


In the absence of  $\text{Ca}^{++}$  synaptotagmin may serve as a brake for release.  $\text{Ca}^{++}$  removes the brake

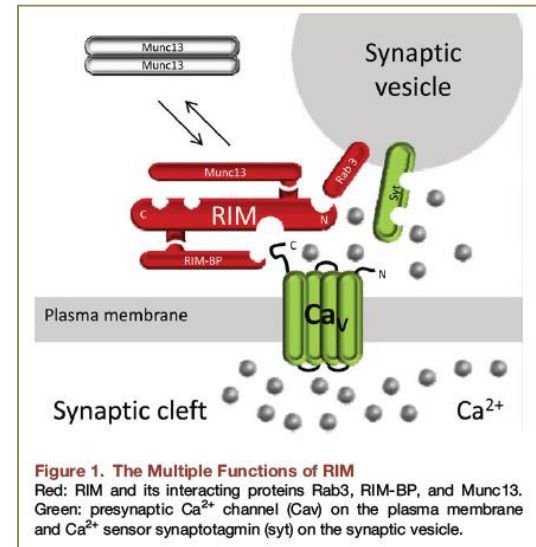
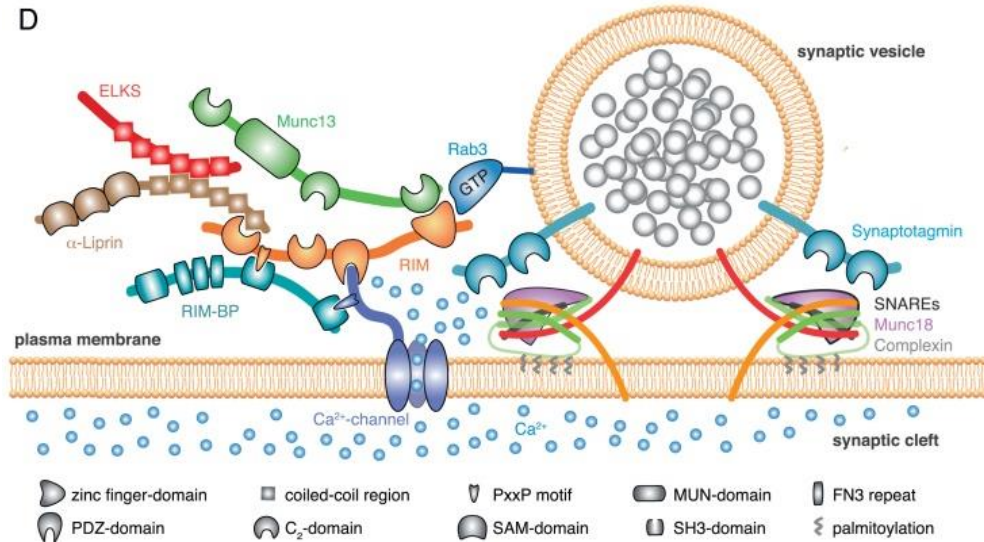
# Critical questions in neurotransmitter release

- How do the synaptic vesicle and the plasma membrane fuse during transmitter release?
- How does calcium trigger synaptic vesicle fusion?
- How is calcium influx localized to release sites in order to enable the fast coupling of an action potential to transmitter release?

What type of molecular structure links Ca<sup>++</sup> channels to membrane docked synaptic vesicles?



**RIMs tether Ca<sup>2+</sup> channels to presynaptic active zones for fast, synchronous release**



**Figure 15.8** Models of the presynaptic active zone.

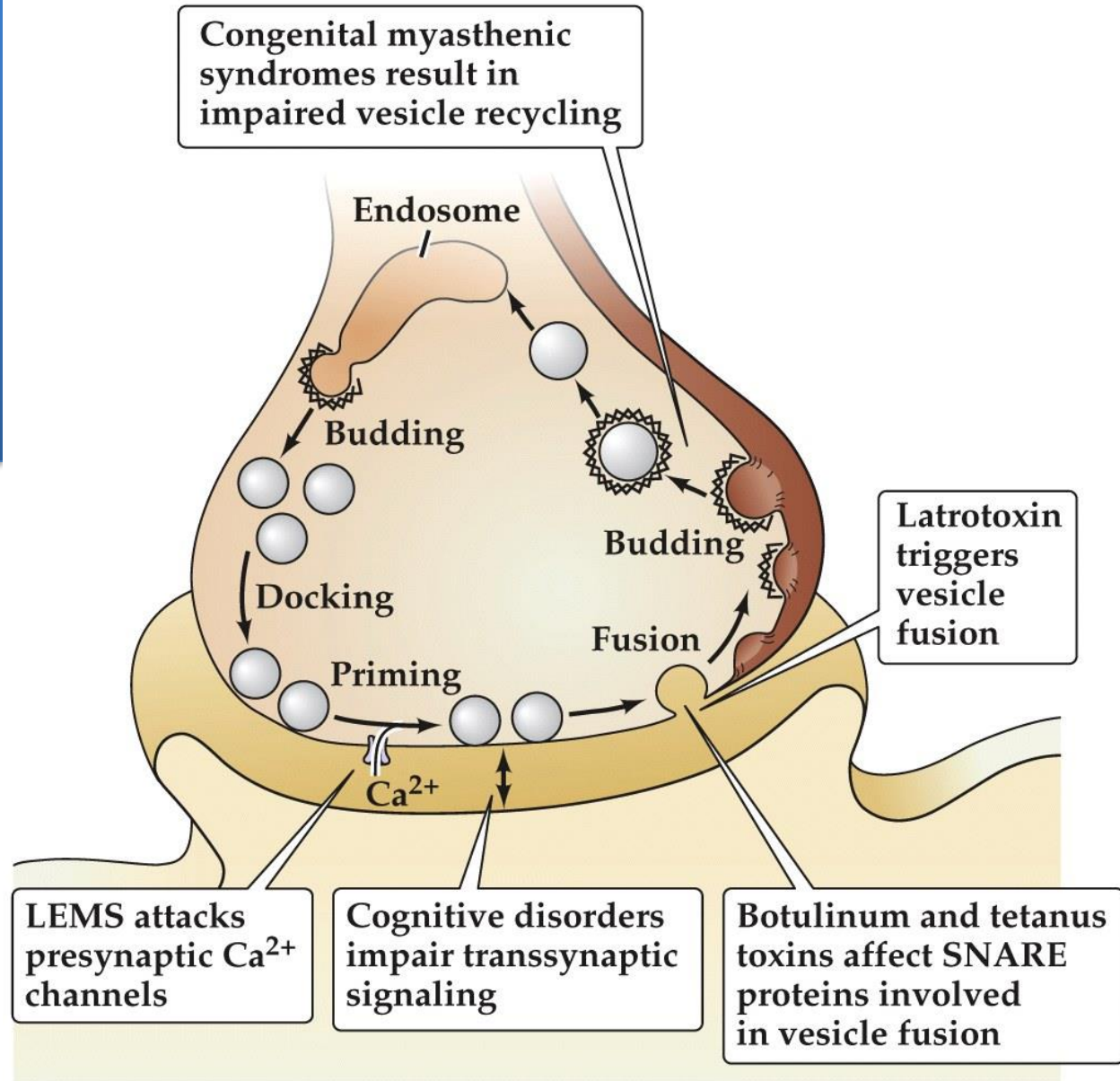
(A–C) Fine structure of the active zone at a neuromuscular junction. (A) Active zone (yellow) as revealed by electron tomography, viewed from the side of the plasma membrane with adjacent synaptic vesicles (blue). Active zone protein material extends from the vesicles and connects to a central beam in an organized fashion. (B) Transverse view of active zone material and docked synaptic vesicles adjacent to the plasma membrane (orange). (C) Schematic rendering of an active zone based on the tomographic analysis. An ordered structure aligns the vesicles and connects them to the plasma membrane and to one another. (D) Protein interactions in the active zone of a central nervous synapse. Several families of large multidomain proteins form a dense network that connects to synaptic vesicles and tethers Ca<sup>2+</sup> channels close by.

(A–C) Adapted with permission from Macmillan Publishers Ltd. (Harlow et al., 2001). (D) Adapted from Kaeser et al. (2011).

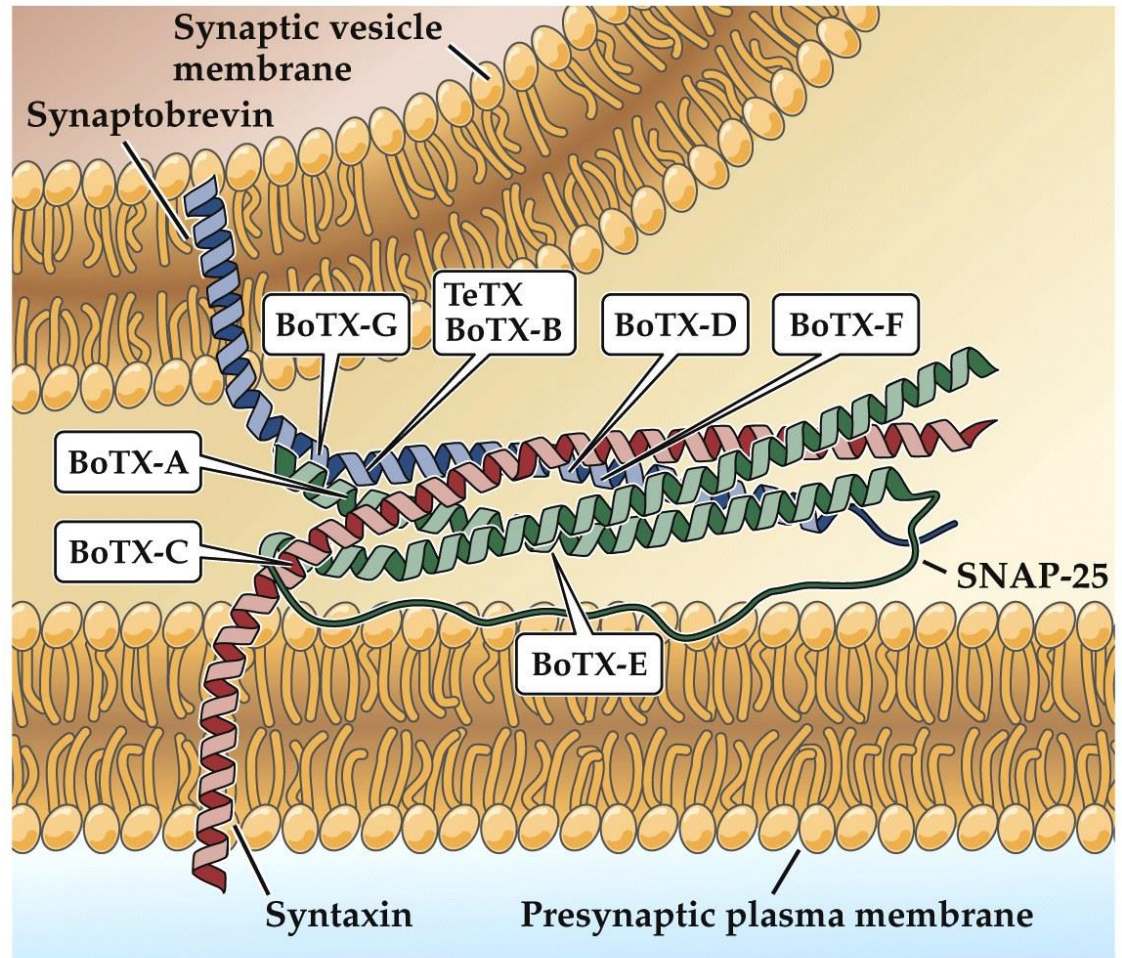


The presynaptic terminal is the target of numerous neurological diseases

LEMS = Lambert-Eaton myasthenic syndrome



# Neurotoxins affecting synaptic vesicle exocytosis



NEUROSCIENCE 5e, Box 5B (Part 2)  
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**FIGURE 6** SNARE proteins and the action of clostridial neurotoxins. The SNARE complex shown at the left brings the vesicle and plasma membranes into close proximity and likely represents one of the last steps in vesicle fusion. Vesicular VAMP, also called synaptobrevin, binds with syntaxin and SNAP-25 that are anchored to the plasma membrane. Tetanus toxin and the botulinum toxins, proteases that cleave specific SNARE proteins as shown, can block transmitter release.