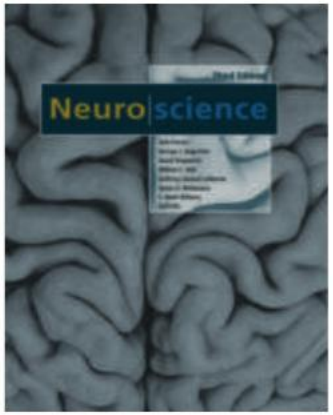
Synaptic Transmission (II)

It Is All About Communication...

Topics I	Topics II
Introduction	Synaptic Transmission
Electrochemical Gradients	Electrophysiology Techniques
Passive Membrane Properties	Basic Circuits (Spinal Cord)
Action Potential	Sensory Systems Overview
Voltage-Gated Ion Channels	Synaptic Plasticity
Ligand-Gated Ion Channels	Recapitulation

Study Material

- NEUROSCIENCE Third Edition
  - Dale Purves
- Chapter 5



THE COVER
Dorsal view of the human brain.
(Courtesy of S. Mark Williams.)

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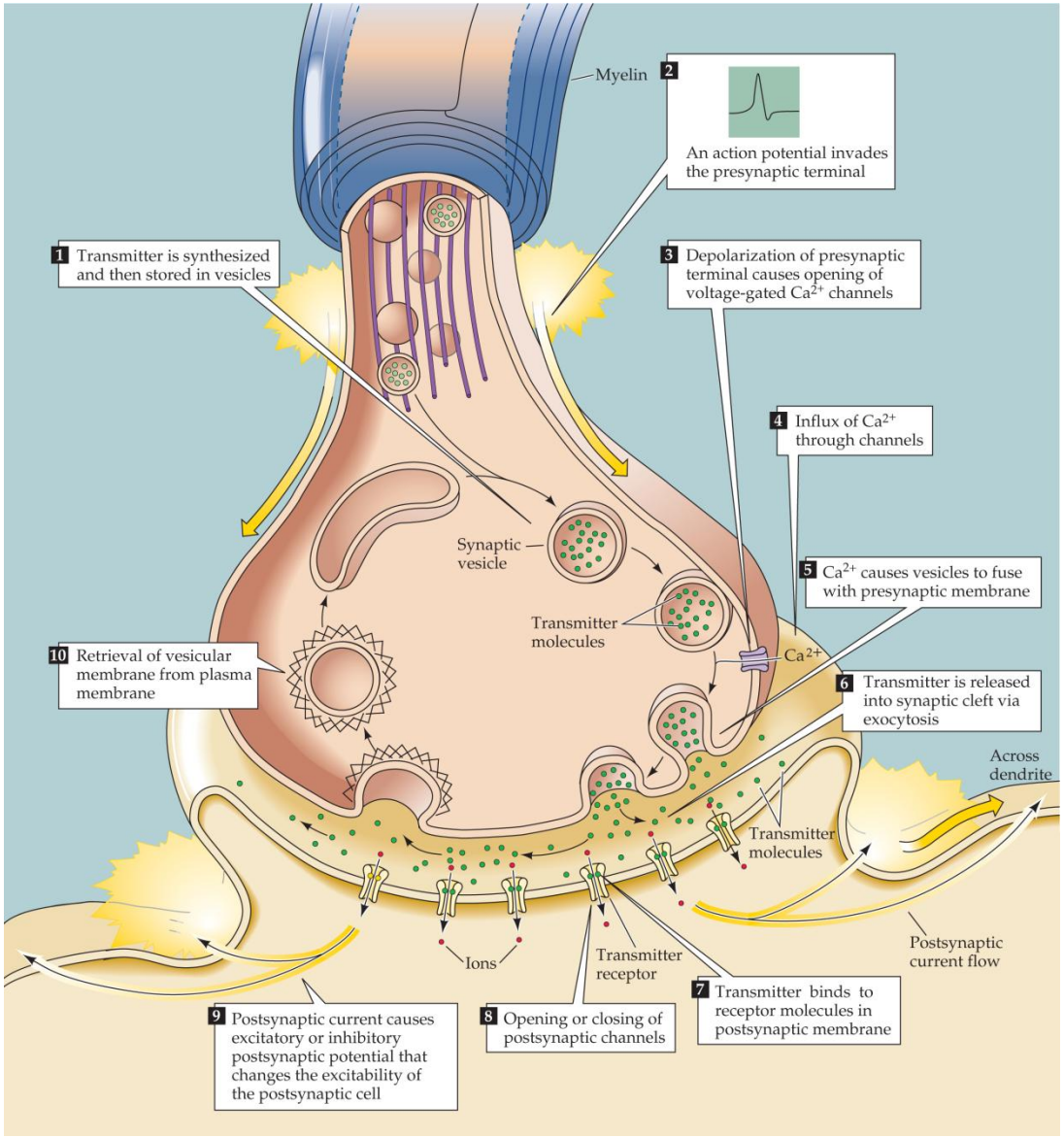
Aims for this Lecture

- Understand the dynamics of vesicular release.
- Understand the restrictions these dynamics impose on synaptic transmission.
- Understand short-term synaptic plasticity under these circumastances.
- The role of clostridial toxins in uncovering synapse function.

Recapitulation L7

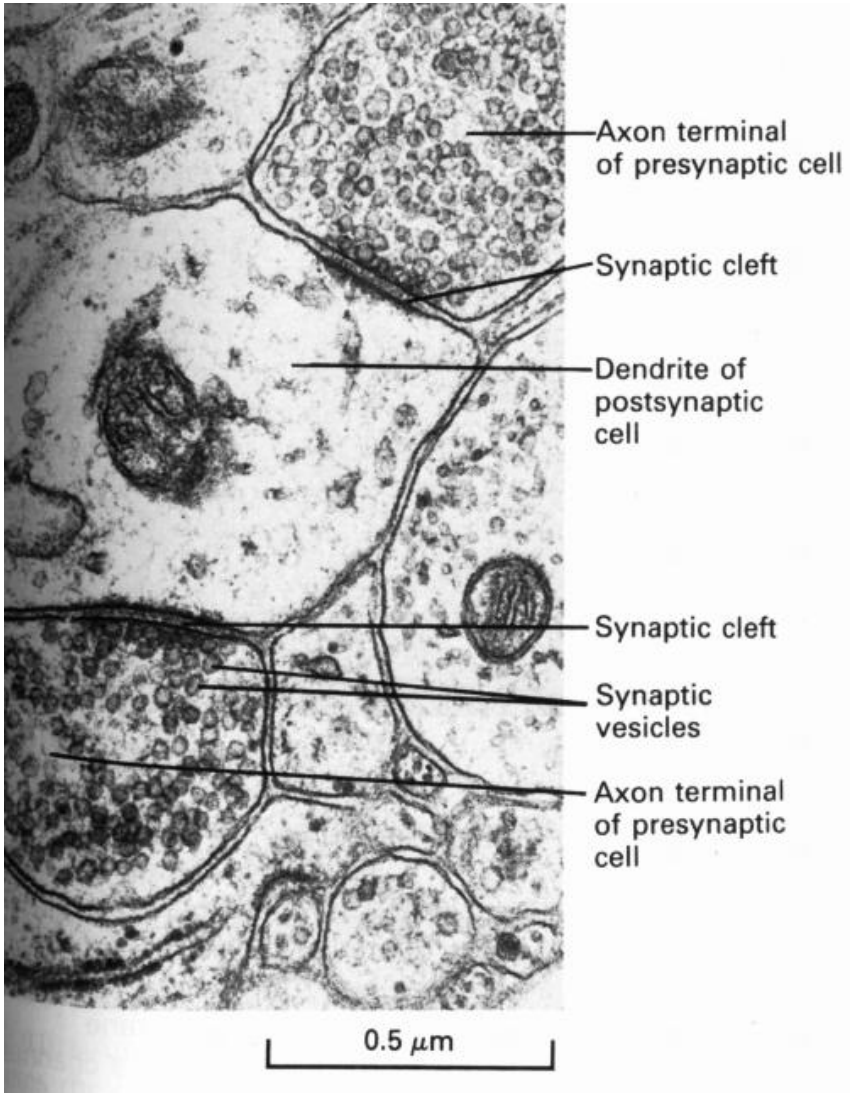
- Synaptic transmission allows communication between individual neurons.
- Electrical and chemical synapses exist. Chemical synapses are more widespread
- Fast chemical transmission uses a special apparatus that allows rapid, activity dependent fusion of vesicles with the plasma membrane
- The transmitter then immediately interacts with postsynaptic receptors.
- Transmitter is rapidly removed from the cleft and recycled (or cleaved in the case of ACh).

A Chemical Synapse



This is the most basic schematic of how chemical synaptic transmission in CNS functions.

Chemical Synapse Structure



Note the tight packing of cellular components in CNS tissue
This is epithelial tissue without much extracellular structural proteins.
Whatever space is not filled by neuronal tissue is taken up by glial processes

Vesicular Transport

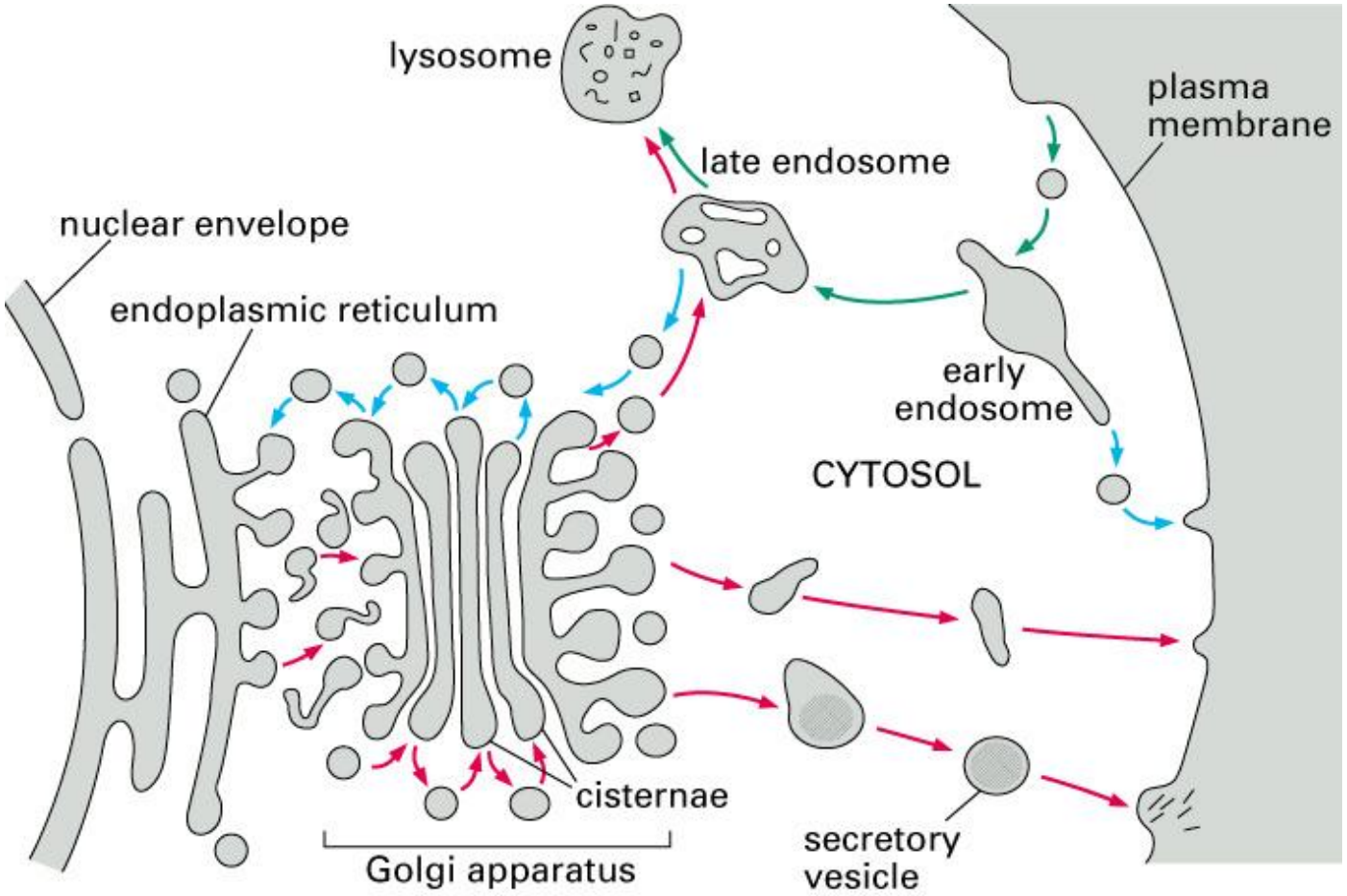


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Vesicular transport is everywhere!

Common Mechanisms

Synapse

Yeast

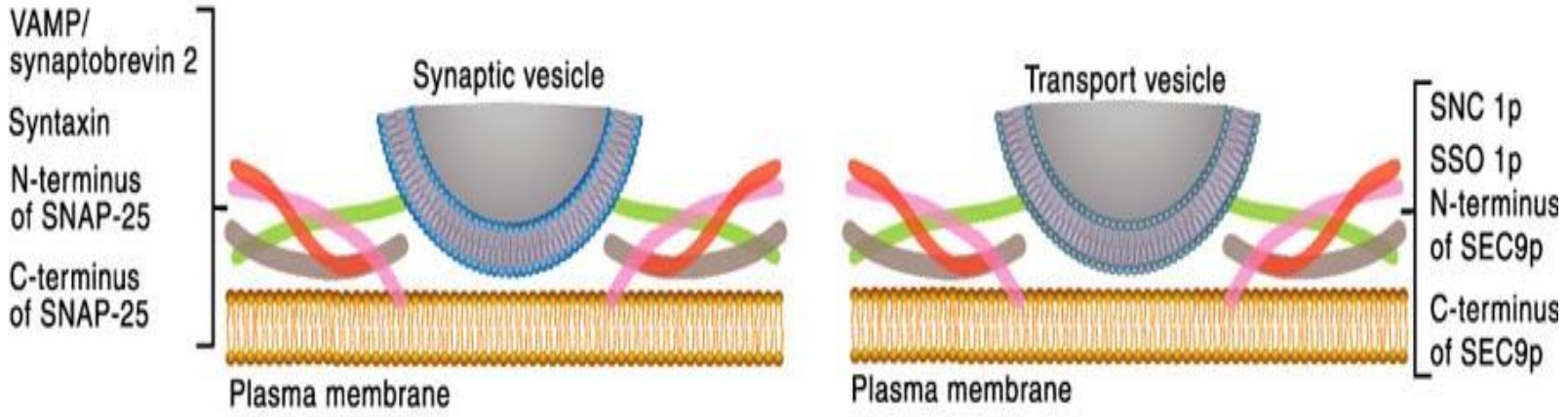
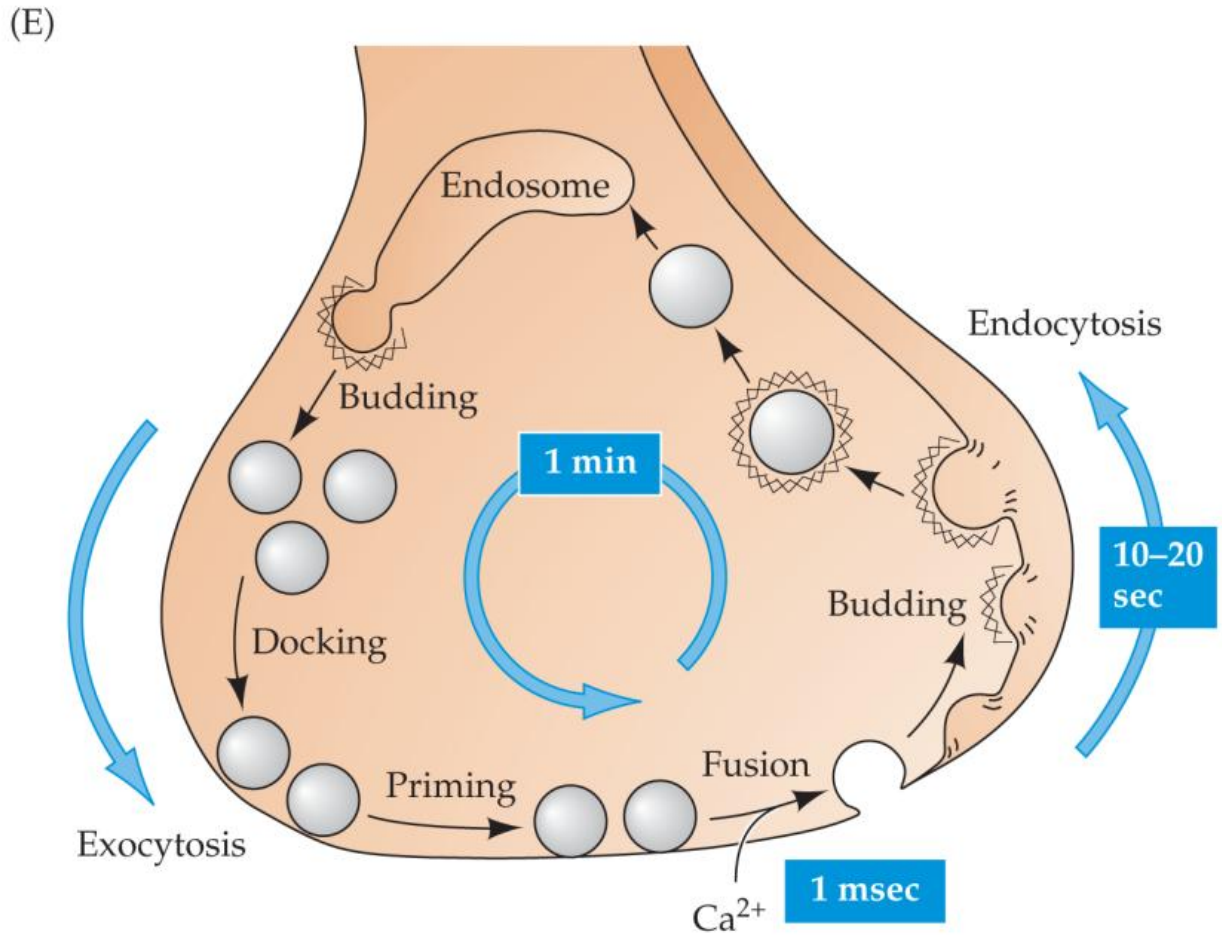


FIGURE 7 Neurotransmitter release shares a core mechanism with many membrane fusion events within eukaryotic cells. The fusion of synaptic vesicles (A) is driven by a particular complex of four coiled-coil domains contributed by three different proteins. Exocytosis in yeast (B), the fusion of late endosomes 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.

Vesicle Dynamics

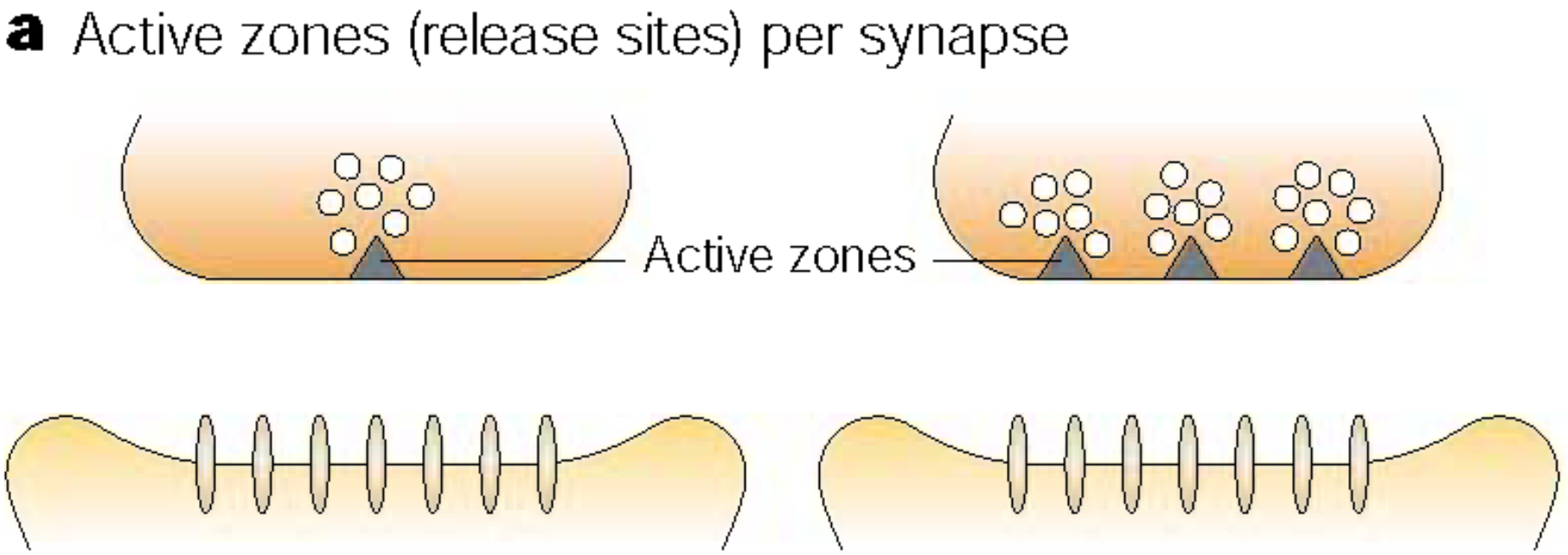


Not all the processes in the synapse progress at the same speed.

Presynaptic Regulation

- The strengths of a synaptic connection can vary dramatically depending on pre- and postsynaptic factors.
- On the presynaptic side these are primarily:
  - Active zone number
  - Calcium channel type and position
  - Quantal size (diameter and transmitter concentration)
  - Readily releasable pool size
  - Residual calcium

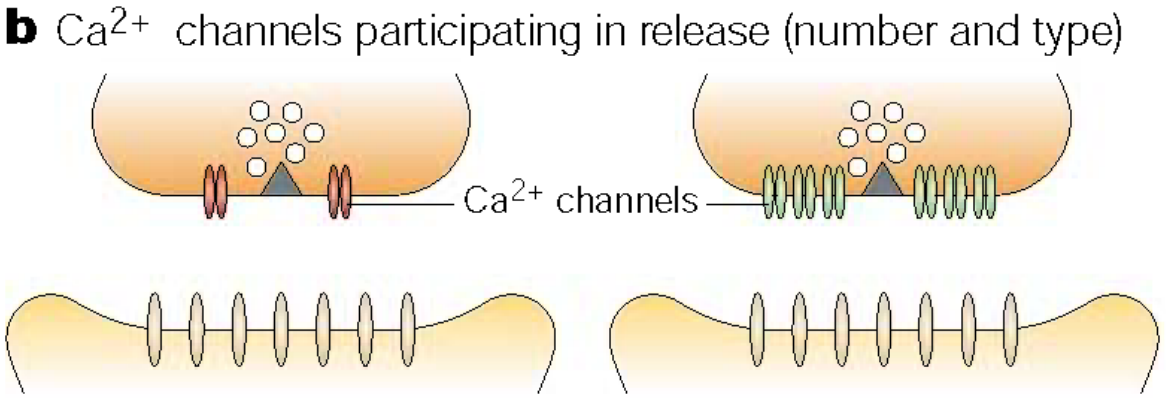
Active Zones

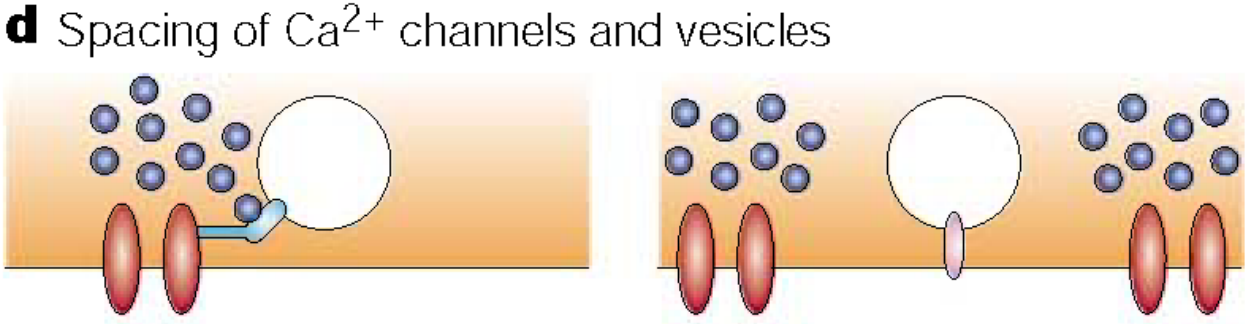


Different synapses have different numbers of active zones. Typical excitatory synapse in the CNS may have only a few, a neuromuscular endplate has hundreds. The number of release sites is indicative of the 'failure mode' the synapse is in.

Atwood and Karunanithi 2002 Nature Rev. Neurosci.

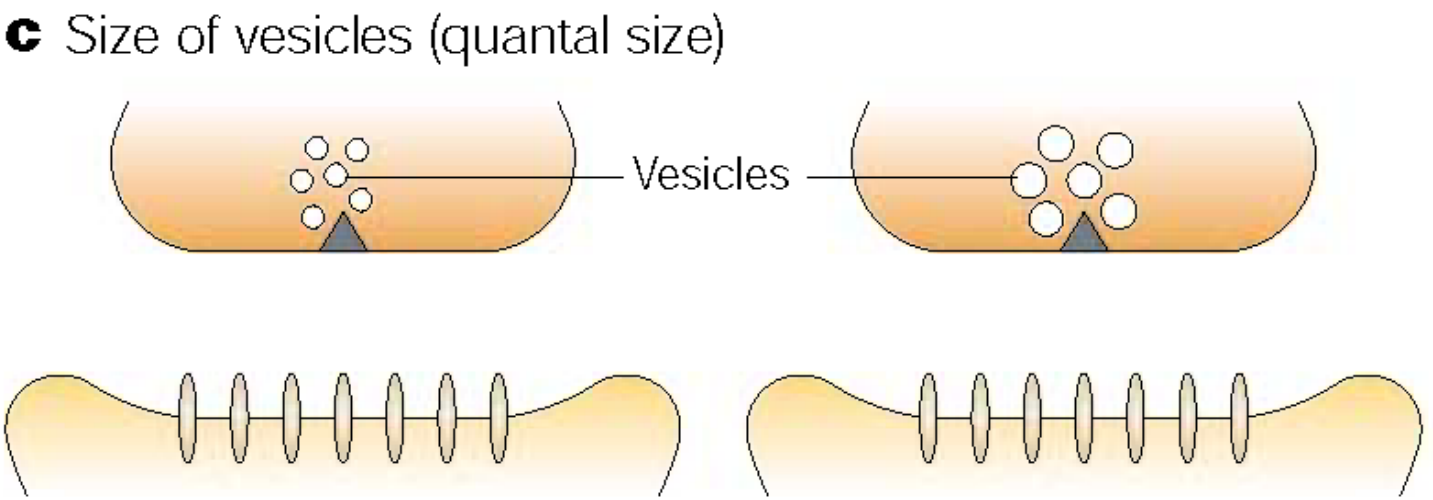
Ca Channels





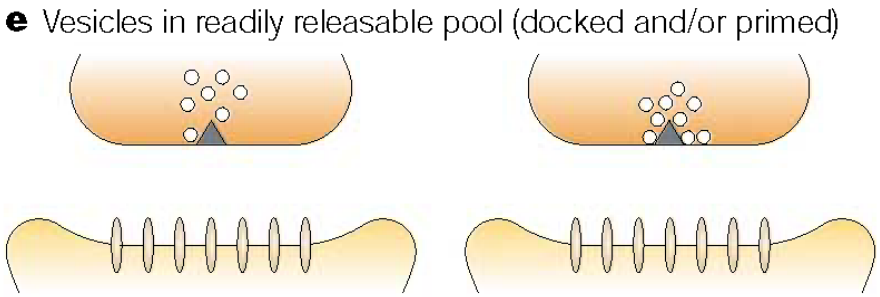
Atwood and Karunanithi 2002 Nature Rev. Neurosci.

Quantal Size



Atwood and Karunanithi
2002 Nature Rev. Neurosci.

The Readily Releasable Pool



Atwood and Karunanithi
2002 Nature Rev. Neurosci.

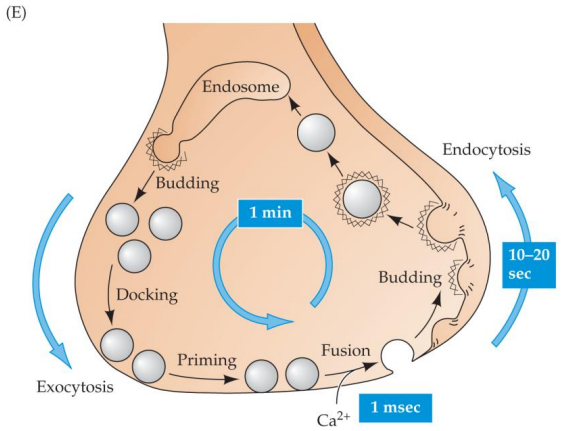
In CNS neurons, vesicles are divided into

Reserve pool (80-95%)

Recycling pool (5-20%)

Readily-releasable pool (0.1-2%; 5-10 synapses per active zone)

Rizzoli, Betz (2005). Nature Reviews Neuroscience 6:57-69)

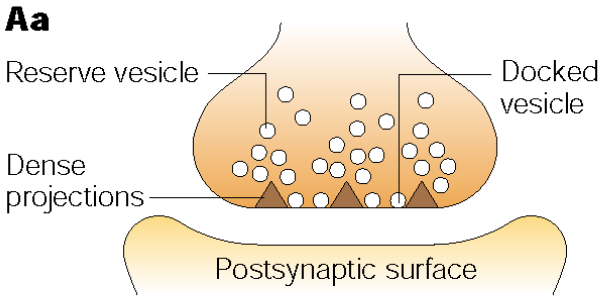


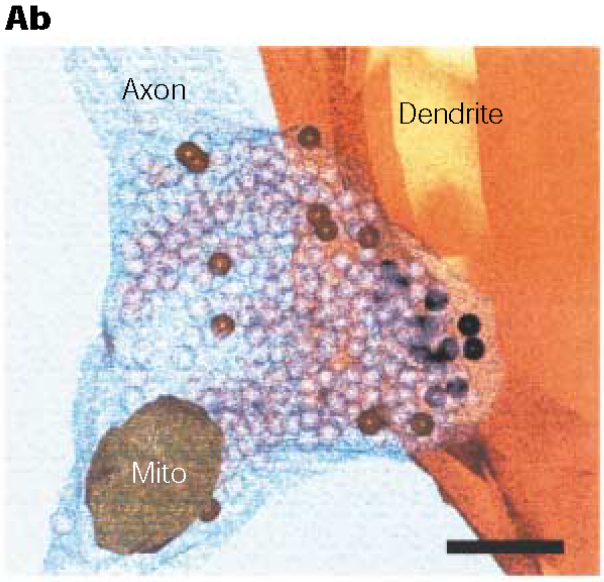
The number of docked and primed vesicles is only a fraction of the total number of vesicles in the terminal.

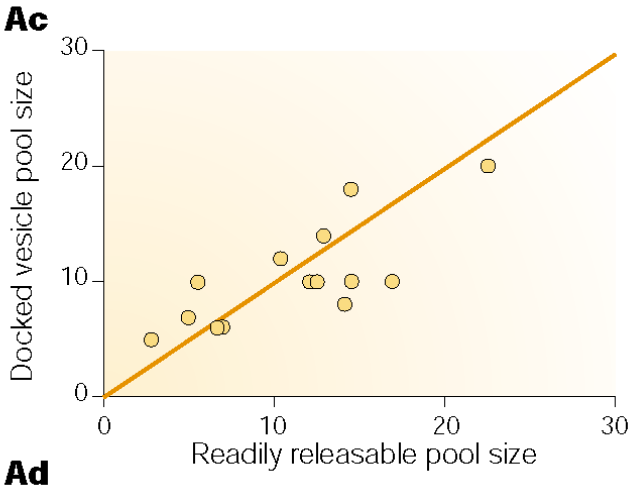
During release the readily releasable pool is depleted and fills up slowly.

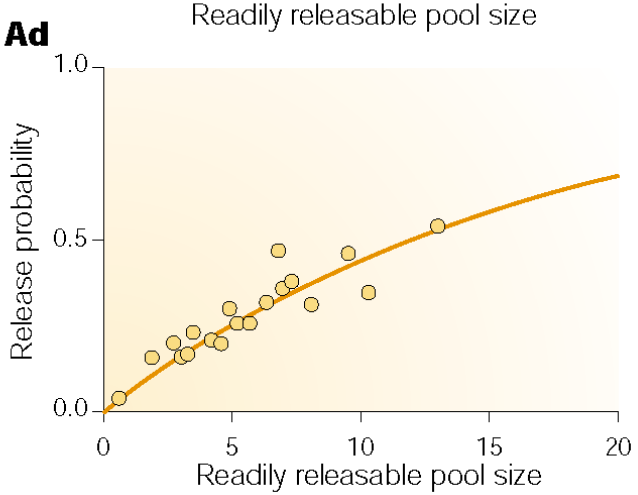
The Readily Releasable Pool

..and the initial probability of release



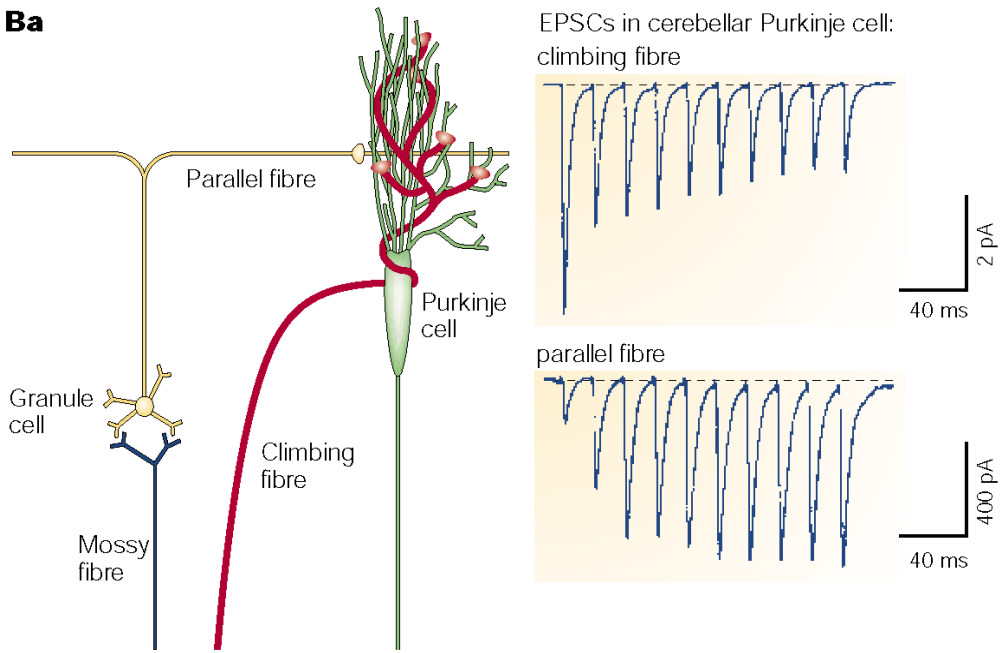






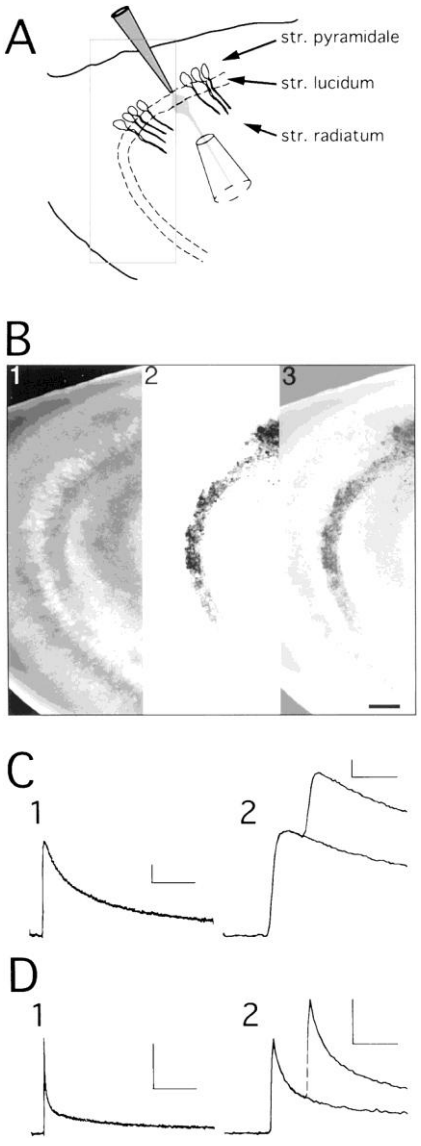
Chuck Steven's lab (Schikorski et al.).

RRP Depletion

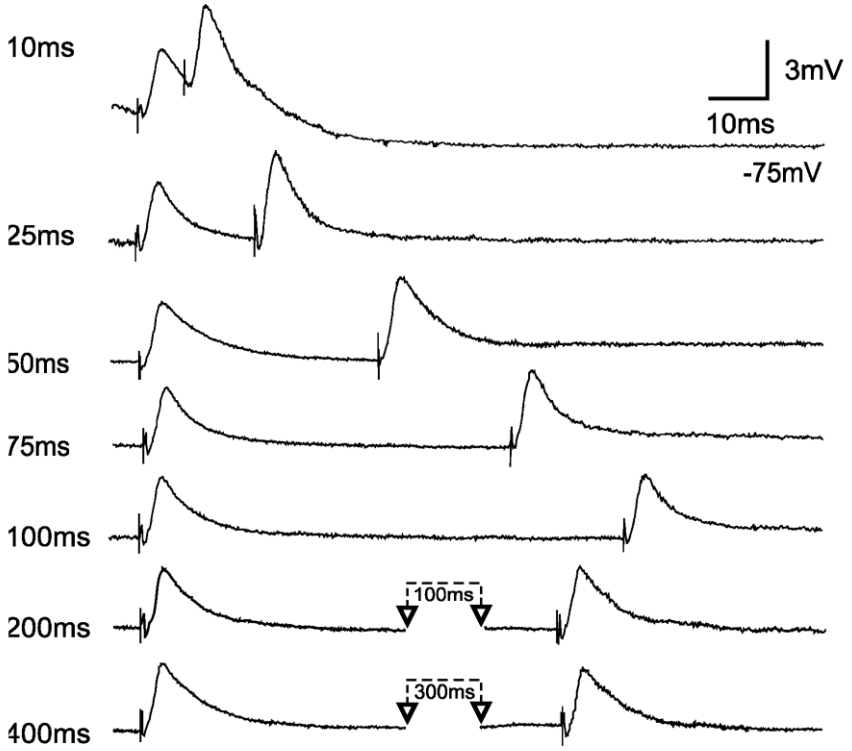


Synapses with a high initial probability of release lose many vesicles during the first stimulus and therefore tend to depress.

Residual Calcium



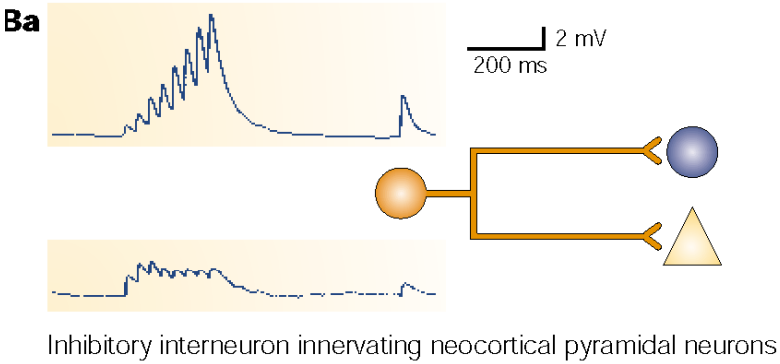
50 ms



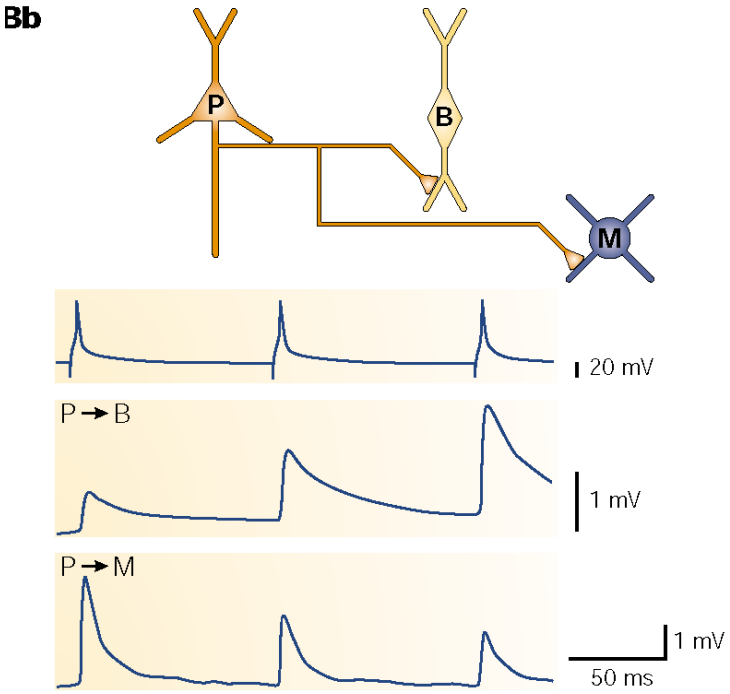
Paired activations of a synapse onto a Layer 2/3 cortical neuron. "Residual Ca^{2+}" in terminal for 10 to 100 msecs after first stimulus increases probability of release.

Kaspar E. Vogt, and Wade G. Regehr J. Neurosci. 2001;21:75-83

Different Synapse Types



Inhibitory interneuron innervating neocortical pyramidal neurons



Excitatory transmission from pyramidal cortical neurons to bitufted and multipolar neurons

A single presynaptic Neuron can have varied transmitter release at its different synapses.

Atwood and Karunanithi 2002 Nature Rev. Neurosci.

Synapse SNARE Model

- Synaptic vesicles have a specific protein that directs them to a receptor on the plasma membrane (with the aid of other proteins SNAPs etc.).
- The vesicle associated SNAP receptor (v-SNARE) in neurons is believed to be synaptobrevin (VAMP).
- Target sites such as the plasma membrane (nerve terminal) would have a corresponding t-SNARE (syntaxin and SNAP-25).
- At sites other than the nerve terminal specific vesicle and target SNAREs would function to target vesicles to specific compartments.

Synapse SNAREs

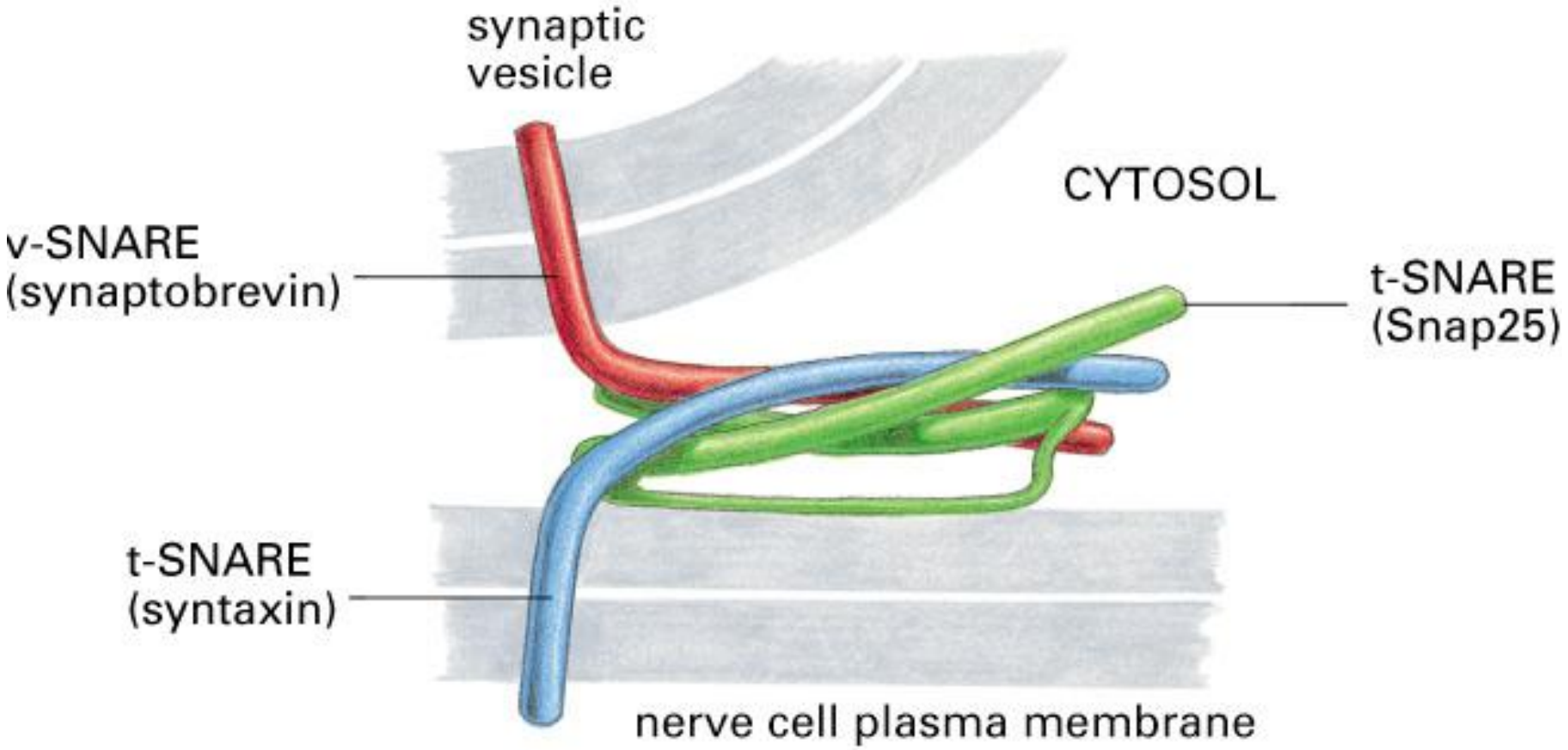


Figure 13–12. Molecular Biology of the Cell, 4th Edition.

Tightening the SNARE

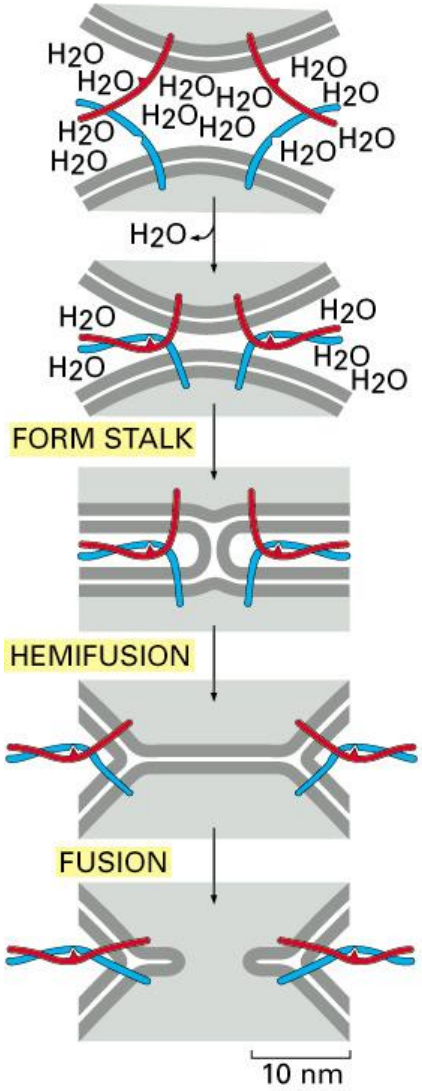
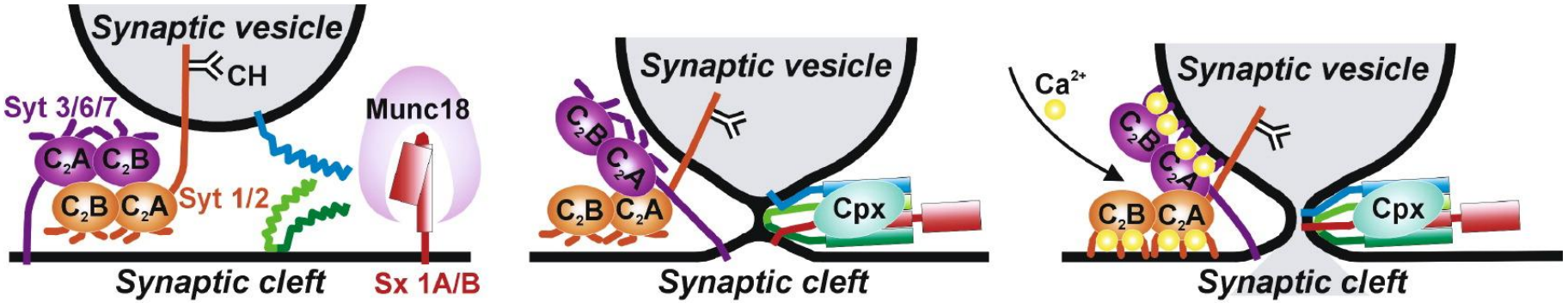


Figure 13–15. Molecular Biology of the Cell, 4th Edition.

For generic fusion processes all you need is tightening the SNAREs and you have fusion.

In synapses the process stops just before fusion.

What about Ca?



Cell, Vol 112, 519-533, 21 February 2003

Review

Membrane Fusion

Reinhard Jahn* [1], Thorsten Lang [1], and Thomas C. Südhof* [2]

What about Ca?

Synaptotagmins

- Single polypeptide, one transmembrane spanning region and large cytoplasmic domain (Syt1,2 vesicle membrane, Syt3,6,7 plasma membrane).
- Protein kinase C like homology in carboxyl terminal (C2 domain), involved in calcium and phospholipid binding. Injection of C2 peptide can block release after the vesicle is docked (squid; Augustine lab).
- Forms tetramers with each binding calcium (possible) cooperatively consistent with 3-4 power of Ca dependence of release (Sudhof and others).
- In vitro studies it binds Ca cooperatively at concentrations in the physiological range (10-100 μM) for release.
- In the absence of Ca^{2+} synaptotagmin may serve as a brake for release. Ca^{2+} removes the brake. May explain how release in nonneuronal cells occurs without synaptotagmin.

Synaptotagmin I functions as a calcium regulator of release probability

Rafael Fernández-Chacón*†‡, Andreas Königstorfer‡§, Stefan H. Gerber*, Jesús García‖, Maria F. Matos*, Charles F. Stevens¶, Nils Brose§, Josep Rizo‖, Christian Rosenmund† & Thomas C. Südhof*

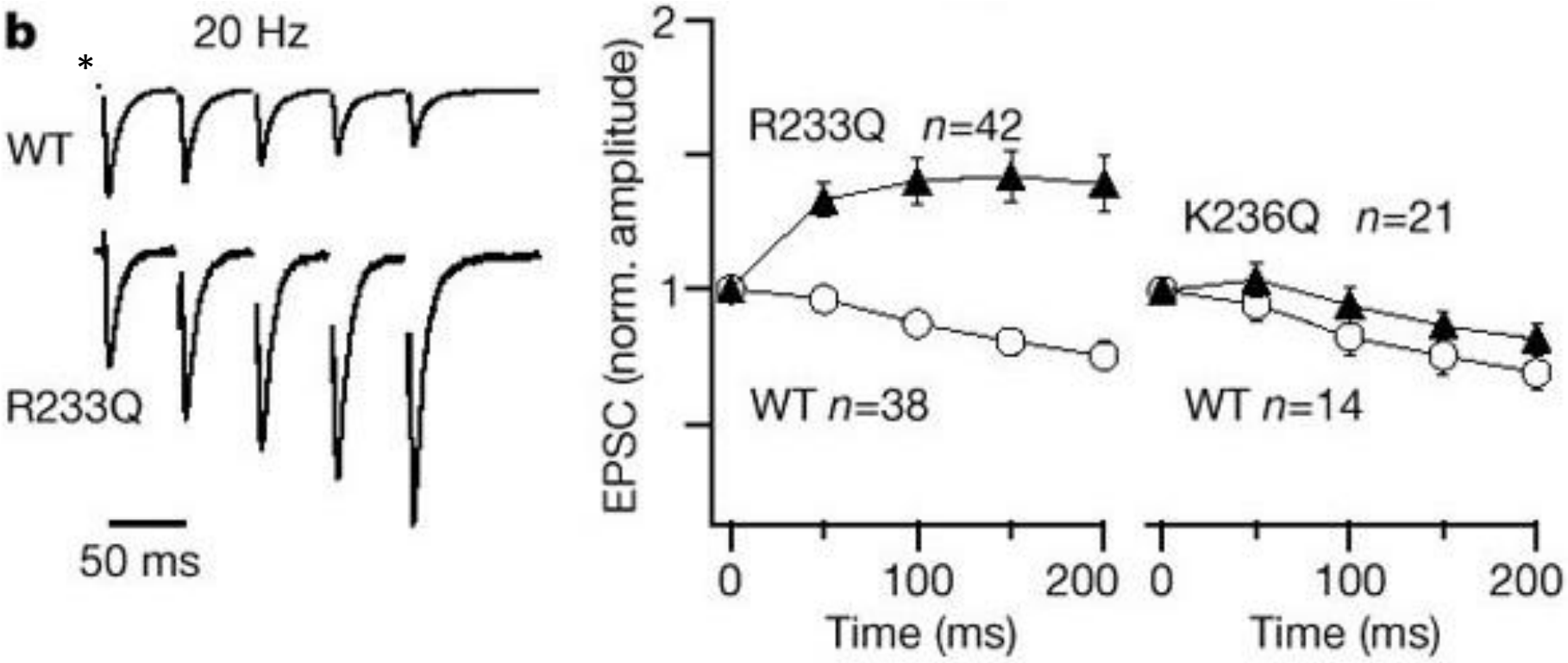
* *Center for Basic Neuroscience, Department of Molecular Genetics, and Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, Dallas, Texas 75390-9111, USA*
§ *Max-Planck-Institut für experimentelle Medizin, and † Max-Planck-Institut für biophysikalische Chemie, 37070 Göttingen, Germany*
‖ *Departments of Biochemistry and Pharmacology, The University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA*
¶ *The Salk Institute, and Howard Hughes Medical Institute, La Jolla, California 92037, USA*
‡ *These authors contributed equally to this work*

In all synapses, Ca^{2+} triggers neurotransmitter release to initiate signal transmission. Ca^{2+} presumably acts by activating synaptic Ca^{2+} sensors, but the nature of these sensors—which are the gatekeepers to neurotransmission—remains unclear. One of the candidate Ca^{2+} sensors in release is the synaptic Ca^{2+}-binding protein synaptotagmin I. Here we have studied a point mutation in synaptotagmin I that causes a twofold decrease in overall Ca^{2+} affinity without inducing structural or conformational changes. When introduced by homologous recombination into the endogenous *synaptotagmin I* gene in mice, this point mutation decreases the Ca^{2+} sensitivity of neurotransmitter release twofold, but does not alter spontaneous release or the size of the readily releasable pool of neurotransmitters. Therefore, Ca^{2+} binding to synaptotagmin I participates in triggering neurotransmitter release at the synapse.

Critical experiment was to the change affinity of Ca for synaptotagmin in vivo

Fernandez-Chacon et al. Nature 2001

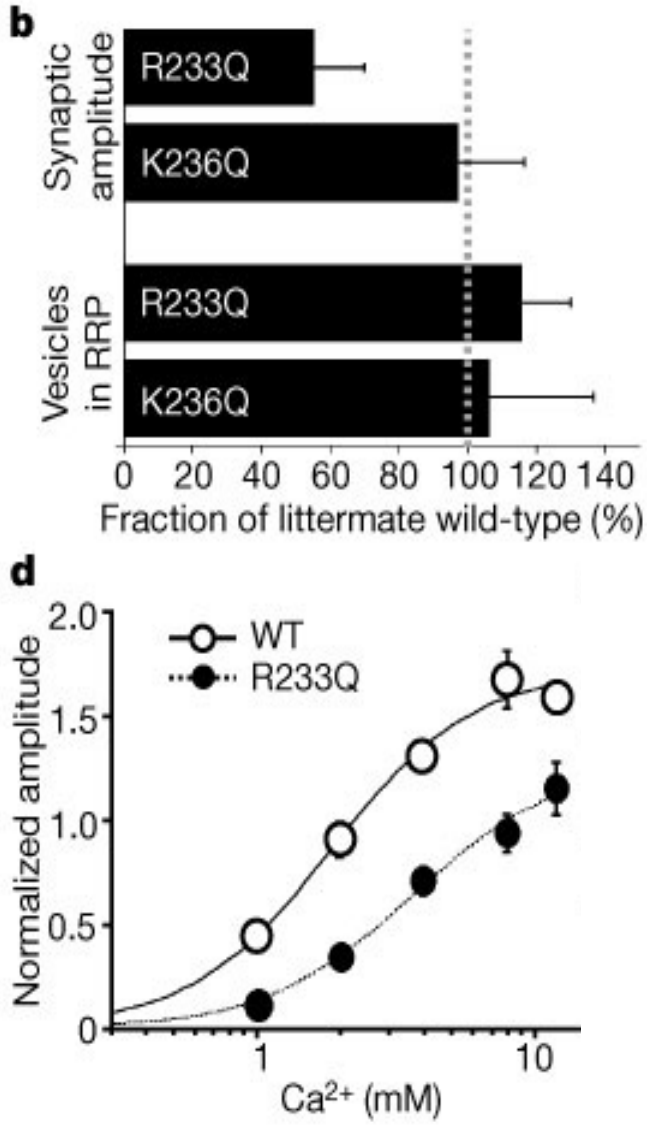
Knock in mutation of synaptotagmin with lower Ca sensitivity results in lower release probability and facilitating responses.



*Note the reduction in wild-type current amplitude is not a consistent finding.

Fernandez-Chacon et al. Nature 2001

Knock in mutation of synaptotagmin with lower Ca sensitivity reduces average response amplitude but not RRP size.



Fernandez-Chacon et al. Nature 2001

Clostridial Neurotoxins

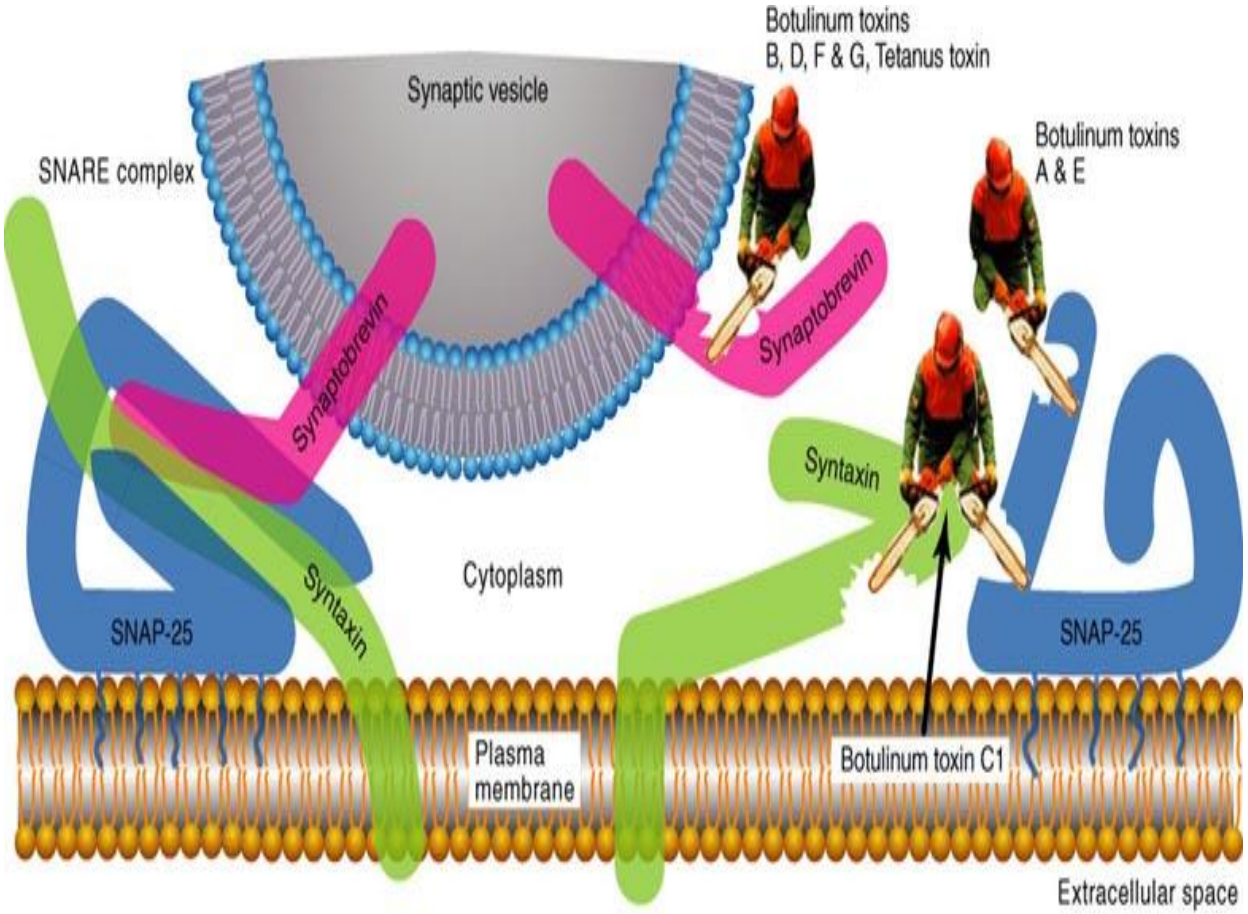
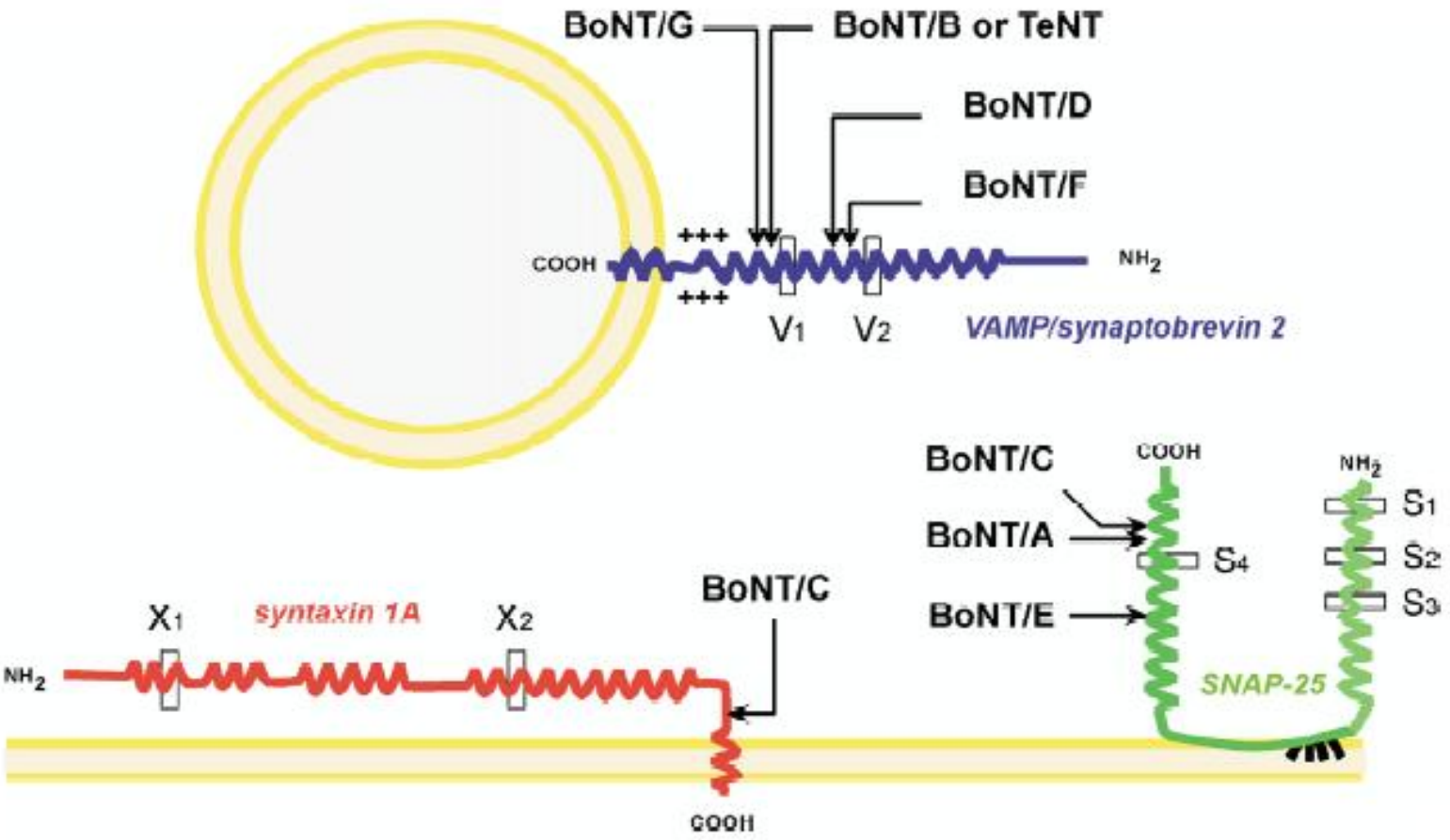


FIGURE 6 SNARE proteins and the action of clostridial neurotoxins. The SNARE complex shown at the left brings the vesicle and plasma membranes into close proximity and likely represents one of the last steps in vesicle fusion. Vesicular VAMP, also called synaptobrevin, binds with syntaxin and SNAP-25 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.

Cleaving SNAREs



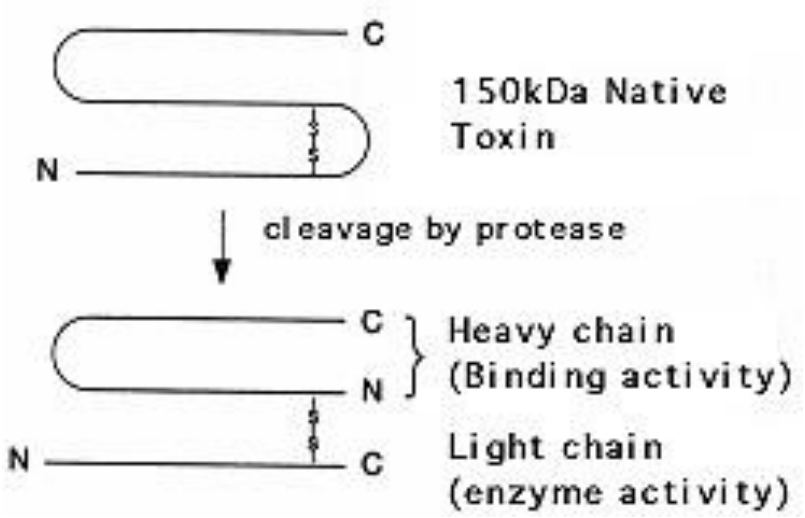
Humeau, Y., F. Doussau, et al. (2000). "How botulinum and tetanus neurotoxins block neurotransmitter release." *Biochimie* 82: 427-446.

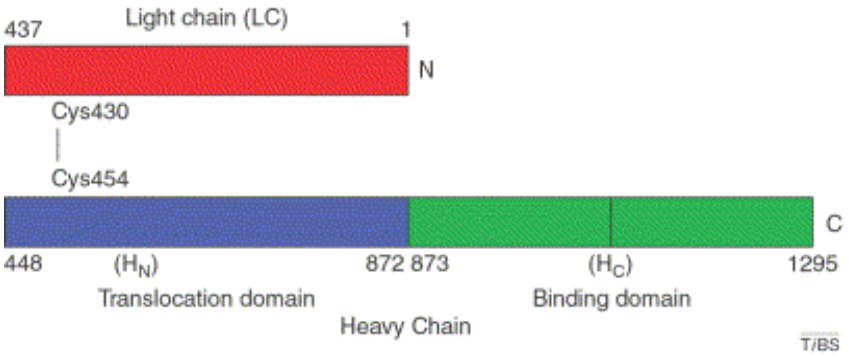
What They Look Like

Clostridial neurotoxins
have domain structure homology

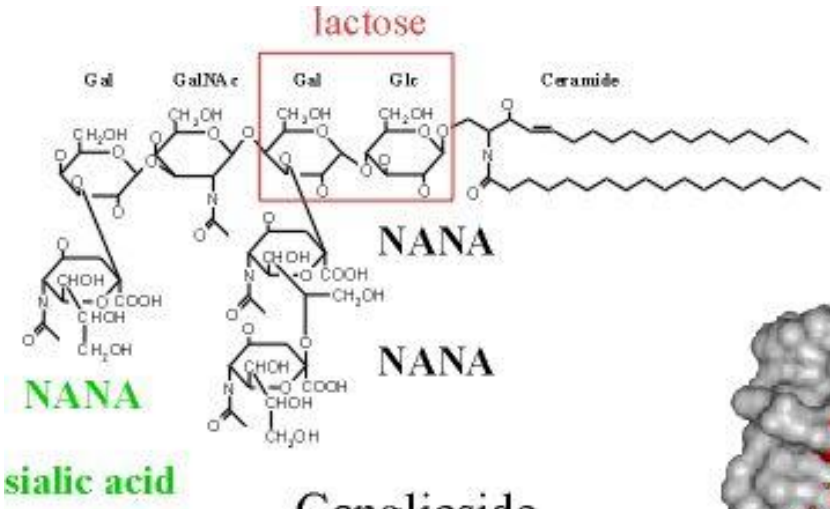
They are secreted as 150kD single chain proteins with a single disulfide bond.

The toxins are cleaved into a heavy chain (H-chain) and a light chain (L-chain).

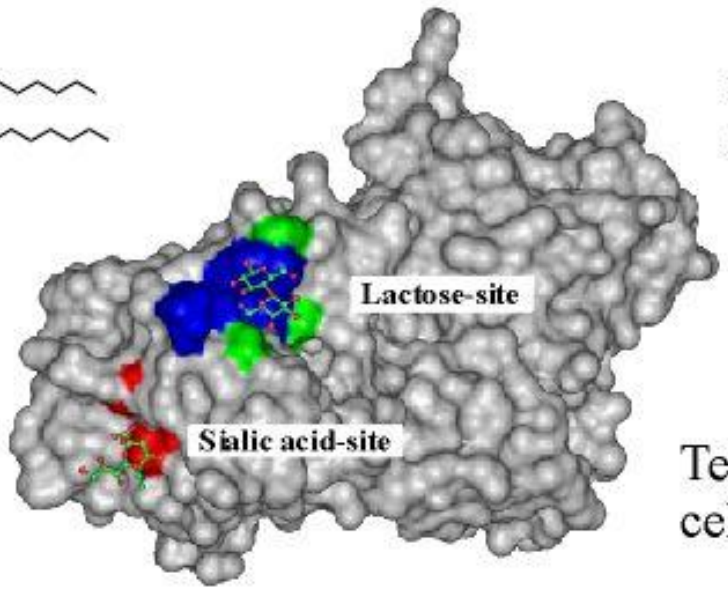




Getting them into Neurons



Ganglioside
GT1b



TeNT
cell binding domain

Emsley et al., 2000
JBC 275, 8889-94

The Clostridial toxins bind to their target cells via the HC Domain. The HC domain binds to ganglioside targets

Gangliosides are carbohydrate modified sphingolipids found
on the external leaflet of the plasma membrane

Clostridium Bacteria

***Clostridium* spp.** Anaerobic Gram-Positive Spore-Forming Bacilli

Four broad types of pathogenesis:

1. **Histotoxic group** — tissue infections
   (***C. perfringens* type A**, exogenously acquired more commonly than endogenously)
   (**C. *septicum***; endogenously-acquired)
   a. cellulilitis
   b. myonecrosis
   c. gas gangrene
   d. fasciitis
2. **Enterotoxigenic group** — gastrointestinal disease
   a. clostridial foodbome disease (8-24h after ingestion of large numbers of organisms on con-taminated meat products, spores germinate, enterotoxin produced (***C. perfringens* type A**)
   b. necrotizing enteritis (beta toxin-producing ***C.perfringens* type C**)

   (***C. difficile*** endogenously-acquired or exogenously-acquired person-to-person in hospital)
   c. antibiotic-associated diarrhea
   d. antibiotic-associated pseudomembrane colitis

3. **Tetanus** (exogenously acquired) — C. *tetani* neurotoxin
   a. generalized (most common)
   b. cephalic(primary infection in head, comnnonly ear)
   c. localized
   e. neonatal (contaminated umbilical stump)

4. **Botulism** (exogenously acquired) — *C. botulinum* neurotoxin
   a. foodborne (intoxication,1-2days incubation period)
   b. infant (ingestion of spores in honey)
   c. wound (symptoms similar to foodborne, but 4 or more days incubation)

Clostrodium and Disease

Species	Human Disease	Frequency
C. difficile	Antibiotic-associated diarrhea, pseudomembranous colitis	Common
C. perfringens	Soft tissue infections (i.e., cellulitis, suppurative myositis, myonecrosis or gas gangrene), food poisoning, enteritis necroticans, septicemia	Common
C. septicum	Gas gangrene, septicemia	Uncommon
C. tertium	Opportunistic infections	Uncommon
C. botulinum	Botulism	Uncommon
C. tetani	Tetanus	Uncommon
C. barati	Botulism	Rare
C. butyricum	Botulism	Rare
C. histolyticum	Gas gangrene	Rare
C. novyi	Gas gangrene	Rare
C. sordellii	Gas gangrene	Rare

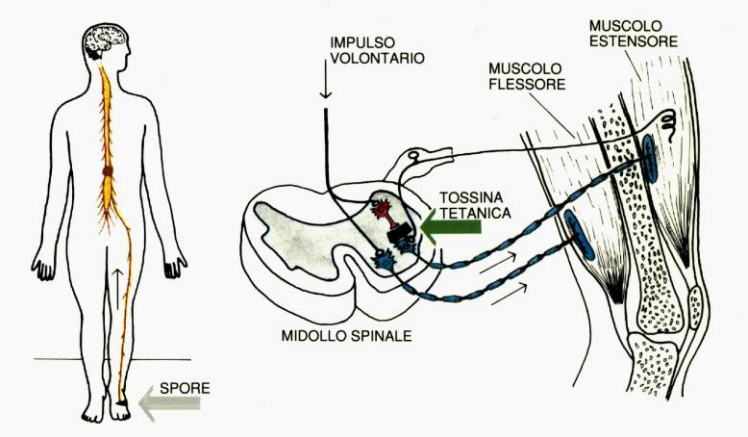
Clostridial Neurotoxins

C. tetani		**Tetanus toxin (TeTx)**
C. Botulinum	**strain A**	**Botulinum toxin A (BoTxA)**
	strain B	**Botulinum toxin B (BoTxB)**
	strain C	**Botulinum toxin C (BoTxC)**
	strain D	**Botulinum toxin D (BoTxD)**
	strain E	**Botulinum toxin E (BoTxE)**
	strain F	**Botulinum toxin F (BoTxF)**
	strain G	**Botulinum toxin G (BoTxG)**

A,B,E and F affect humans
C and D affect birds and some mammals
G has never been identified as causing disease. Isolated from soil in Argentina

Tetanus

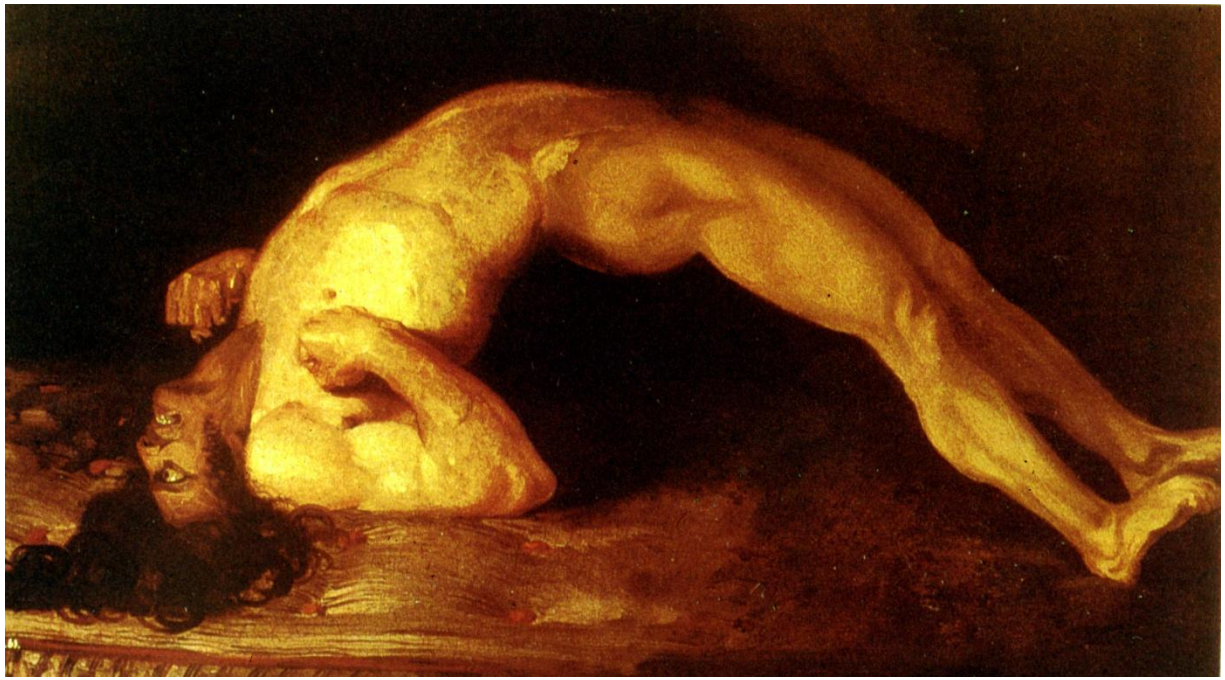
Disease	Clinical Manifestations
Generalized	Involvement of bulbar and paraspinal muscles (trismus or lockjaw, risus sardonicus, difficulty swallowing, irritability, opisthotonos); involvement of autonomic nervous system (sweating, hyperthermia, cardiac arrhythmias, fluctuations in blood pressure)
Cephalic	Primary infection in head, particularly ear; isolated or combined involvement of cranial nerves, particularly seventh cranial nerve; very poor prognosis
Localized	Involvement of muscles in area of primary injury; infection may precede generalized disease; favorable prognosis
Neonatal	Generalized disease in neonates; infection typically originates from umbilical stump; very poor prognosis in infants whose mothers are nonimmune



Tetanus toxin preferentially targets inhibitory synaptic transmission in the spinal cord

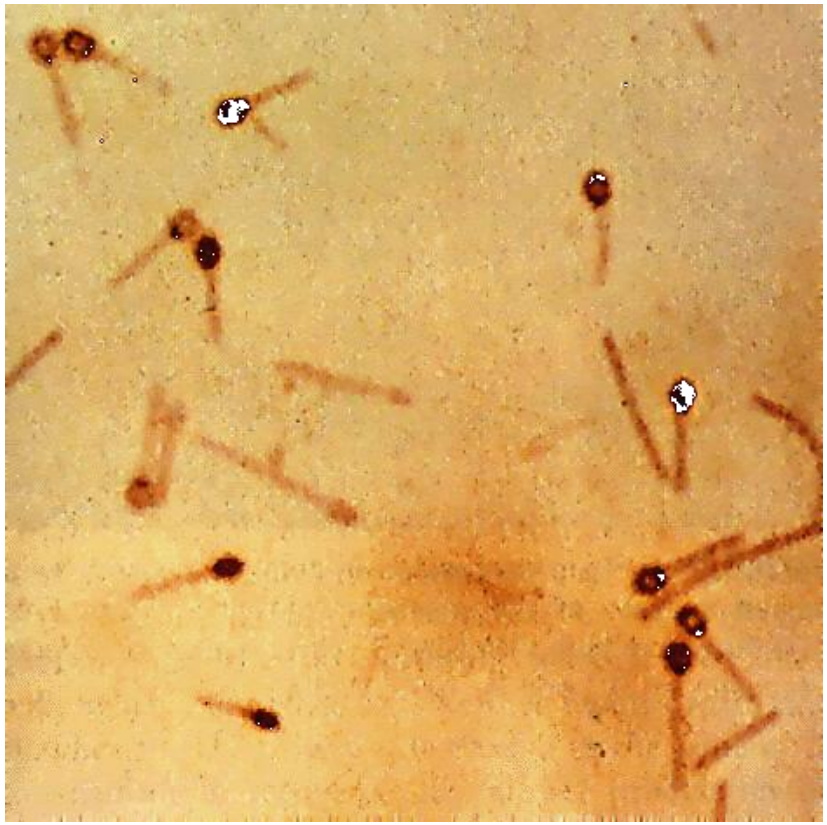
Clinical Manifestations

Risus sardonicus



Opistotonus

Clostridium Tetani Stain



The terminal round spores give them a ‘tennis racket’ or ‘drumstick’ appearance.

Botulism

Physiology and Structure

Gram-positive, spore-forming bacillus.

Strict anaerobe (vegetative cells extremely oxygen-sensitive).

Fastidious growth requirements.

Can produce one of seven distinct botulinum toxins (A–G).

Strains associated with human disease produce lipase, digest milk proteins, hydrolyze gelatin, and ferment glucose.

Virulence

Spore formation.

Botulinum toxin (prevents release of neurotransmitter acetylcholine).

Binary toxin.

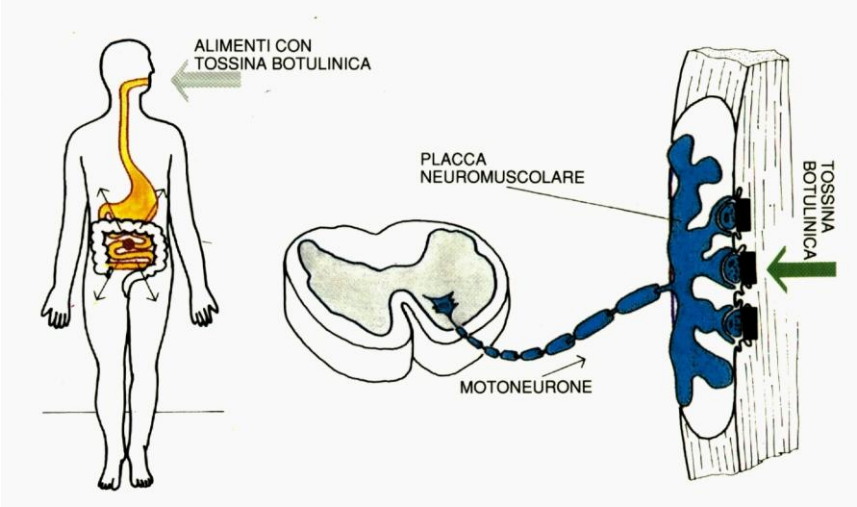
Epidemiology

Ubiquitous; *C. botulinum* spores are found in soil worldwide

Human diseases associated with toxins A, B, E, and F.

Relatively few cases of botulism in the United States.

Infant botulism more common than other forms.



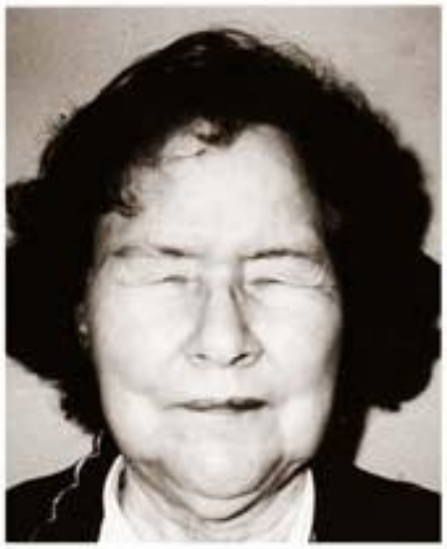
Botulinum toxin preferentially tragets the neuromuscular endplate

From the latin word for sausage

Clinical Manifestation and Use

Infant Botulism

Spores (found e.g. in honey) germinate in intestinal tract



Botox can be an important therapeutic agent to control muscle spasms

Use in Cosmetic Medicine