Lecture 09. Introduction to Synapses: Release of Transmitter

Dr. Bruce Johnson

Pre-lecture preparation

Watch Video 9-1, "Release of neurotransmitter"

Reading

Bear et al.: be able to explain the following figures: p. 115, Fig. 5.4; p. 123, Fig. 5.12; p. 125, Box 5.3; p. 126, Fig. 5.13. If you have difficulty with these, read the associated text.

Lecture Objectives

1. Be able to describe the steps involved in chemical synaptic transmission, from the action potential in the pre-synaptic axon to the post-synaptic potential in the post-synaptic dendrite.

2. Explain the evidence showing that calcium entry is the ultimate trigger for release of transmitter.

3. Describe how synaptic vesicles filled with neurotransmitter are the basis for the quantal release of transmitter, and how the EPSP arises from the nearly simultaneous fusion of many quanta and the exocytosis of their transmitter contents into the synaptic cleft.

4. Begin to understand the many molecules that are involved in the regulation and control of exocytosis, with a focus on the central roles of the SNARE complex and synaptotagmin.

Lecture outline

1. Introduction to synaptic transmission
   - Chemical synapses involve release of neurotransmitters from the pre-synaptic nerve terminal to the post-synaptic cell (typically onto a dendrite)
   - Action potential triggers the entry of calcium to cause fusion of synaptic vesicles that then release their transmitter into the synaptic cleft
   - The transmitters diffuse across the cleft and bind to specialized receptors which evoke the post-synaptic voltage response.
2. Types of synapses
   - Excitatory: try to depolarize post-synaptic neuron above spike threshold
   - Inhibitory: try to prevent the post-synaptic neuron from depolarizing to threshold
   - Modulatory: alter the properties of the neuron or the strength of the synapse
3. Types of molecules that act as neurotransmitters
   - Amino acids: glutamate, GABA

- Monoamines: dopamine, norepinephrine, serotonin
- Other small molecules: acetylcholine
- Peptides: endorphins, Oxytocin, Substance P

4. Release of transmitter
   - Essential role for increase in intracellular calcium in pre-synaptic terminal to evoke release
   - Release occurs by vesicular exocytosis, which can be observed by electron microscopy
5. Steps to release of transmitter
   - Transport of vesicles to active zones: role of synapsins
   - Docking of vesicles close to the membrane: role of Rab3a and others
   - Priming of the vesicles for release: formation of SNARE-Complexin complex
   - Calcium-evoked fusion: role of synaptotagmin
   - Recycling of the vesicle membrane: "kiss-and-run" vs. clathrin mechanisms

Study Questions

1. You have found that khayat, a strange drug used by Siberian shamen, increases the frequency of spontaneous MEPPs at the human neuromuscular junction. Which of the following is a possible action of this drug?

   a) It blocks acetylcholine receptors.
   b) It increases the resting level of Ca^{2+} in the presynaptic terminal.
   c) It increases the number of voltage-sensitive Na^{+} channels in the pre-synaptic terminal.
   d) It decreases the spike-evoked fusion of vesicles from the pre-synaptic terminal.
   e) It increases the rate of synthesis of acetylcholine.

2. If someone challenged the existence of chemical synapses as "only a theory," how would you reply?

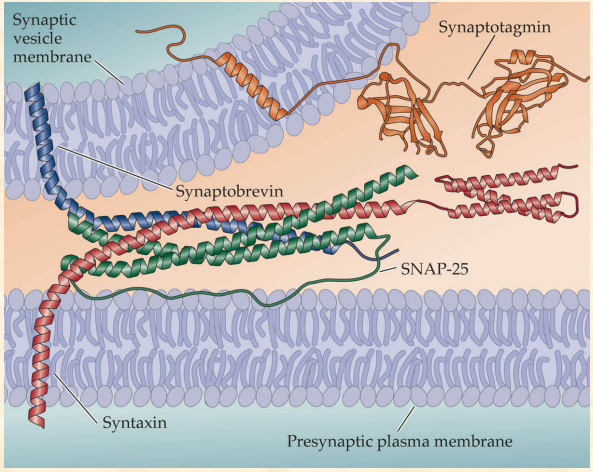
3. Indicate how each of the following are involved in neurotransmitter secretion:
   NSF
   Complexin
   SNAREs (synaptobrevin, synataxin, SNAP-25)
   Synaptotagmin
   Synapsin

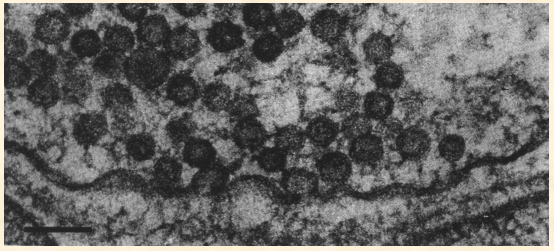
   Which one is key in the regulation of transmitter release by Ca^{2+}?

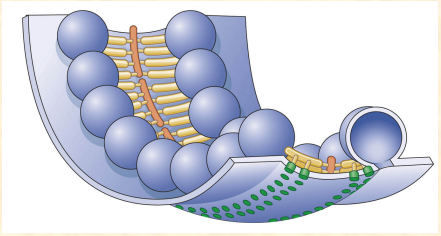
4. What lines of evidence suggest that neurotransmitters are released from synaptic vesicles?

Lecture 9: Introduction to chemical synapses and mechanisms of release of transmitter

Bruce Johnson







Office hours: Wednesday and Thursday, 3-5 PM in B150 Comstock Hall

https://www.youtube.com/watch?v=mItV4rC57kM

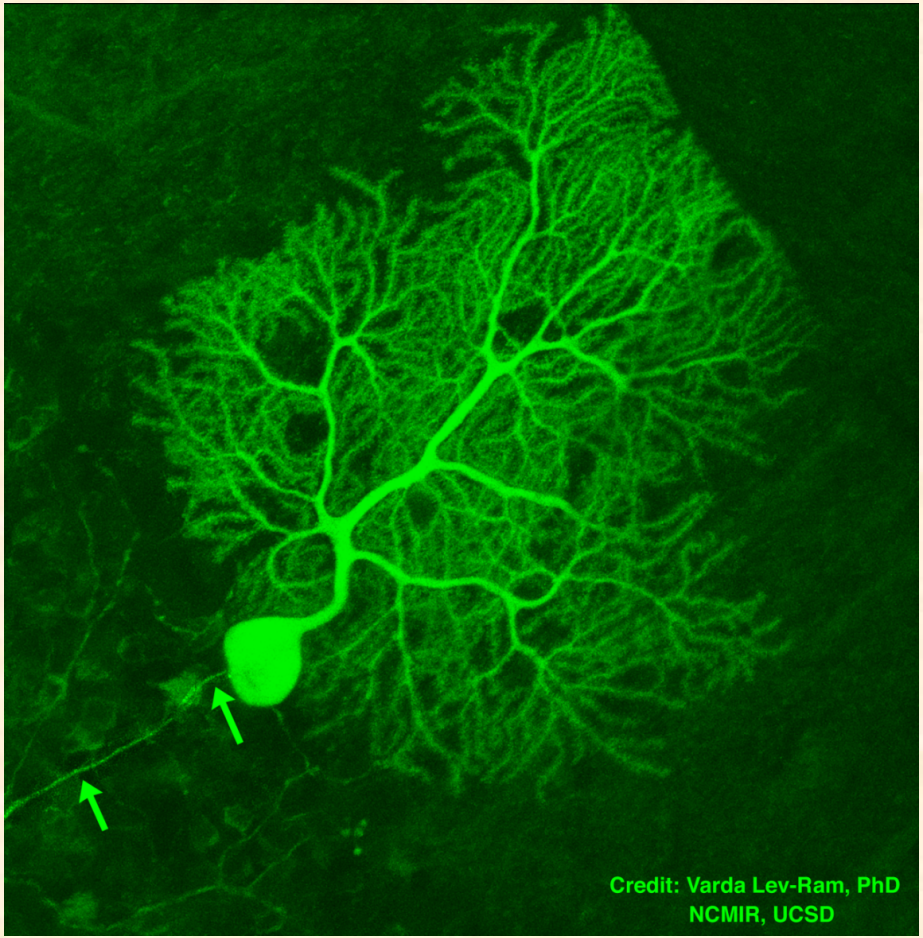
Goals for today

- Introduce synapses: excitatory and inhibitory

- Introduce the main fast neurotransmitters

- Understand the steps leading up to transmitter release from nerve terminals: Video

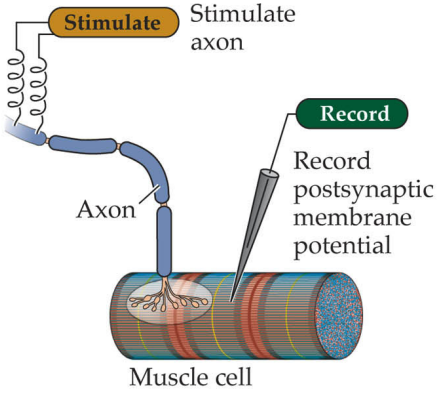
- Discuss the life cycle of a synaptic vesicle and how calcium entry triggers the release of transmitter

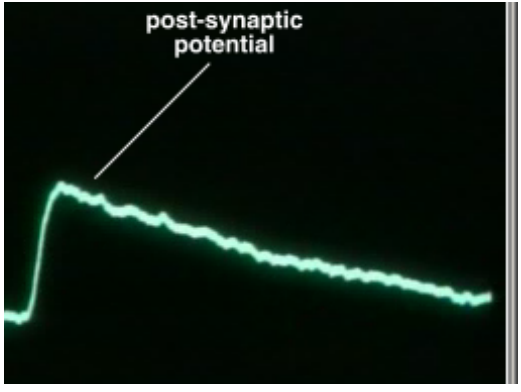


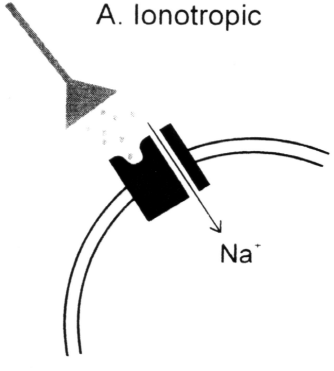
Synaptic transmission

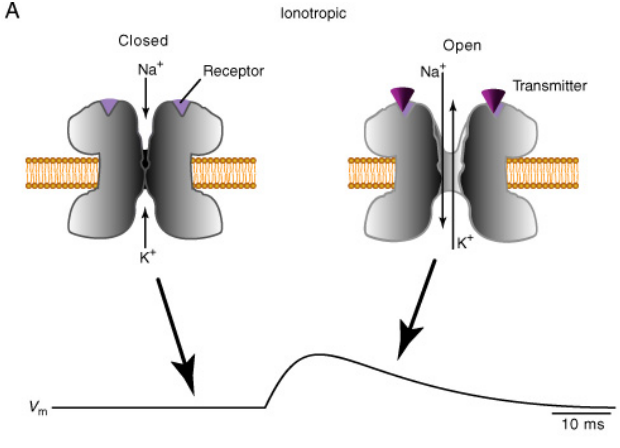
1) signal transmission between NS components and effectors
2) A site of plasticity for learning and memory
3) A site of action for disease, psychoactive drugs

Fast Chemical Synaptic Transmission



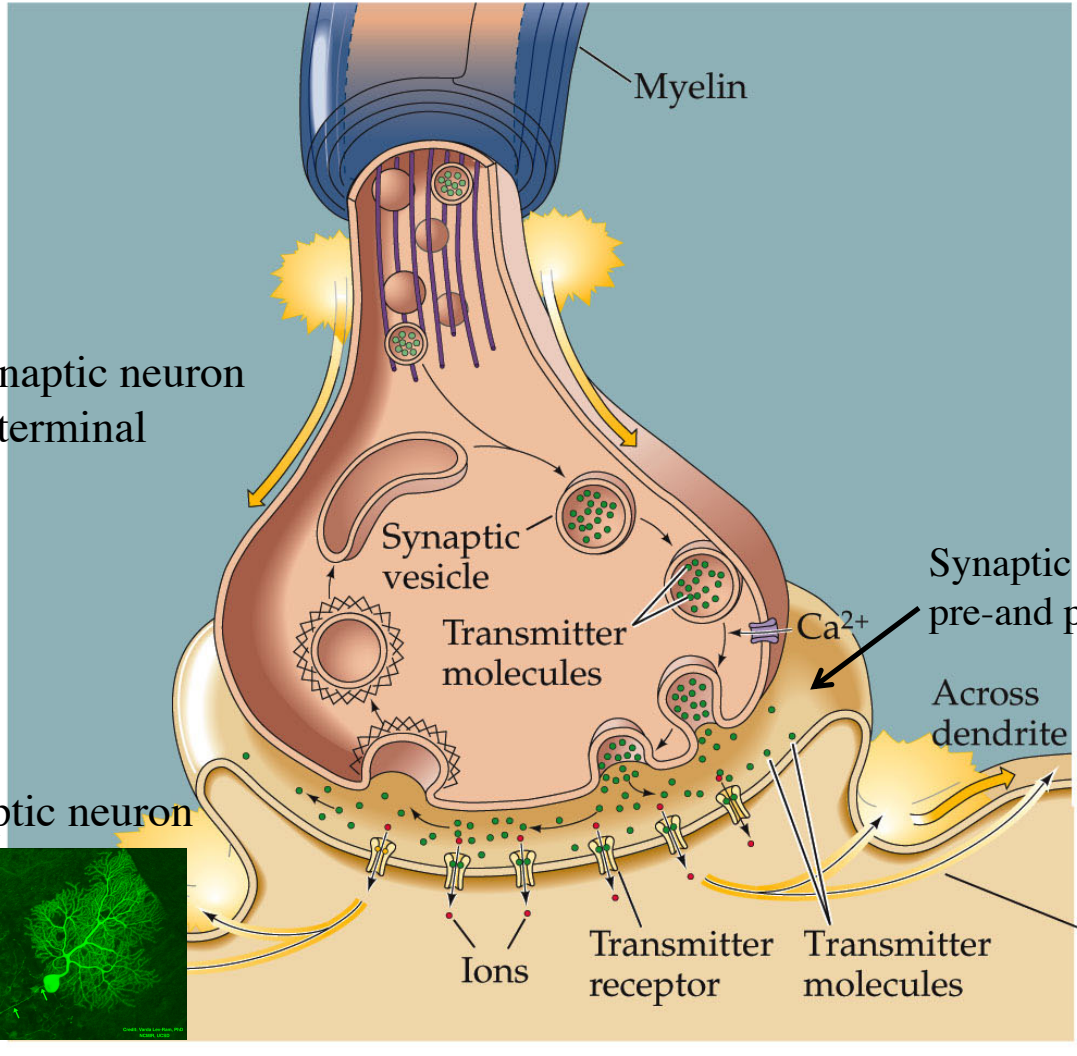






direct, fast
ionotropic

Overview of Chemical Synapses



Pre-Synaptic neuron
Nerve terminal

Synaptic cleft separating the pre-and post-synaptic neurons

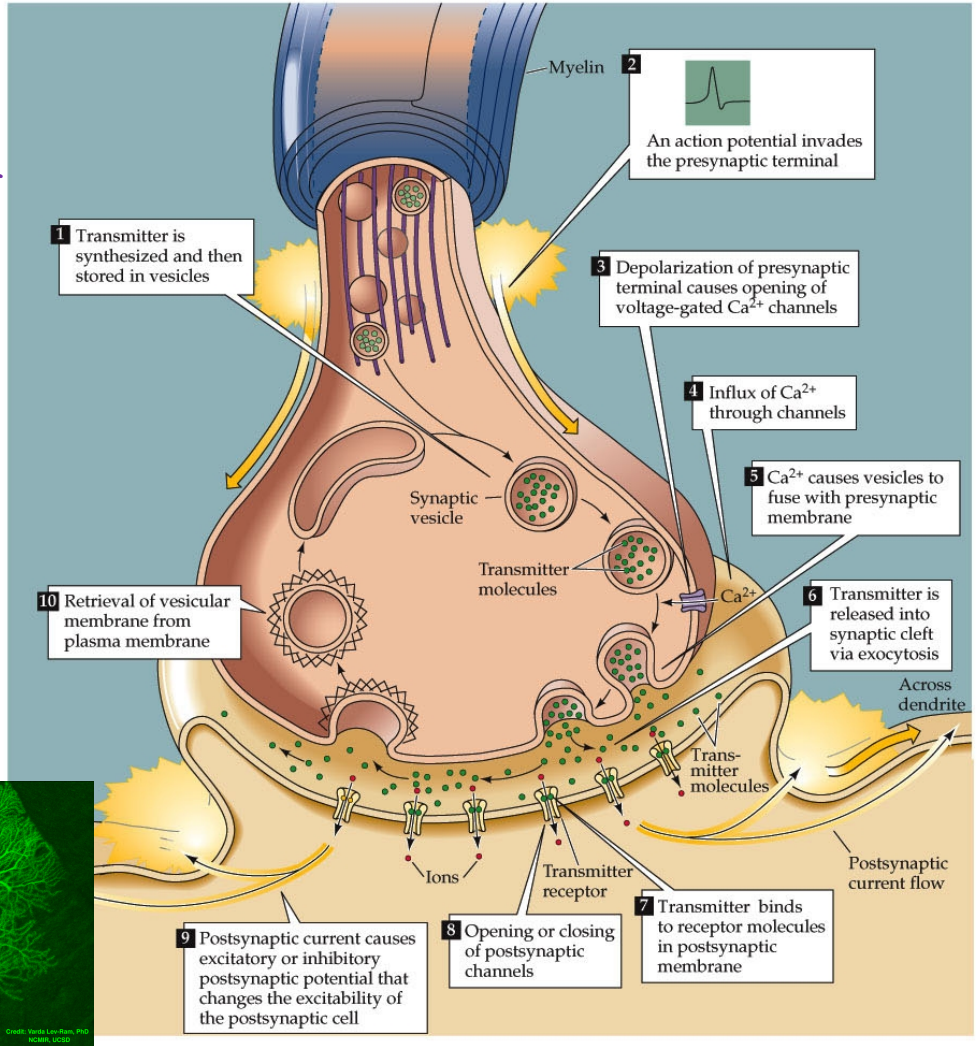
Across dendrite

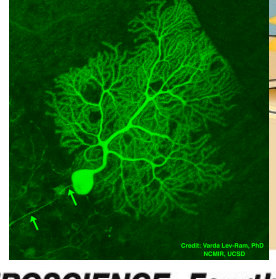
Post-synaptic neuron

Steps in ionotropic chemical synaptic transmission

Today: Release of Transmitter

Weds: Reception of Transmitter

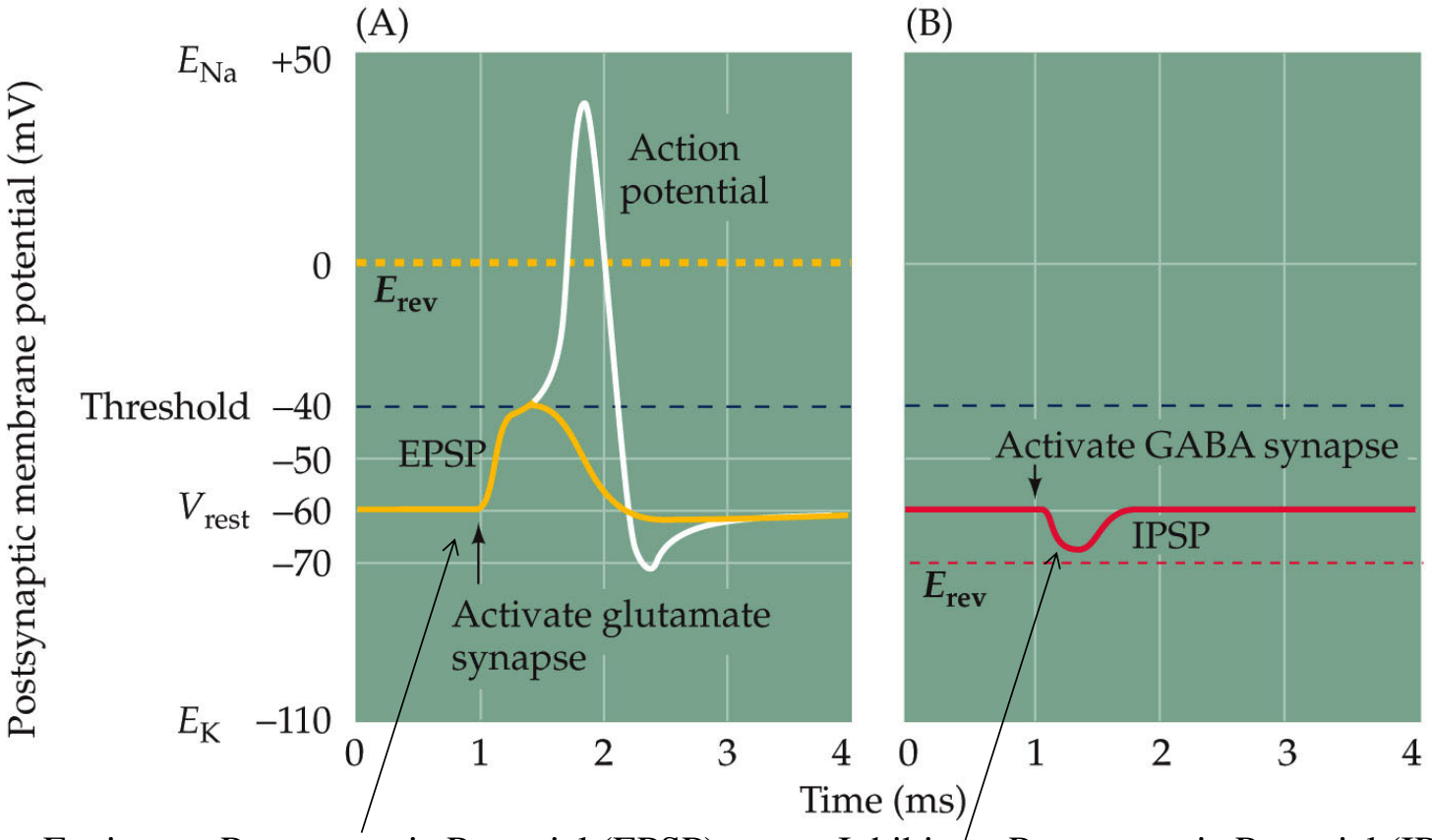




NEUROSCIENCE, Fourth Edition, Figure 5.3

Excitatory synapse: Tries to depolarize cell above threshold for AP

Inhibitory synapse: Tries to keep cell below threshold for AP



Excitatory Post-synaptic Potential (EPSP)

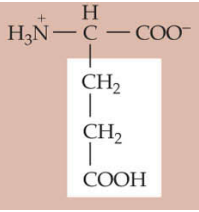
Inhibitory Post-synaptic Potential (IPSP)

Many molecules serve as neuro transmitters and/or neuromodulators.

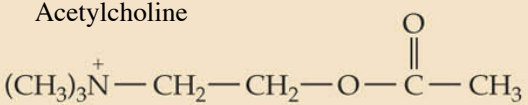
Each one can bind to many different types of receptors

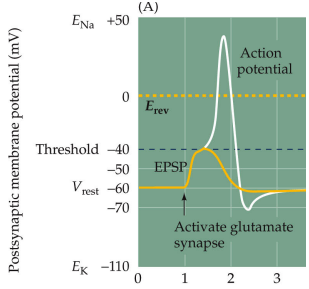
Excitatory

Glutamate



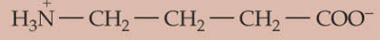
Acetylcholine





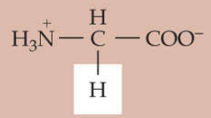
Inhibitory

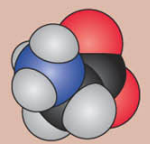
GABA

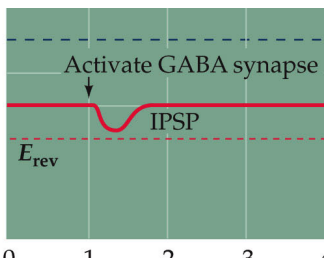


γ-aminobutyric acid

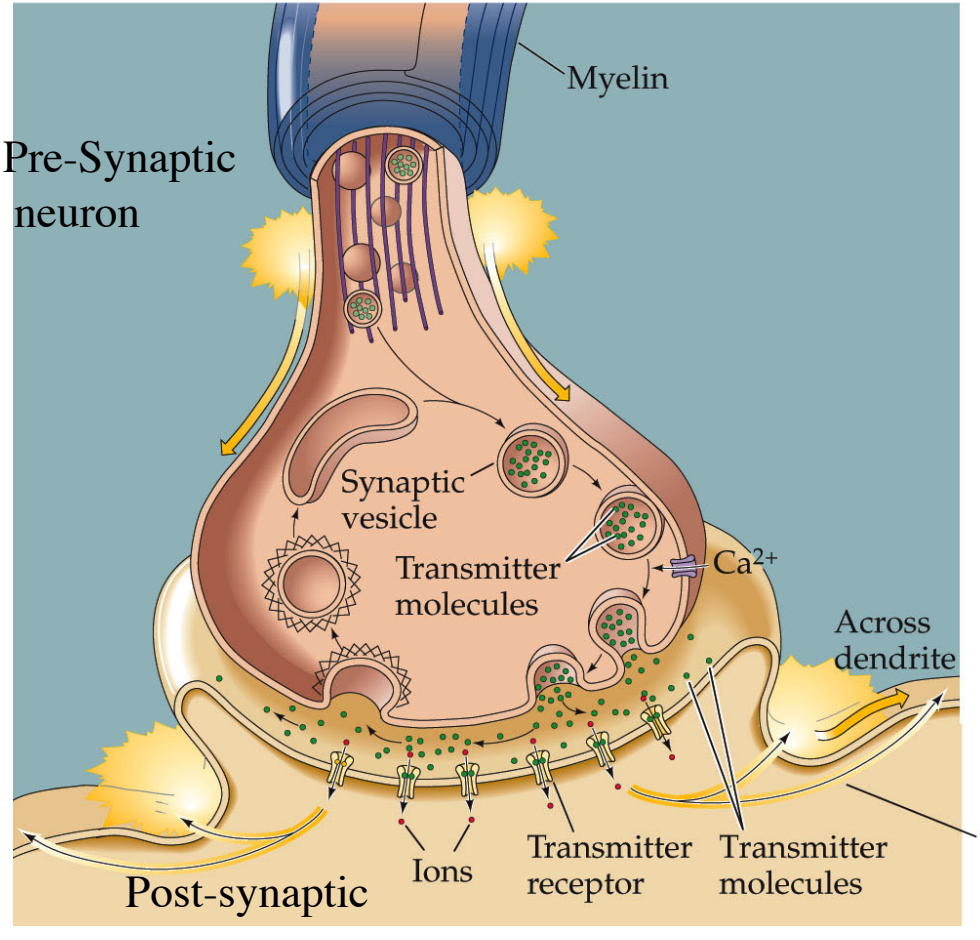
Glycine







Video: Mechanisms of release of neurotransmitter



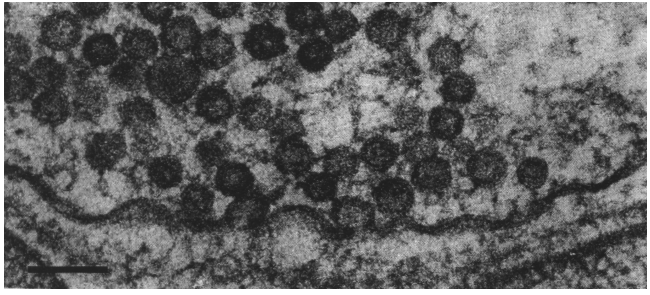
1) Calcium entry is the trigger for release

2) Transmitter is packaged in synaptic vesicles, to form the quanta of release

3) Vesicular exocytosis: the vesicle fuses with the membrane and releases the transmitter into the synaptic cleft

Clicker question

A QUANTUM is:



A. A synaptic vesicle

B. A molecule of neurotransmitter

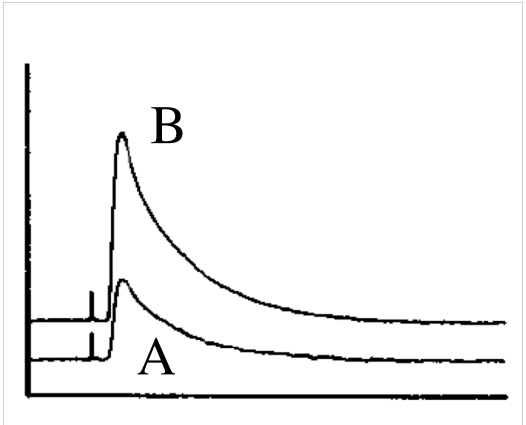
C. The amount of transmitter in a vesicle

D. 0.5 mV in amplitude

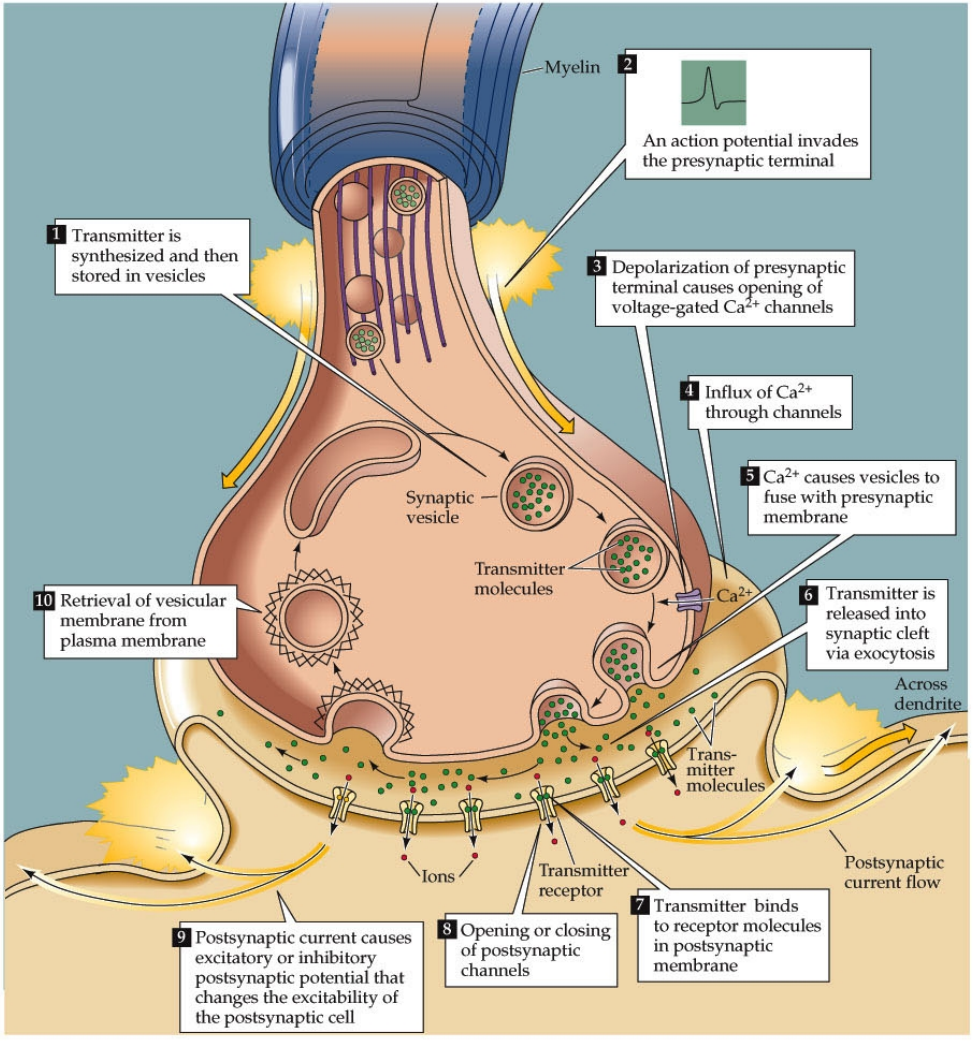
Clicker question

You are studying ACh release from two synapses onto the same post-synaptic neuron, which has an identical concentration of ACh receptors at both synapses. However, when you stimulate an action potential to synapse A, the EPSP is much smaller than response at synapse B. What could differ in the **pre-synaptic terminals A and B**?

A. The density of voltage-activated calcium channels.
B. The size of the readily releasable pool of transmitter
C. The density of voltage-activated potassium channels
D. The density of voltage-activated sodium channels
E. All of the above.



Steps in ionotropic chemical synaptic transmission



***NEUROSCIENCE, Fourth Edition,* Figure 5.3**

Clicker question

You stimulate the pre-synaptic neuron and record a 5 mV EPSP in the post-synaptic neuron. Now you add 50 μM Co^{2+} and stimulate the pre-synaptic neuron again. The EPSP under these conditions will be:

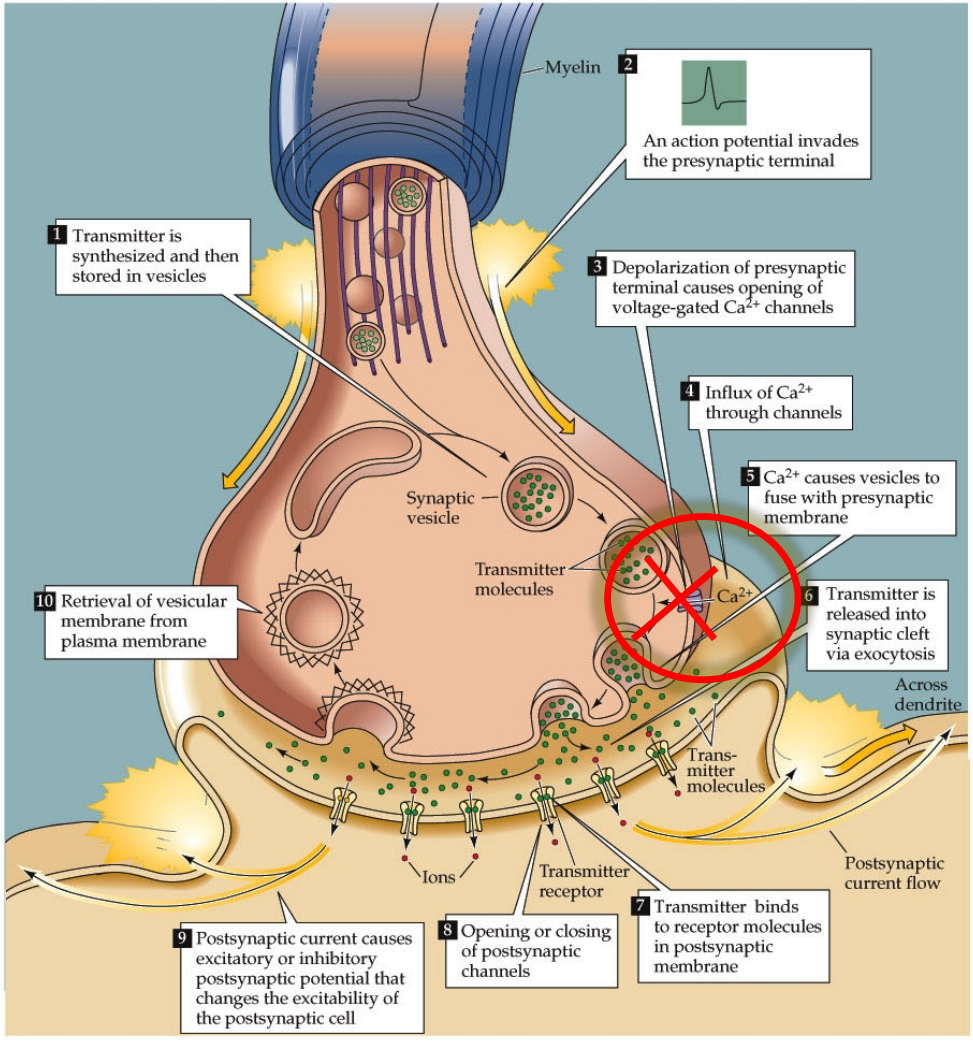
A. Larger

B. Smaller

C. Unchanged

D. Could be either larger or smaller

Steps in ionotropic chemical synaptic transmission



***NEUROSCIENCE, Fourth Edition,* Figure 5.3**

Clicker question:

You have discovered a mutant of the dynamin protein in Drosophila that makes synaptic vesicles twice as large as in wild type controls. What will this do to synaptic transmission?

A. Decrease the amplitude of the EPSPs

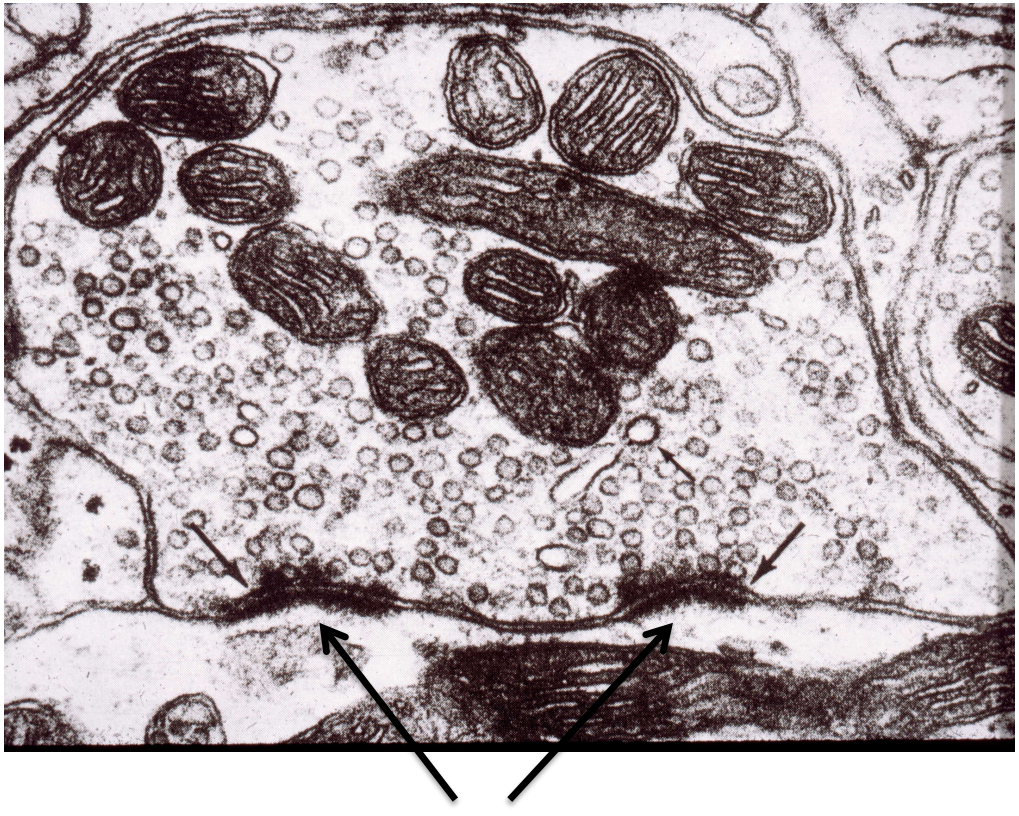
B. Increase the amplitude of the spontaneous m-EPSPs

C. Make the pre-synaptic membrane surface area larger

D. A and C

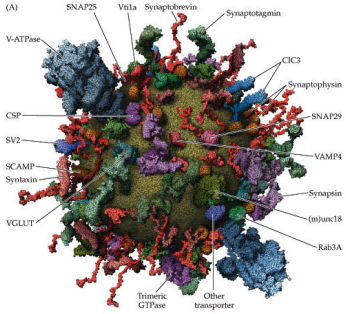
E. It will have no effect.

How does calcium entry trigger vesicle fusion and exocytosis?



Steps leading to release of transmitter

Many, many proteins participate in processes of synaptic transmission



Reserve pool of vesicles

Transport

Endosome

Hsc 70
Auxilin
Synaptojanin

Dynamin

Uncoating

Budding

Synapsin

Budding

Endocytosis

Docking

Readily releasable pool of vesicles

Priming

Fusion

Clathrin

Rab3A, Complexin

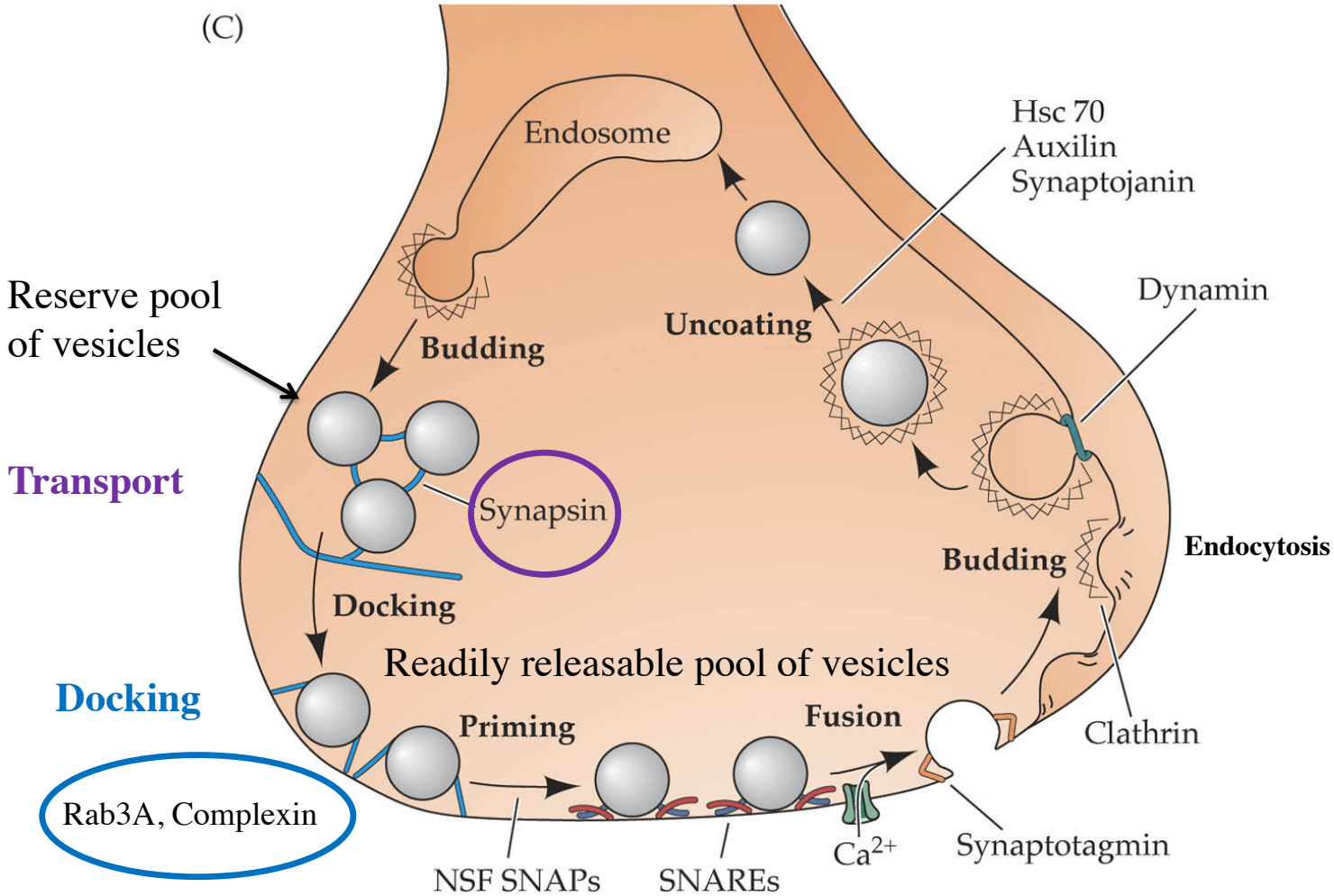
NSF SNAPs

SNAREs

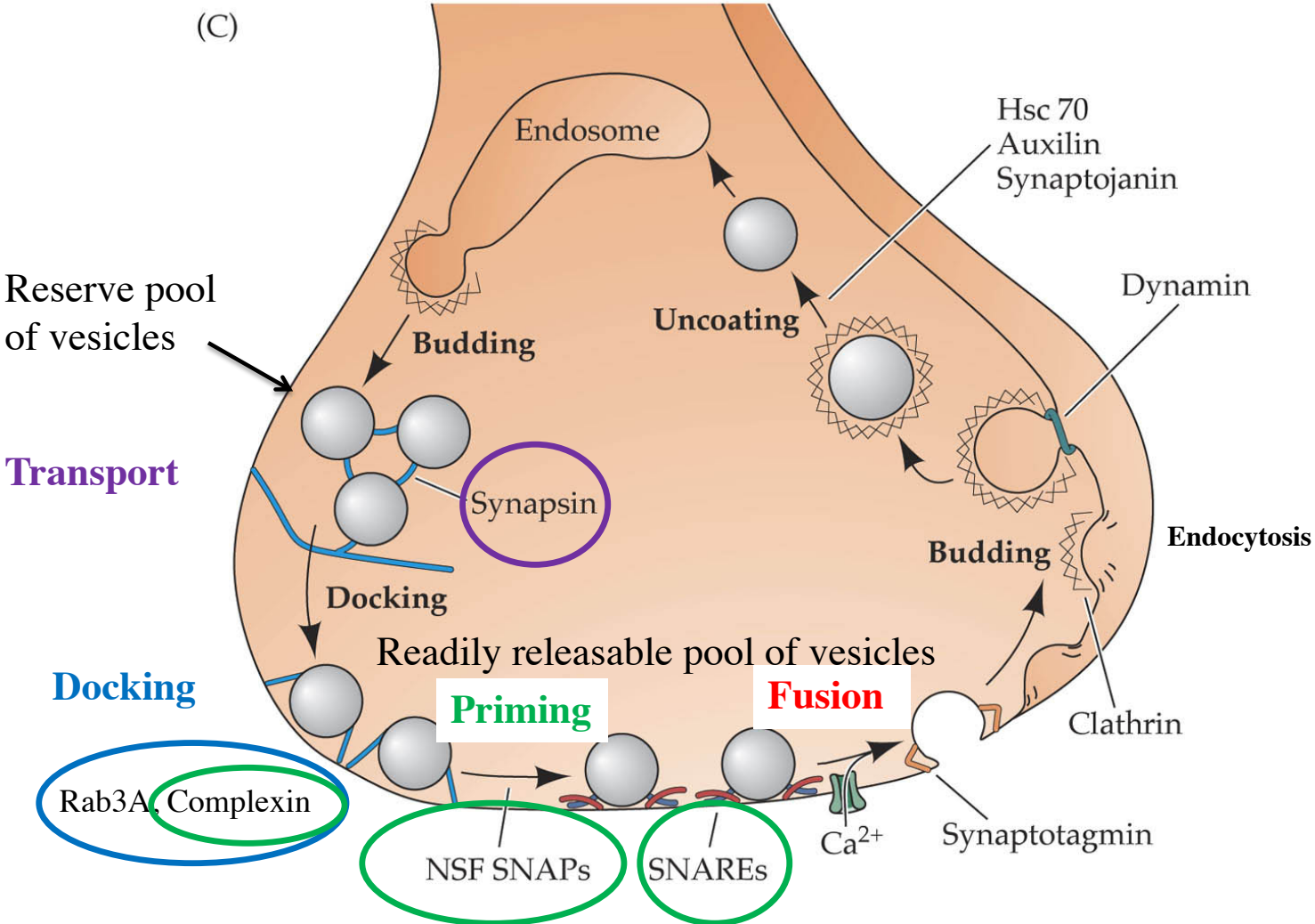
Ca^{2+}

Synaptotagmin

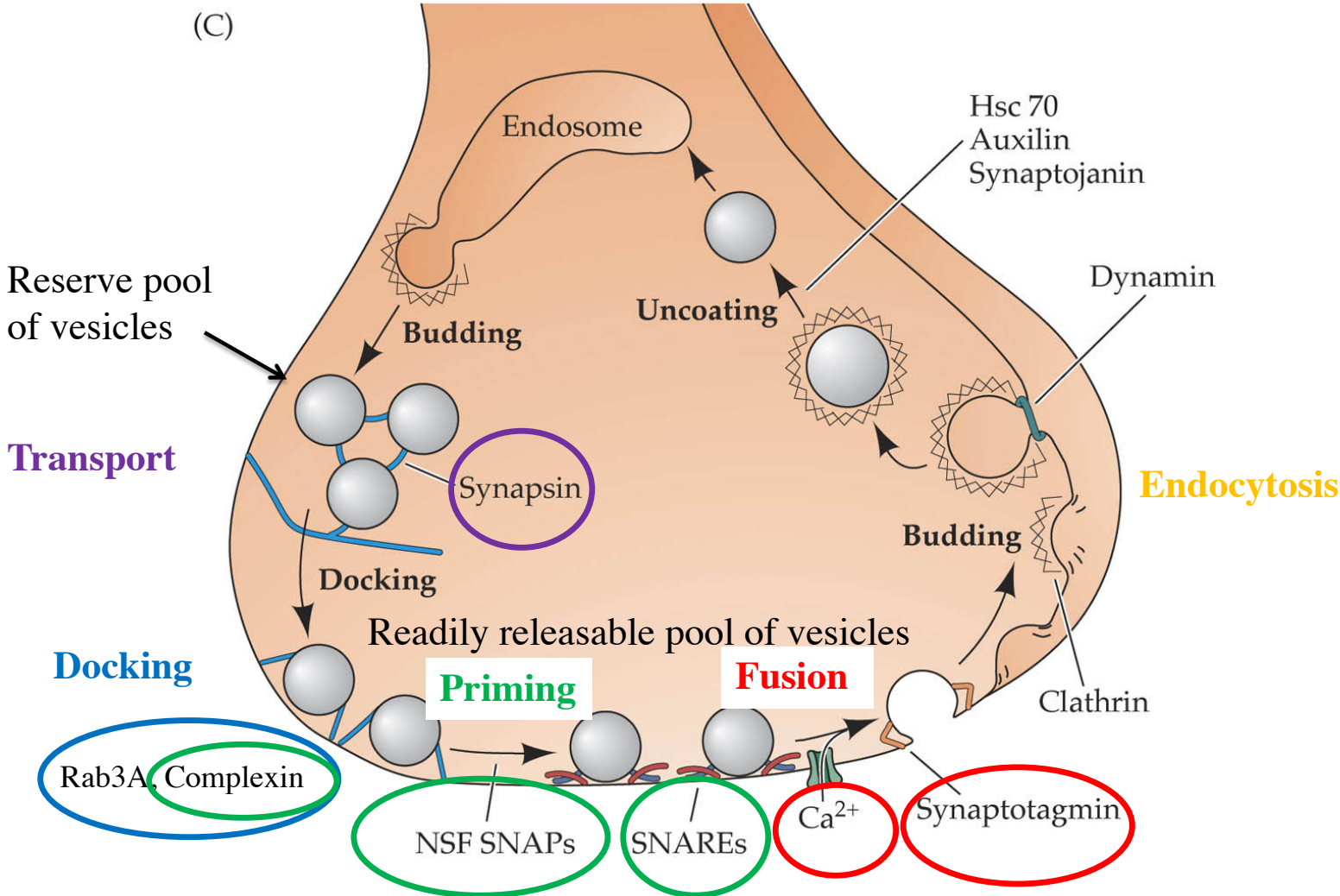
Steps leading to release of transmitter



Steps leading to release of transmitter

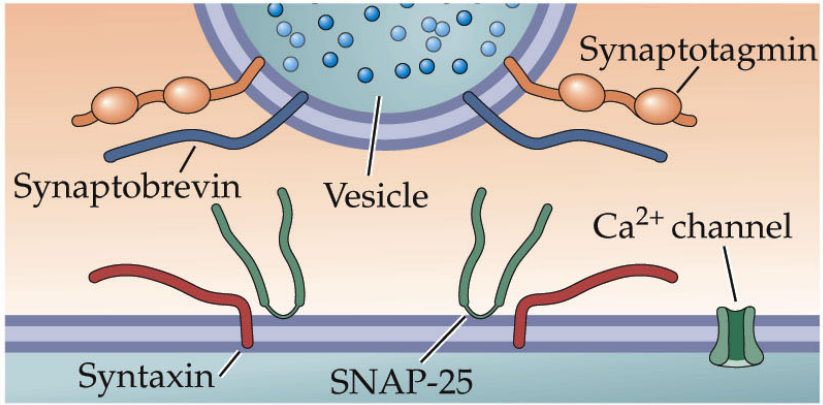


Steps leading to release of transmitter

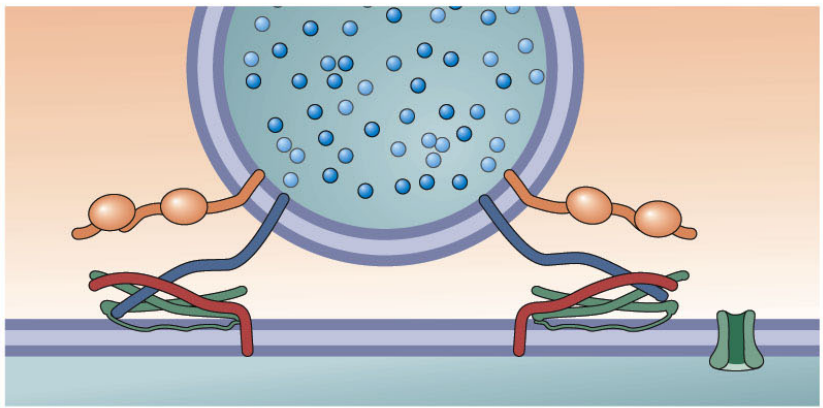


SNARE complex prepares vesicles for fusion when calcium enters

(B) (1) Vesicle docks



(2) SNARE complexes form to pull membranes together to prime the vesicles for release

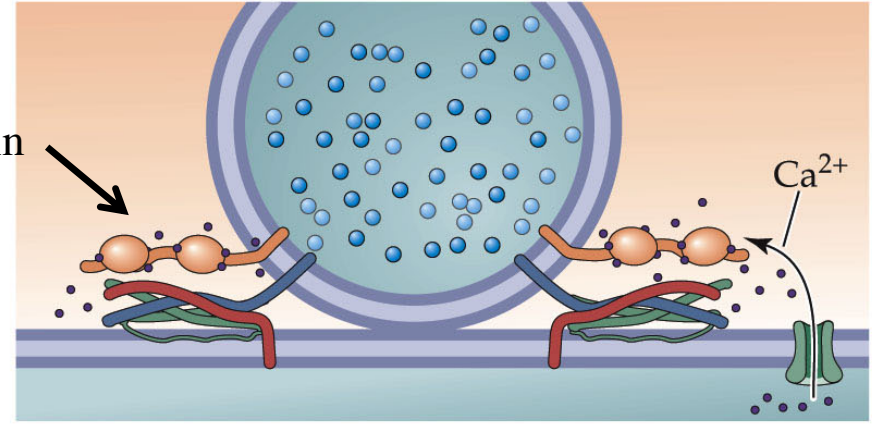


***NEUROSCIENCE, Fourth Edition,* Figure 5.14 (Part 2)**

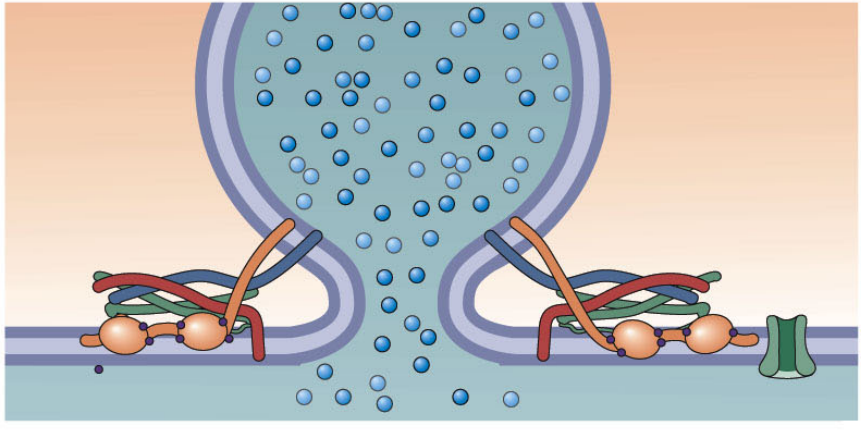
Vesicle fusion and transmitter release

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

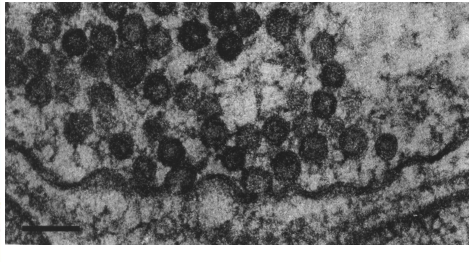
Synaptotagmin



(4) Ca^{2+}-bound synaptotagmin catalyzes membrane fusion

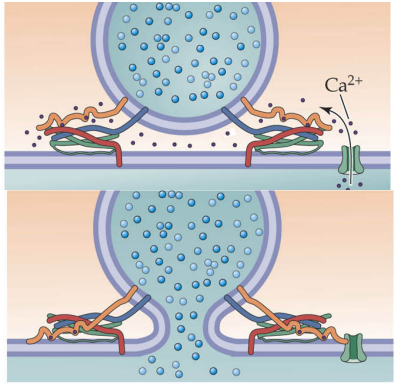


200 microS!



EUROSCIENCE, *Fourth Edition*, Figure 5.14 (Part 3)

Vesicle fusion and transmitter release



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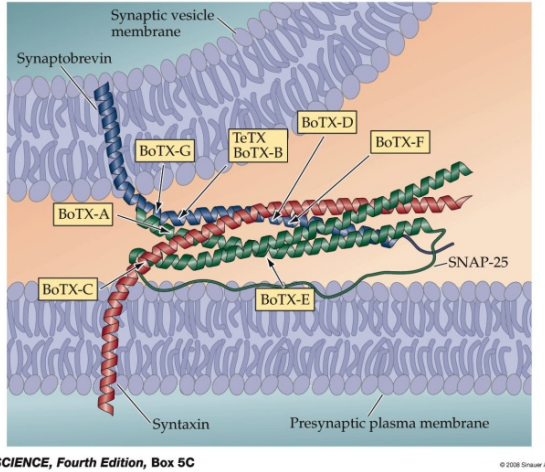
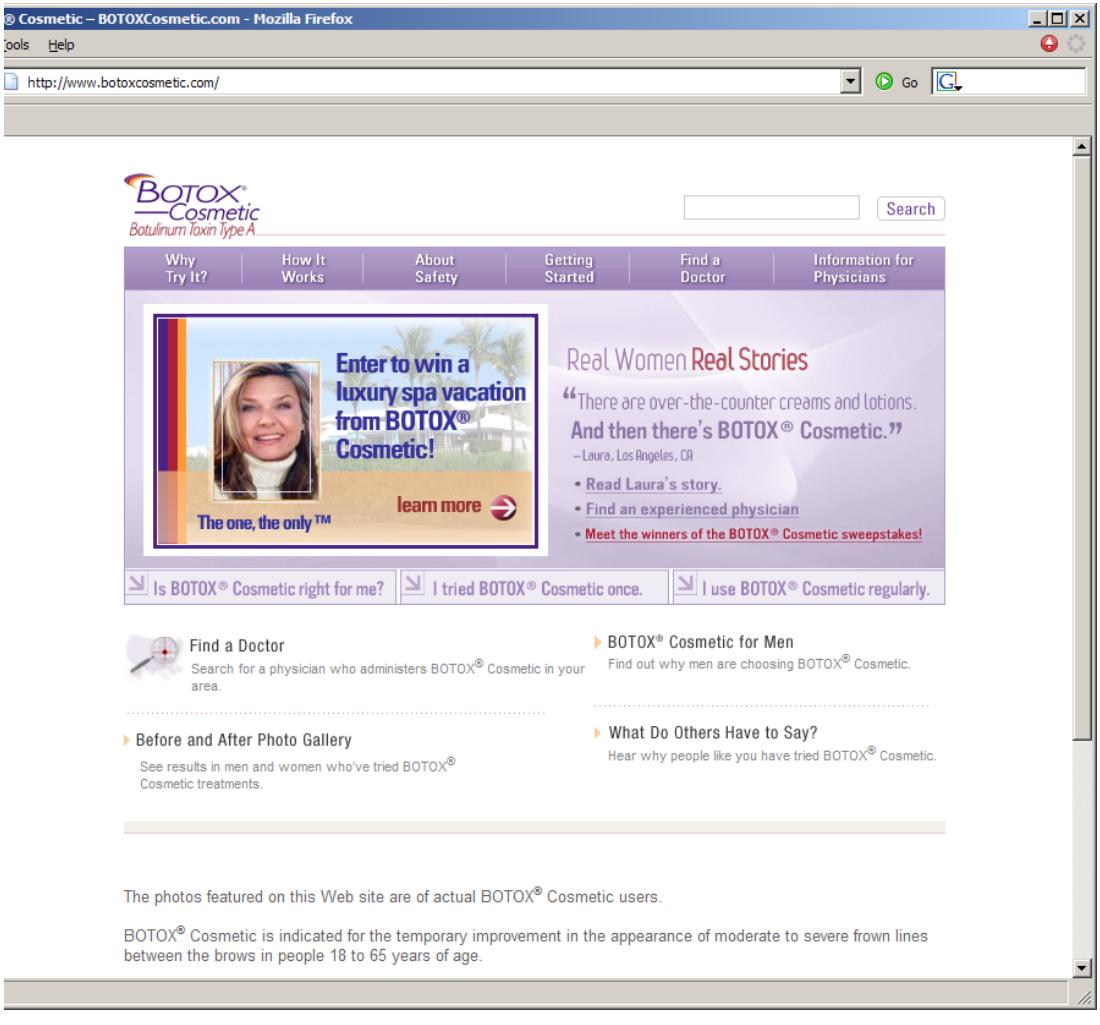


Sites of toxin action

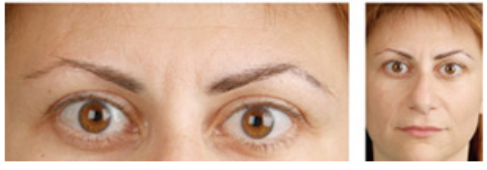
NEUROSCIENCE, Fourth Edition, Box 5C

Botox as a Cosmetic



Botox Testimonials

Before



After

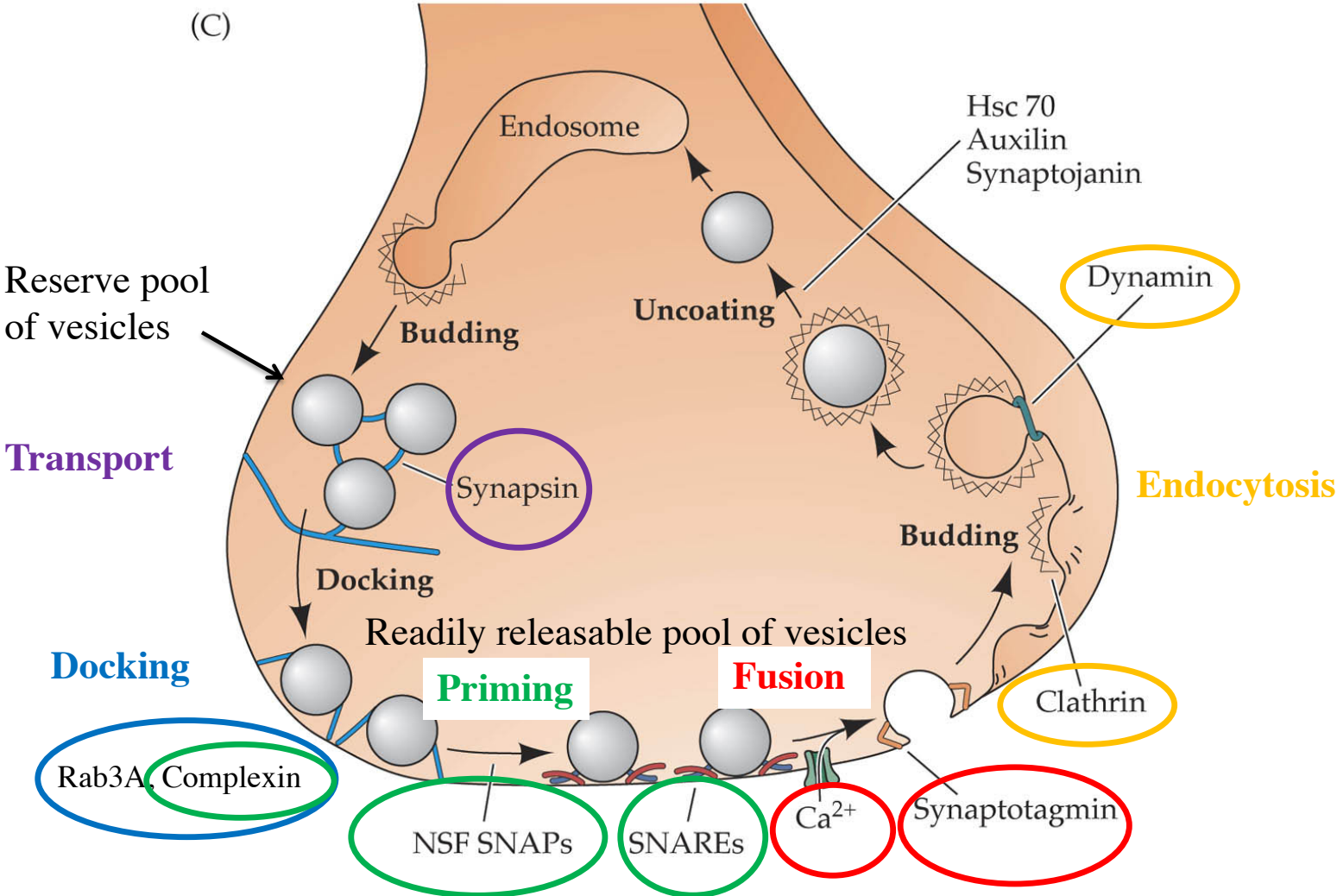
Results may vary.

Before

After

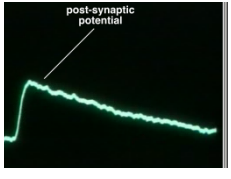
Results may vary.

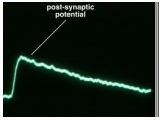
Steps leading to release of transmitter

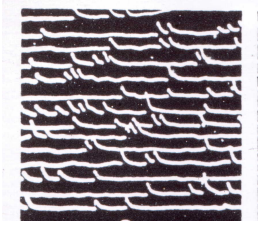


Lambert-Eaton Syndrome as a transmitter release disease
Determining site of action of a synaptic change

https://www.youtube.com/watch?v=grGxcKQypVY







MUSCULAR WEAKNESS — with → MUSCLE CONTRACTIONS NORMAL

MUSCULAR WEAKNESS — symptom → PATIENT 2

PATIENT 2 — physiology shows → REDUCED EPP BUT NOT MEPP AMP

REDUCED EPP BUT NOT MEPP AMP — and also → MEPP FREQUENCY DOWN

REDUCED EPP BUT NOT MEPP AMP — indicates → PRESYNAPTIC PROBLEM

PRESYNAPTIC PROBLEM — determine → QUANTAL CONTENT REDUCED

QUANTAL CONTENT REDUCED — determine by → M = EPP/MEPP

M = EPP/MEPP — or → M = LOGE (N/NF)

M = LOGE (N/NF) — or → Q = (SD2)/EPP THEN M = EPP/Q

QUANTAL CONTENT REDUCED — could be → AP ALTERED

AP ALTERED — with → REDUCED AMPLITUDE — check → NA CURRENT, CHANNELS

AP ALTERED — with → SHORTER DURATION — check → NA CURRENT, CHANNELS

SHORTER DURATION — check → K CURRENT, CHANNELS

QUANTAL CONTENT REDUCED — could be → CA CHANNELS BLOCKED

CA CHANNELS BLOCKED — determine by → CA IMAGING

CA CHANNELS BLOCKED — determine by → CA CURRENT, CHANNELS

Conclusions

- The action potential's depolarization opens voltage-dependent calcium channels which trigger fusion and exocytosis from vesicles

- Calcium interacts with many proteins to cause fusion, with a final interaction between the calcium sensor, synaptotagmin and the SNARE complex that holds the vesicle near to the membrane

Clicker question

A simple form of learning in the marine invertebrate *Aplysia* results from a decrease in potassium channel function at a pre-synaptic nerve terminal. What effect will this have on the amplitude of the spike-evoked EPSP?

A. It will become larger

B. It will become smaller

C. It will not change

D. It will become "pixelated" as you can see the contribution of each m-EPSP on top of the previous one.