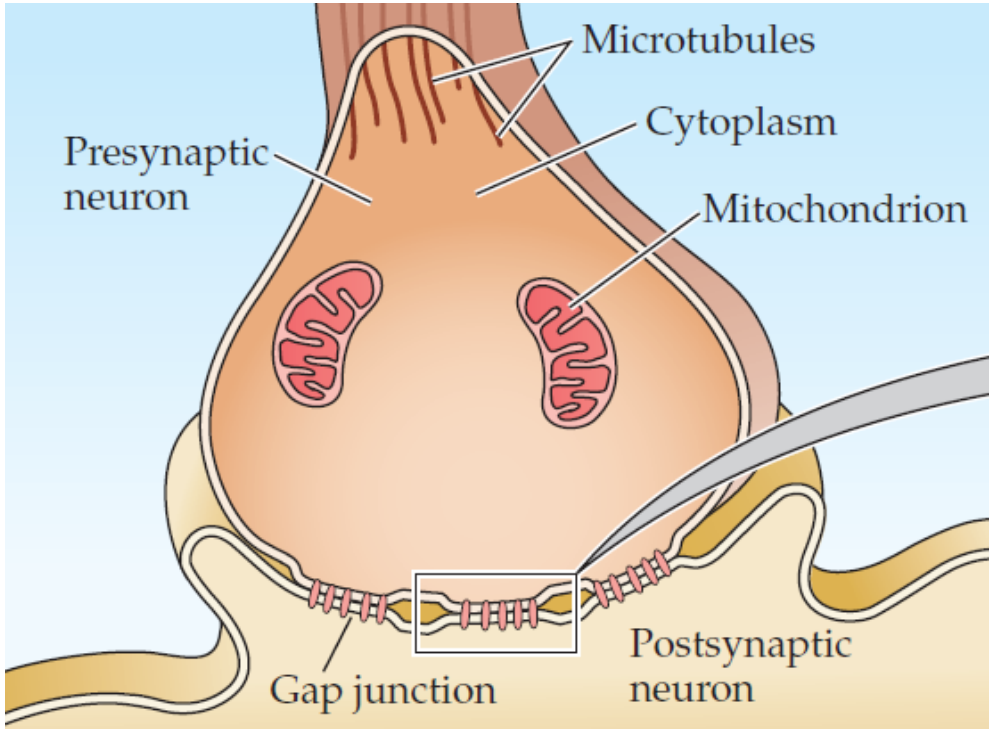
Part 3, Neural Signaling

3.3. Synaptic transmission

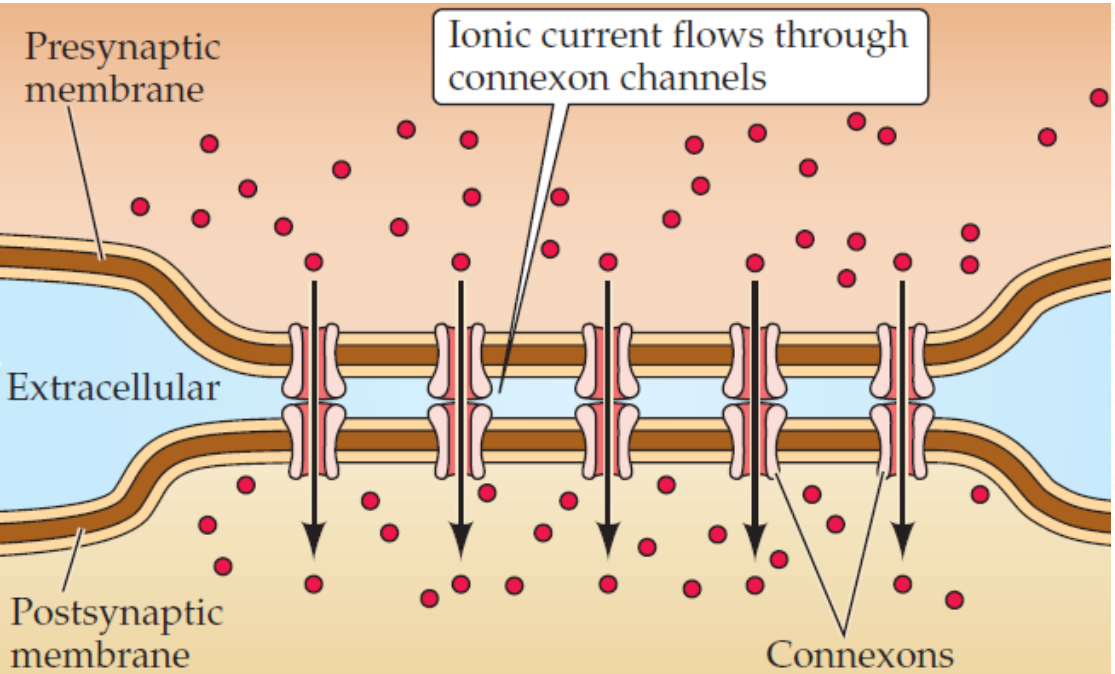
Electrical synapses

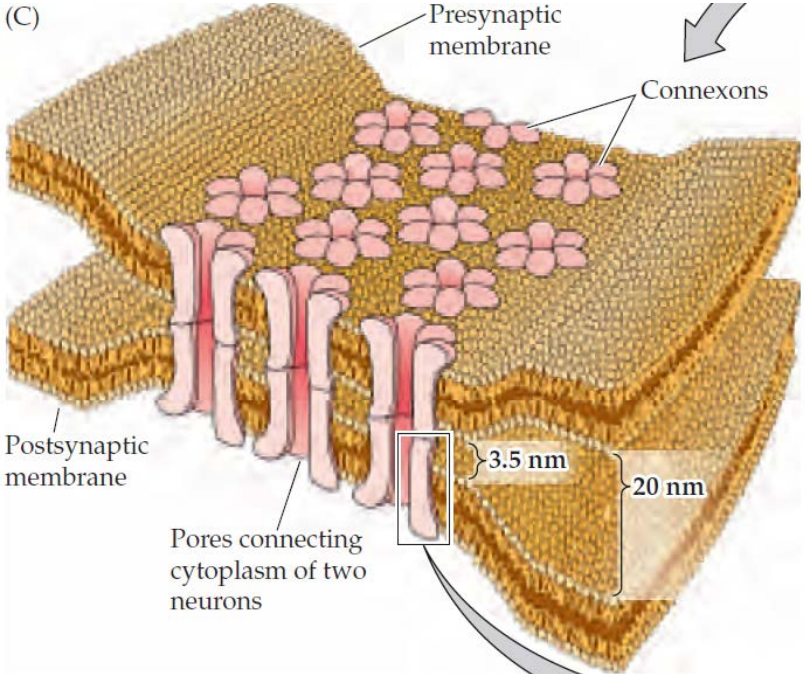
- The many kinds of synapses within the human brain fall into two general classes: **electrical synapses** and **chemical synapses**.
- Although they are a distinct minority, electrical synapses are found in all nervous systems and permit direct, passive flow of electrical current from one neuron to another.
- The membranes of the two communicating neurons (**presynaptic** and **postsynaptic**) come extremely close at the synapse and are actually linked together by an intercellular specialization called a **gap junction**.



Structure of electrical synapses

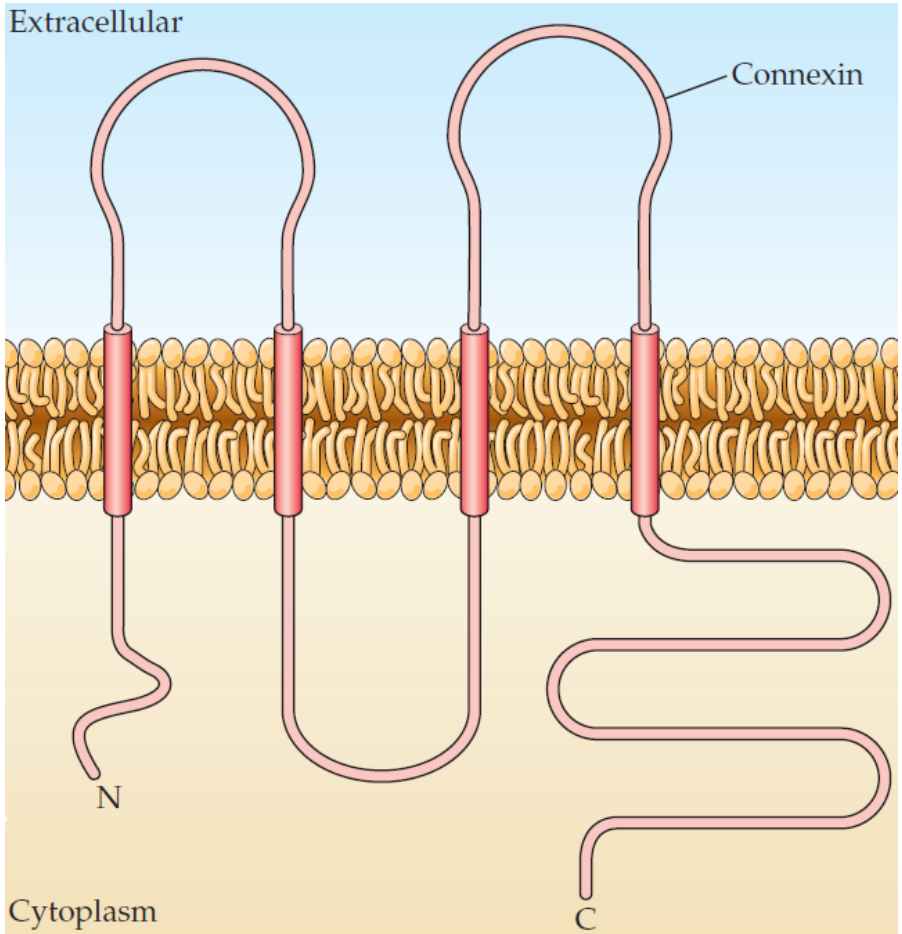
- Gap junctions contain precisely aligned, paired channels called **connexons**.
  - Connexons are present in the membranes of both the pre- and postsynaptic neurons.
  - Six presynaptic **connexins** align with six postsynaptic connexins to form a pore that connects the two cells.
  - The pore of a connexon channel is much larger than the pores of the voltage-gated, which permits ATP and other important intracellular metabolites, such as second messengers, to be transferred between neurons.





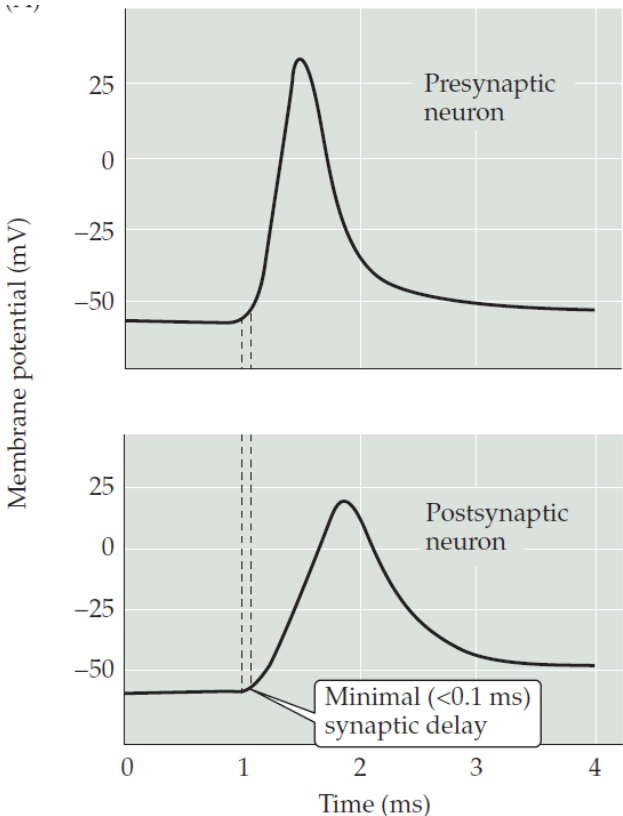
Structure of electrical synapses

- Connexons are composed of a special family of ion channel proteins, the **connexins**.
- There are several different types of connexins, found in different cell types and yielding gap junctions with diverse physiological properties.



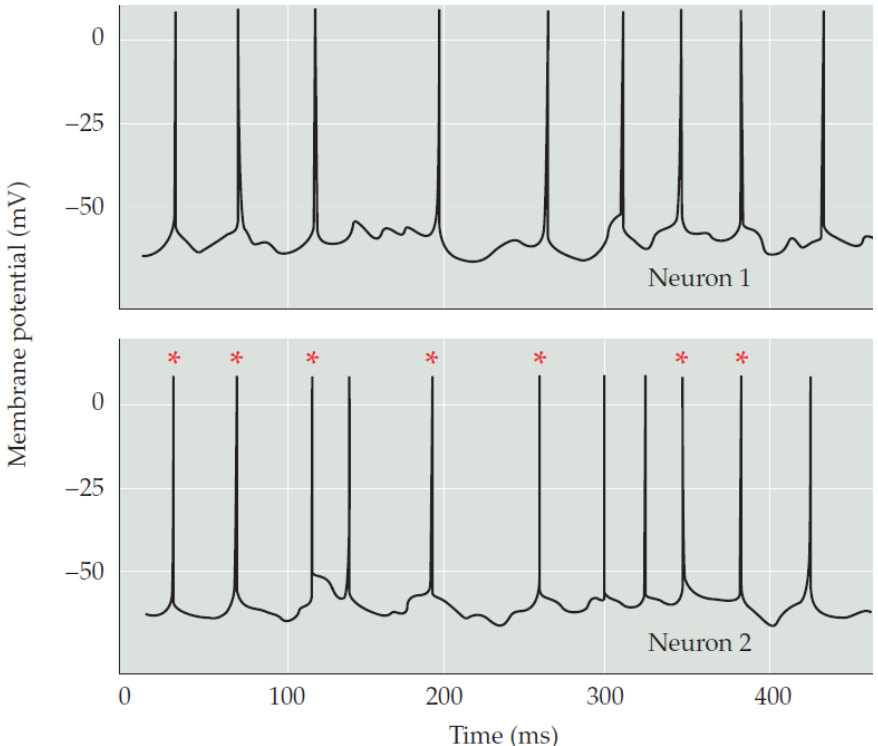
Function of electrical synapses

- Electrical synapses work by allowing ionic current to flow passively through the gap junction pores from one neuron to another.
- The usual source of this current is the potential difference generated locally by the presynaptic action potential.
- This arrangement has a number of interesting consequences:
  - transmission can be bidirectional; that is, current can flow in either direction across the gap junction, depending on which member of the coupled pair is invaded by an action.
  - transmission is extraordinarily fast: because passive current flow across the gap junction is virtually instantaneous, communication can occur without the delay that is characteristic of chemical synapses.
    - These features are apparent in the operation of the first electrical synapse to be discovered, which resides in the crayfish nervous system.
    - A postsynaptic electrical signal is observed at this synapse within a fraction of a millisecond after the generation of a presynaptic action potential.
    - Such synapses interconnect many of the neurons within the circuit that allows the crayfish to escape from its predators, thus minimizing the time between the presence of a threatening stimulus and a potentially lifesaving motor response.



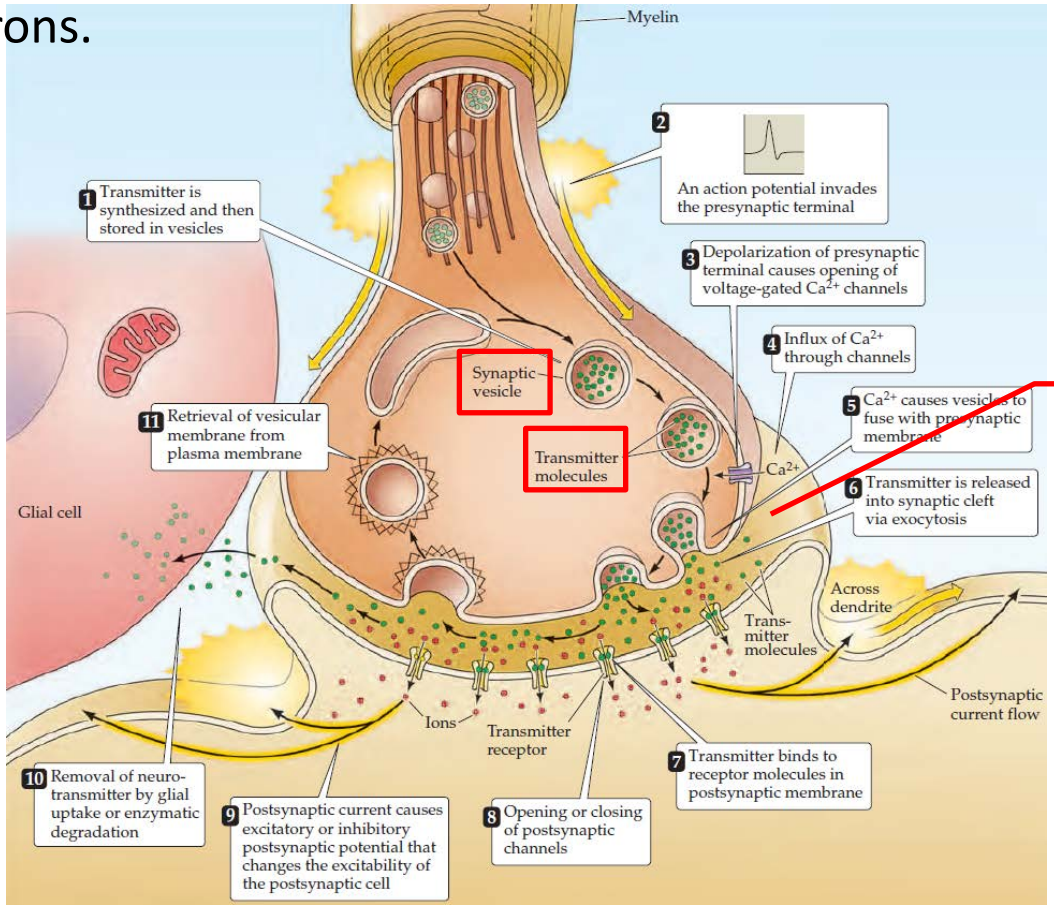
Function of electrical synapses

- A more general purpose of electrical synapses is to synchronize electrical activity among populations of neurons.
  - the brainstem neurons that generate rhythmic electrical activity underlying breathing are synchronized by electrical synapses.
  - Electrical synapses allow synchronization of electrical activity in hippocampal interneurons.
  - Electrical transmission between certain hormone-secreting neurons within the hypothalamus ensures that all cells fire action potentials at about the same time, thus facilitating a burst of hormone secretion into the circulation.

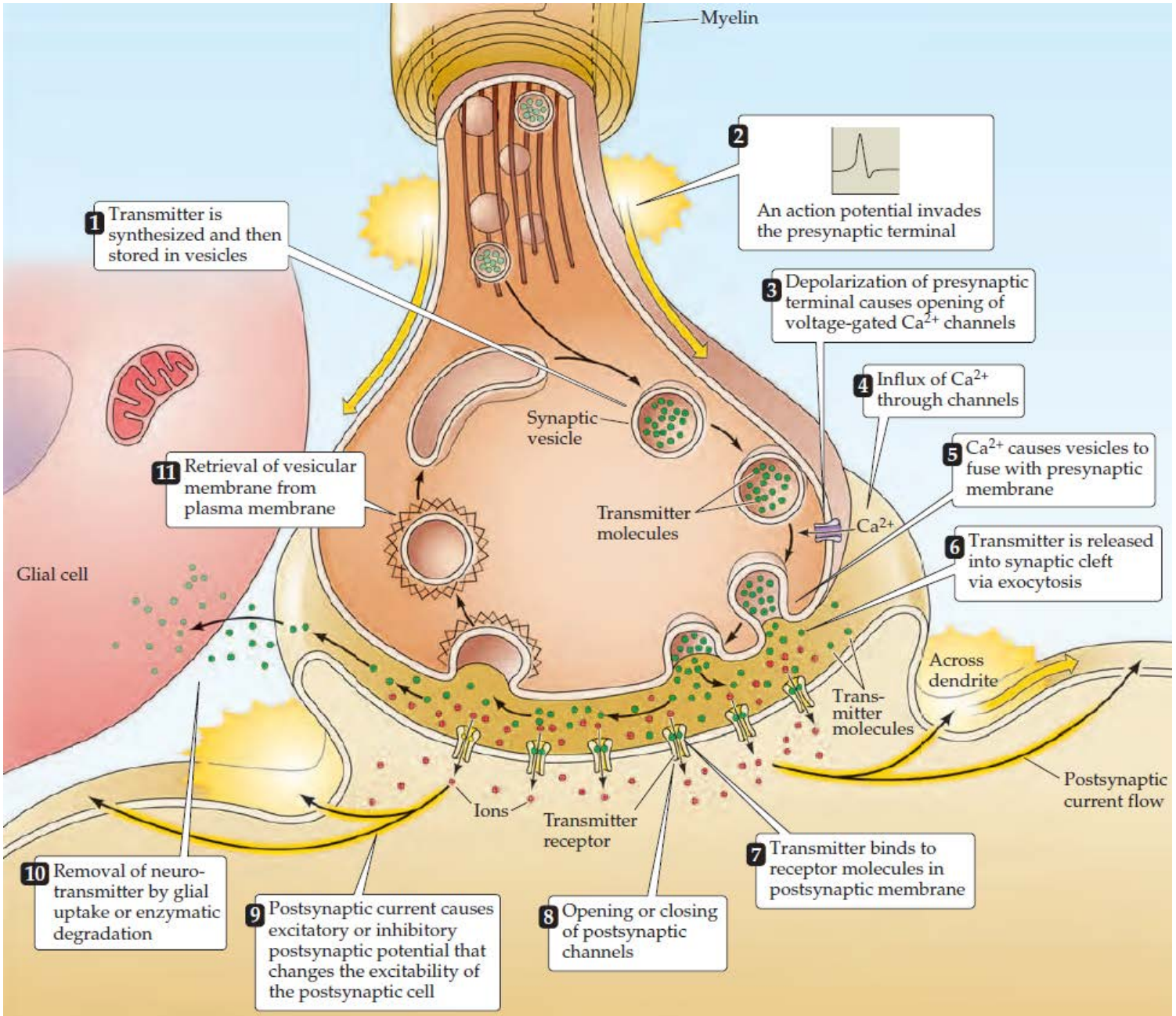


Chemical synapses

- In a **chemical synapse**, the space between the pre- and postsynaptic neurons is substantially greater than at electrical synapses and is called the **synaptic cleft**.
- The key feature of all chemical synapses is the presence of small, membrane-bounded organelles called **synaptic vesicles** within the presynaptic terminal.
- The synaptic vesicles are filled with one or more **neurotransmitters**, which are secreted from the presynaptic neuron, and act as messengers between the communicating neurons.

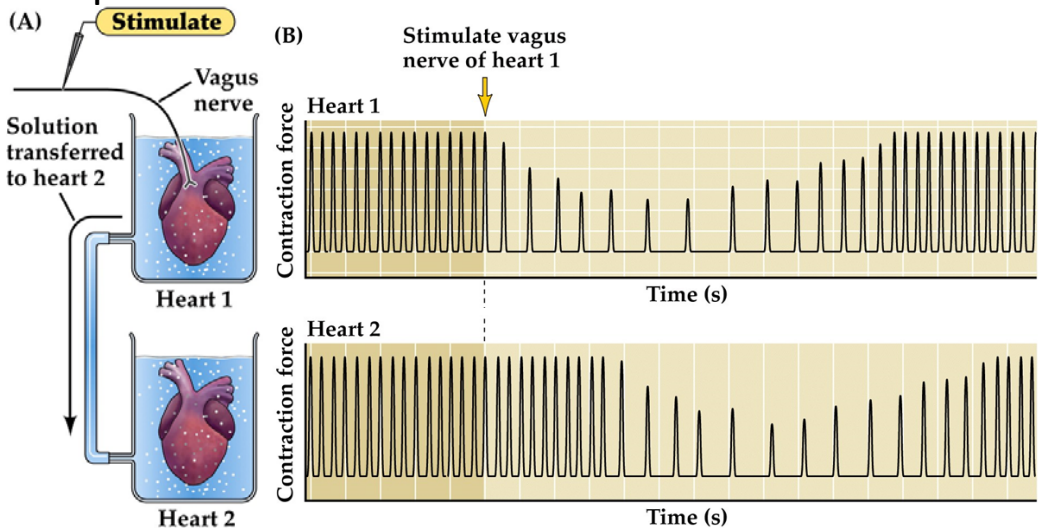


Signal transmission at chemical synapses



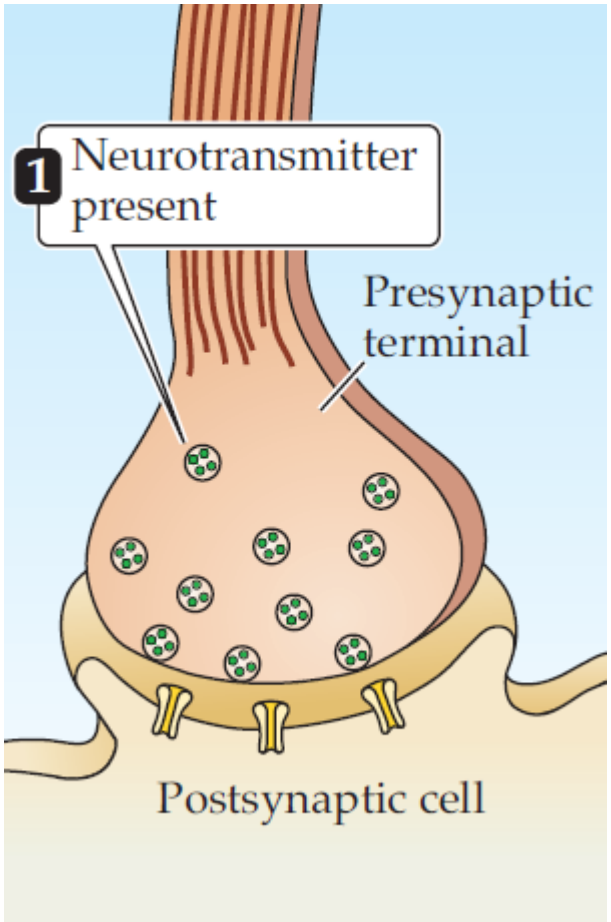
Chemical neurotransmission

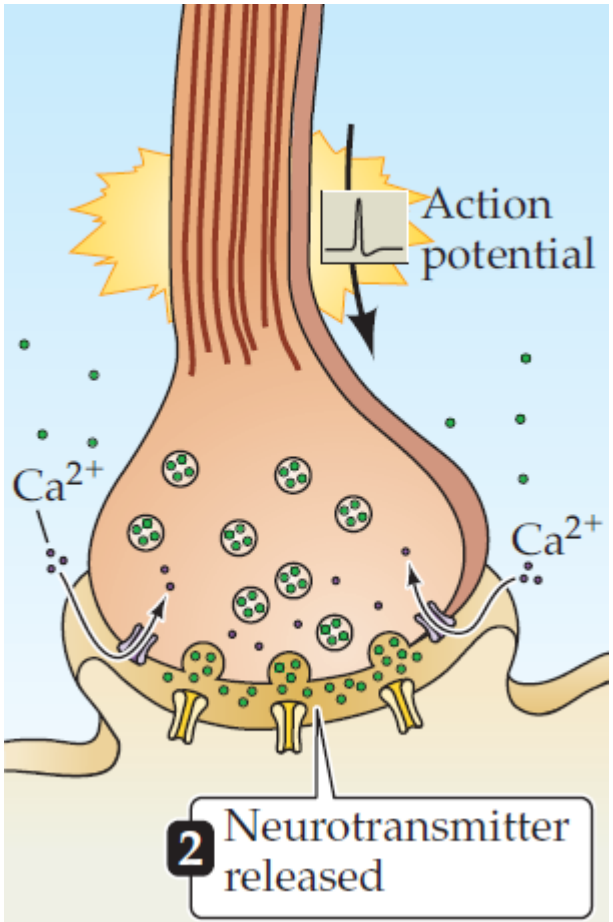
- Can electrical information be transferred from one neuron to the next by means of chemical signaling?
- In 1926, the German physiologist **Otto Loewi** performed a key experiment that supported this idea, which earned for him the Nobel Prize in Physiology or Medicine in 1936.
- According to Loewi, the idea for his key experiment came to him in his sleep.
- Loewi proved that electrical stimulation of the vagus nerve slows the heartbeat by releasing a chemical signal.
  - He isolated and perfused the hearts of two frogs, monitoring their beating.
  - When the vagus nerve innervating the first heart was stimulated, its beating slowed.
  - Remarkably, even though the vagus nerve of the second heart had not been stimulated, its beat also slowed when exposed to the perfusate from the first heart.
- The vagus nerve regulates the heart rate by releasing a chemical that accumulates in the perfusate: "vagus substance," later shown to be **acetylcholine** (ACh)--a neurotransmitter.

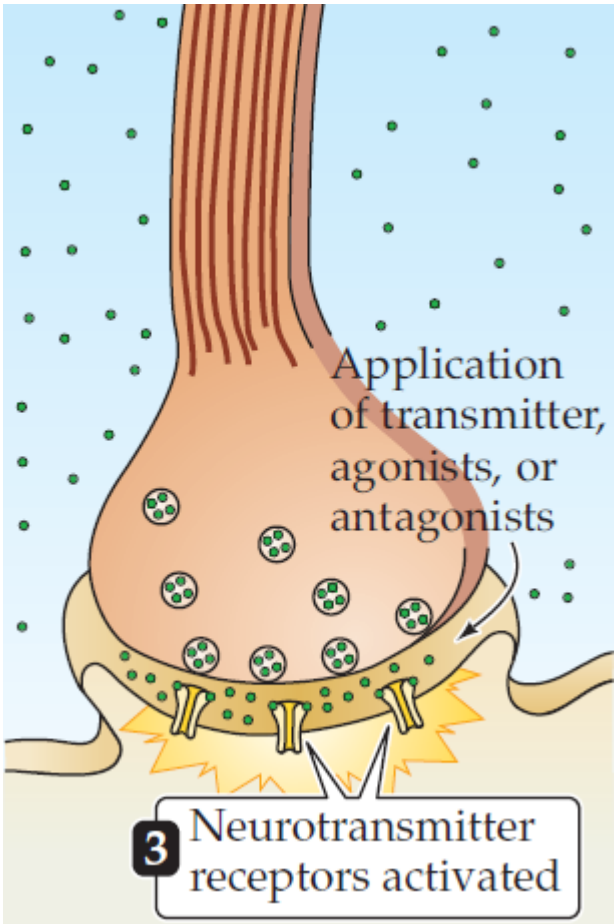


Criteria that define a neurotransmitter

1. The substance must be present within the presynaptic neuron.
2. The substance must be released in response to presynaptic depolarization, and the release must be Ca^{2+}-dependent.
3. Specific receptors for the substance must be present on the postsynaptic cell.







Neurotransmitter

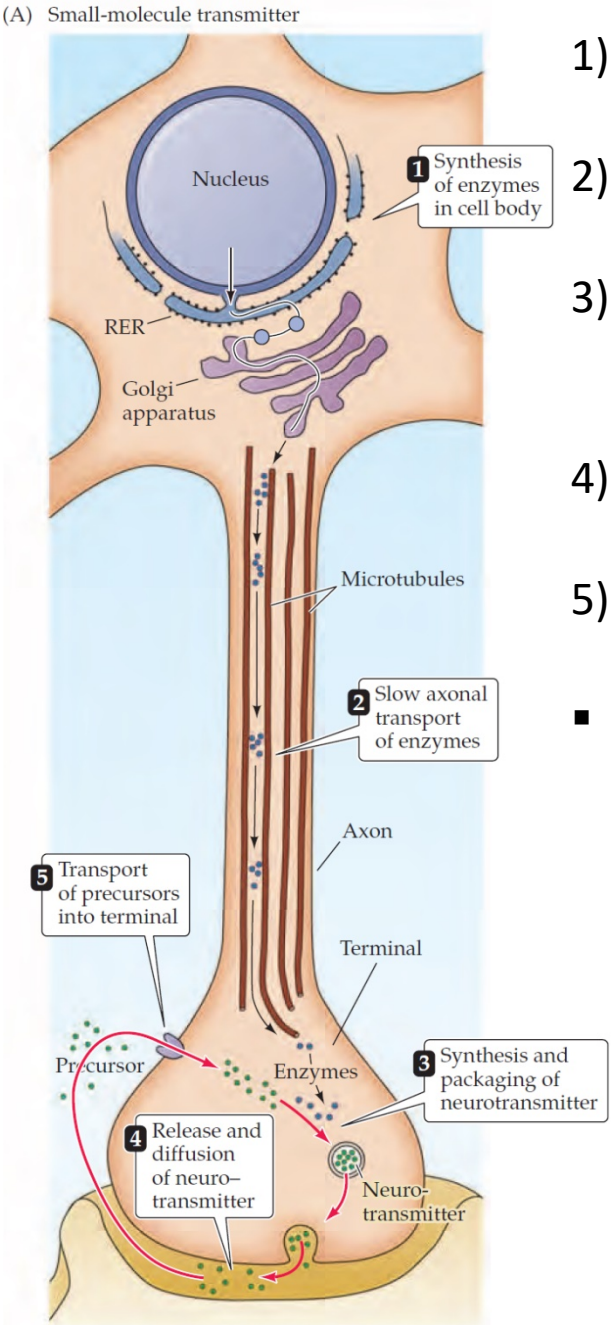
- These have led to the identification of more than 100 different neurotransmitters, which can be classified into two broad categories: **small-molecule neurotransmitters** and **neuropeptides**.

- In general, small-molecule neurotransmitters mediate rapid synaptic actions, whereas neuropeptides tend to modulate slower, ongoing synaptic functions.

- Until relatively recently, it was believed that a given neuron produced only a single type of neurotransmitter.

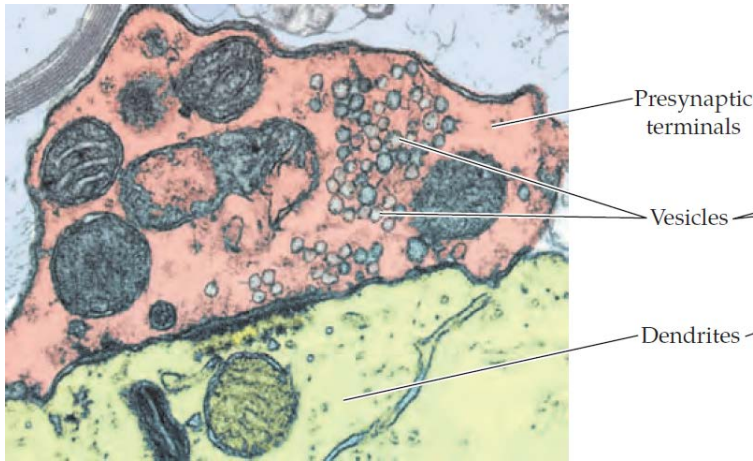
- It is now clear, however, that many types of neurons synthesize and release two or more different neurotransmitters--**co-transmitters**.

Metabolism of small-molecule transmitters

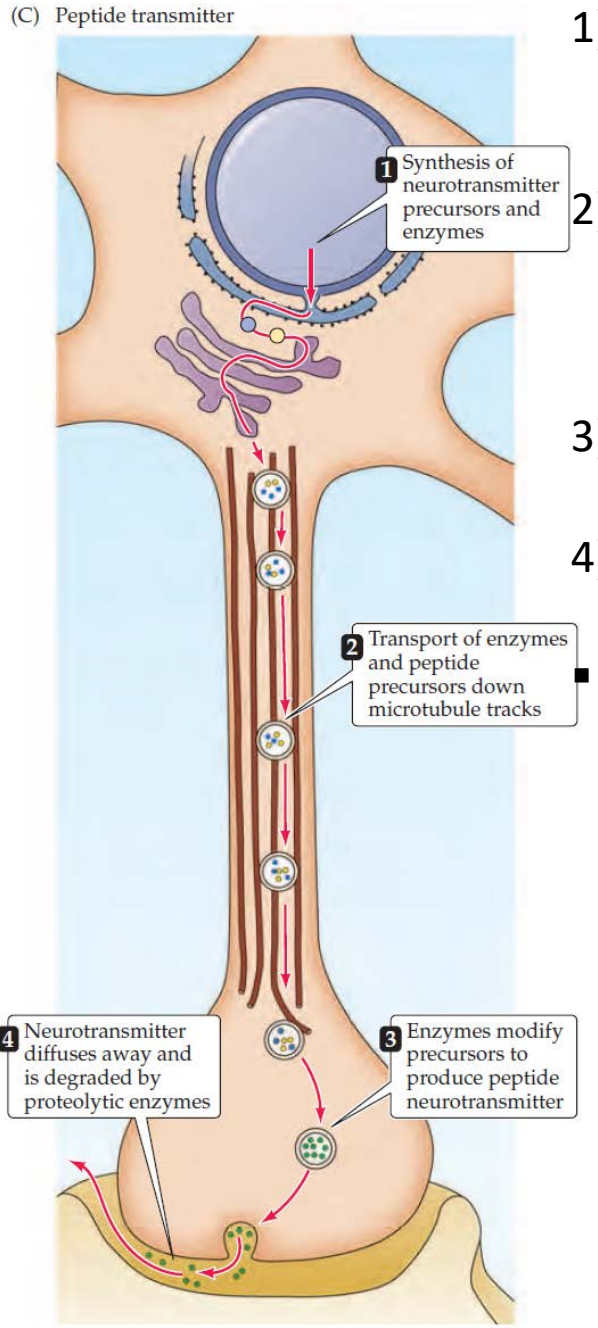


1) The enzymes necessary for neurotransmitter synthesis are made in the cell body of the presynaptic cell.
2) The enzymes are transported down the axon by **slow axonal transport** (0.5-5.0 mm/day).
3) Precursors are taken up into the terminals by specific transporters, and neurotransmitter synthesis and packaging take place within the nerve endings.
4) After vesicle fusion and release, the neurotransmitter may be enzymatically degraded.
5) The reuptake of the neurotransmitter (or its metabolites) begins another cycle of synthesis, packaging, release, and removal.

- Most small-molecule neurotransmitters are packaged in vesicles 40–60 nm in diameter, the centers of which appear clear in electron micrographs; accordingly these vesicles are referred to as **small clear-core vesicles**.

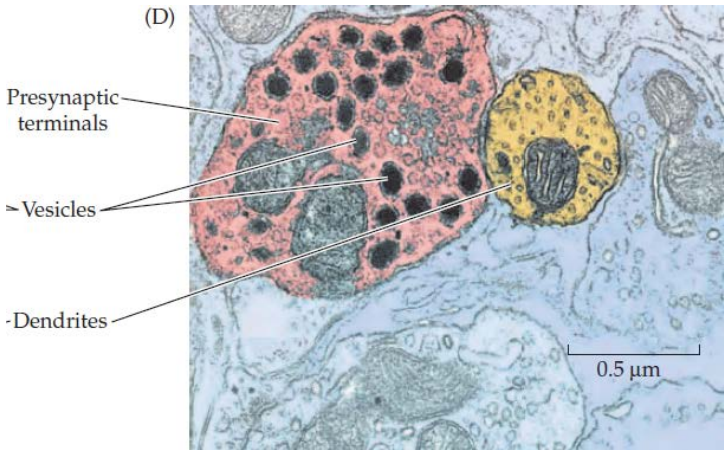


Metabolism of peptide transmitters



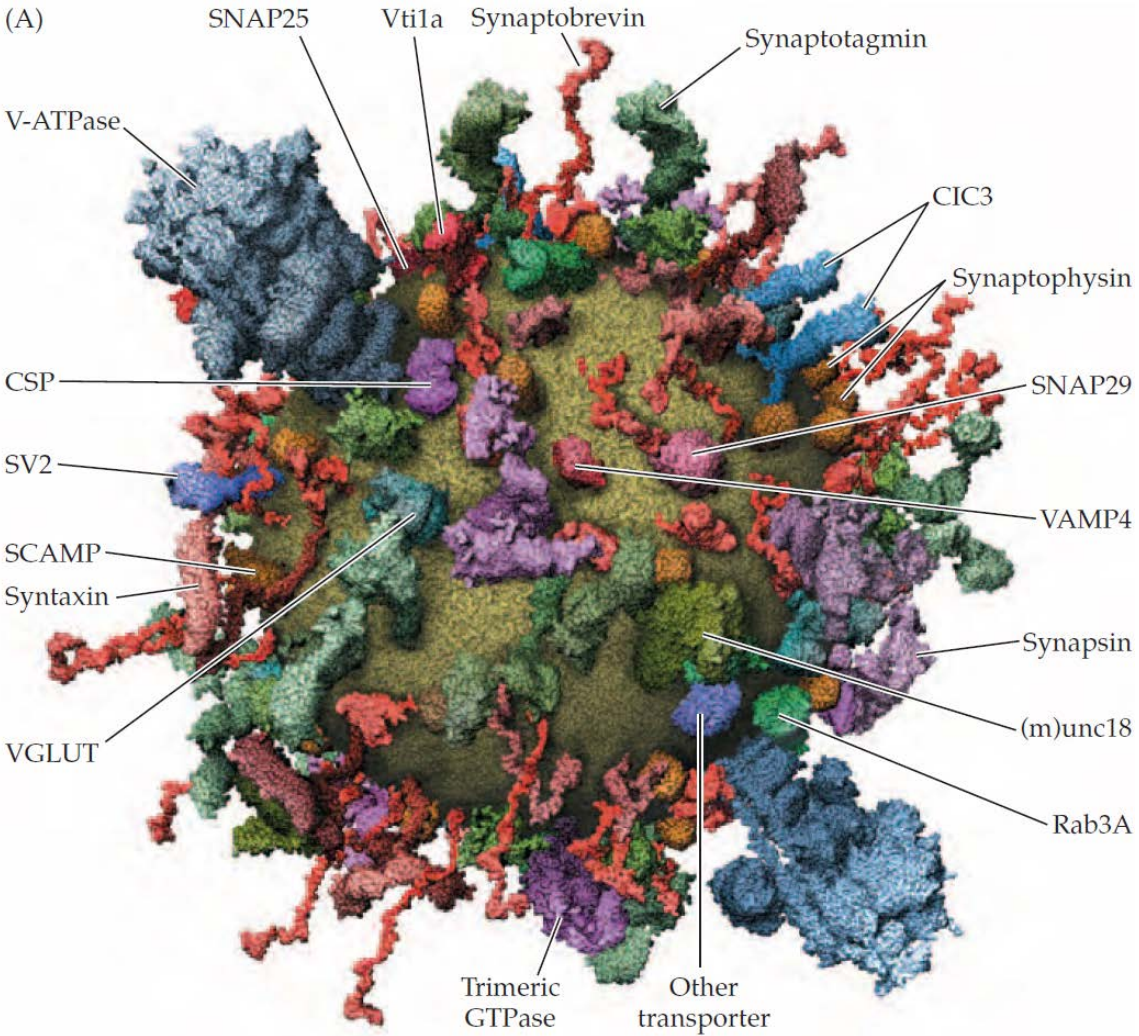
1) Peptide neurotransmitters, as well as the enzymes that modify their precursors, are synthesized in the cell body. Enzymes and propeptides are packaged into vesicles in the Golgi apparatus
2) Vesicles are transported to the nerve terminals via **fast axonal transport** (400 mm/day).
   - Peptide-containing vesicles move along these microtubule "tracks" by ATP requiring "motor" proteins such as kinesin.
3) During the transport, the enzymes modify the propeptides to produce one or more neurotransmitter peptides.
4) After vesicle fusion and exocytosis, the peptides diffuse away and are degraded by proteolytic enzymes.

Neuropeptides are packaged into synaptic vesicles that range from 90–250 nm in diameter. Because the center of these vesicles appear <u>electron-dense</u> in electron micrographs, they are referred to as **large dense-core vesicles**.



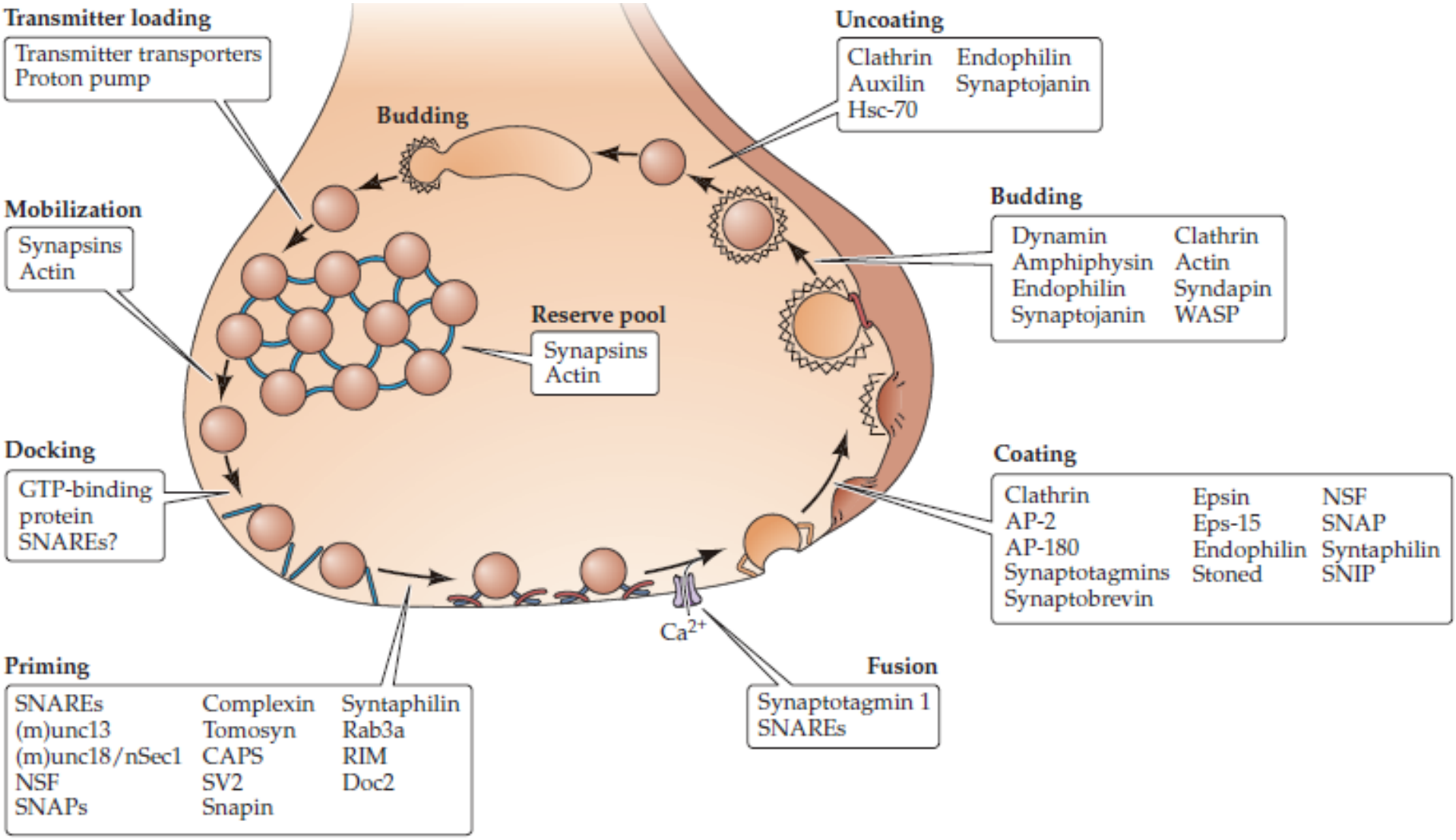
Molecular mechanisms of synaptic vesicle cycling

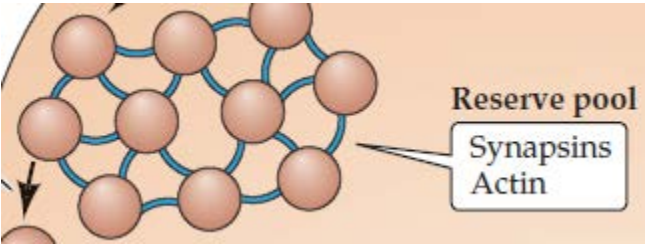
- Precisely how an increase in presynaptic Ca^{2+} concentration goes on to trigger vesicle fusion and neurotransmitter release is not understood.
- However, many important insights have come from molecular studies that have identified and characterized the proteins found on synaptic vesicles.



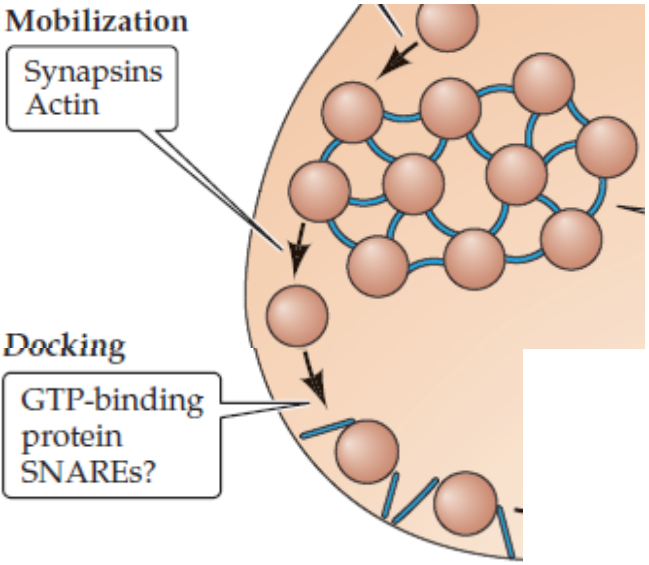
- Model of the molecular organization of a synaptic vesicle.
- The cytoplasmic surface of the vesicle membrane is densely covered by proteins, only 70% of which are shown here.

Molecular mechanisms of synaptic vesicle cycling



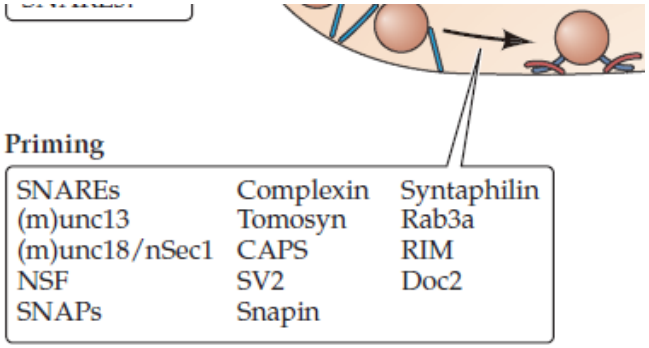


- the protein **synapsin**, which reversibly binds to synaptic vesicles, may keep these vesicles tethered within the reserve pool by crosslink vesicles to each other and to **actin** filaments in the cytoskeleton.

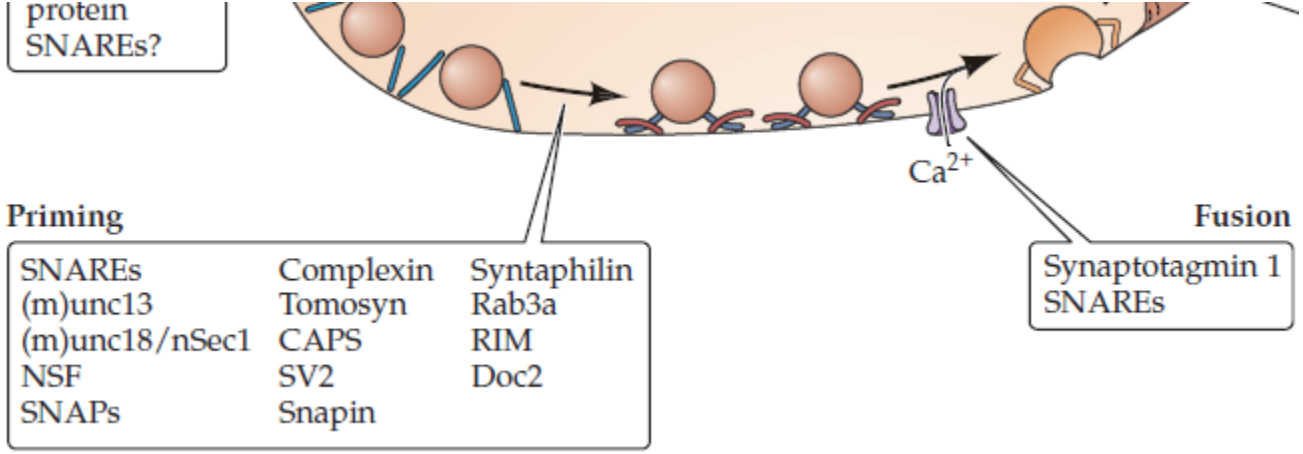


- Mobilization of these reserve pool vesicles is caused by phosphorylation of synapsin by proteins kinases, most notably the **Ca^{2+}/calmodulin-dependent protein kinase, type II** (**CaMKII**), which allows synapsin to dissociate from the vesicles.

- Once vesicles are free from their reserve pool tethers, they make their way to the plasma membrane and are then attached to this membrane by poorly understood docking reactions.

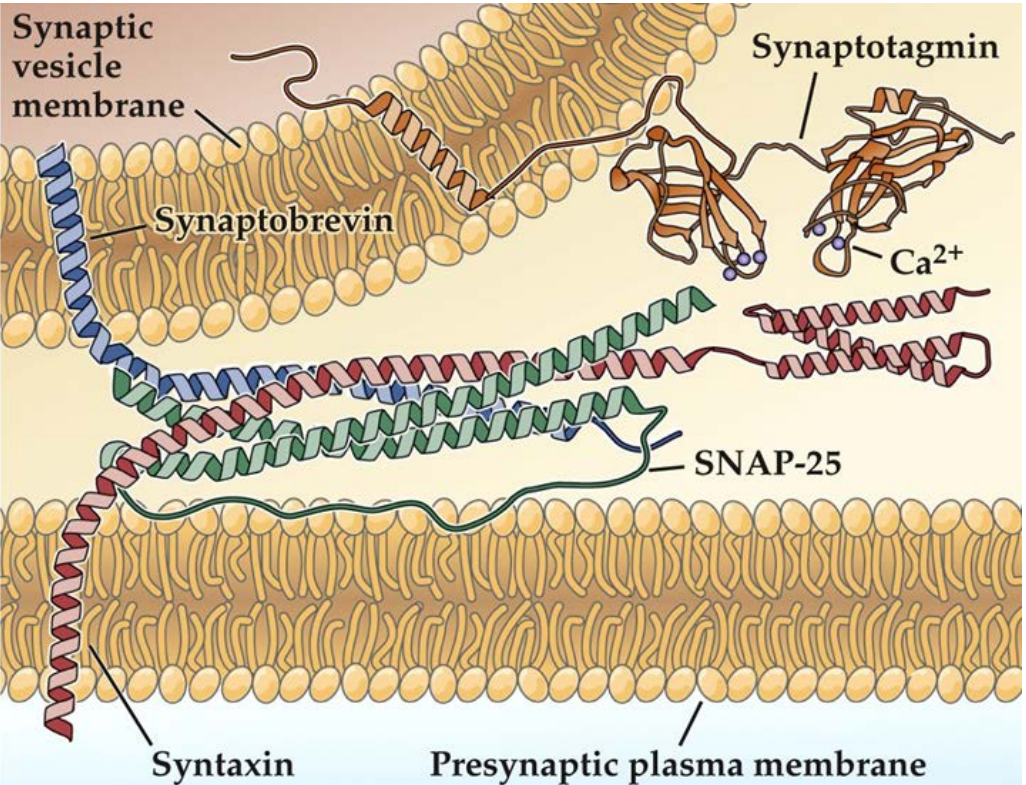


- A series of priming reactions then prepares the vesicular and plasma membranes for fusion. A large number of proteins are involved in priming.
  - the ATPase **NSF** (NEM-sensitive fusion protein) and **SNAPs** (soluble NSF attachment proteins).
  - These two proteins work by regulating the assembly of other proteins that are called **SNAREs** (SNAP receptors).
  - Many of the other proteins involved in priming—such as munc-13, nSec-1, complexin, snapin, syntaphilin, and tomosyn—also interact with the SNAREs.

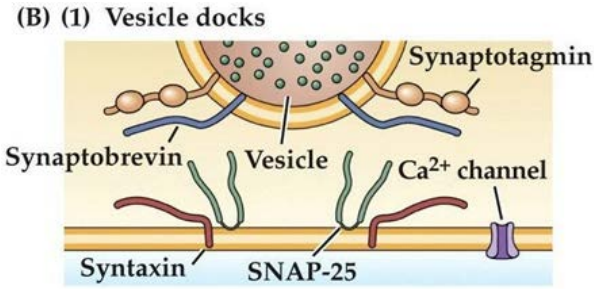


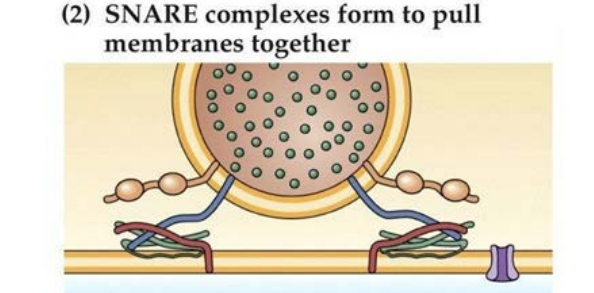
- One of the main purposes of priming seems to organize SNARE proteins into the correct conformation for membrane <u>fusion</u>.

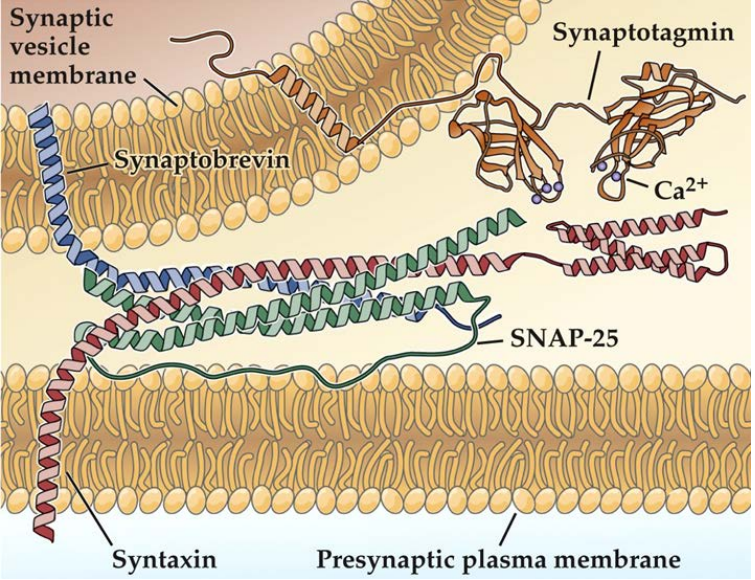
- One of the SNARE proteins, **synaptobrevin**, is in the membrane of synaptic vesicles, while two other SNARE proteins called **syntaxin** and **SNAP-25** are found primarily on the plasma membrane.



- These SNARE proteins can form a macromolecular complex that spans the two membranes, thus bringing them into close apposition.

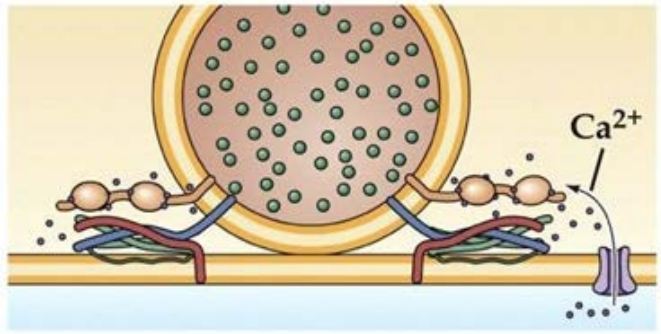




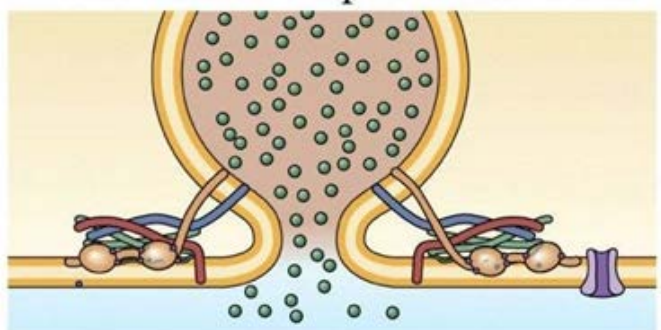


- Ca^{2+} regulation of neurotransmitter release is conferred by **synaptotagmin**, a protein found in the membrane of synaptic vesicles.
- Synaptotagmin acts as a Ca^{2+} sensor, signaling the elevation of Ca^{2+} within the terminal and thus triggering vesicle fusion.
- How Ca^{2+} binding to synaptotagmin leads to exocytosis is not yet clear.

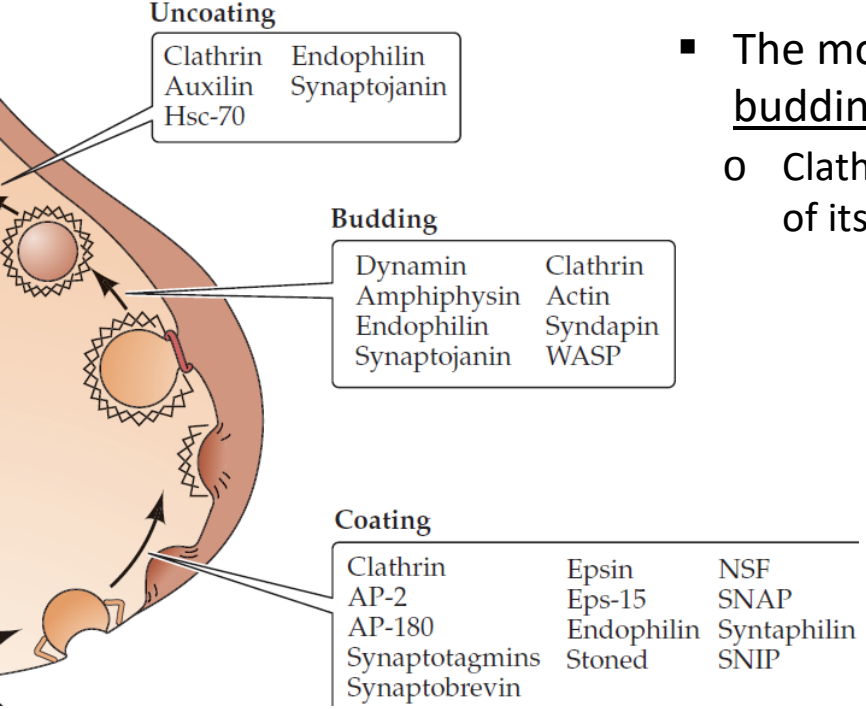
(3) Entering Ca^{2+} binds to synaptotagmin



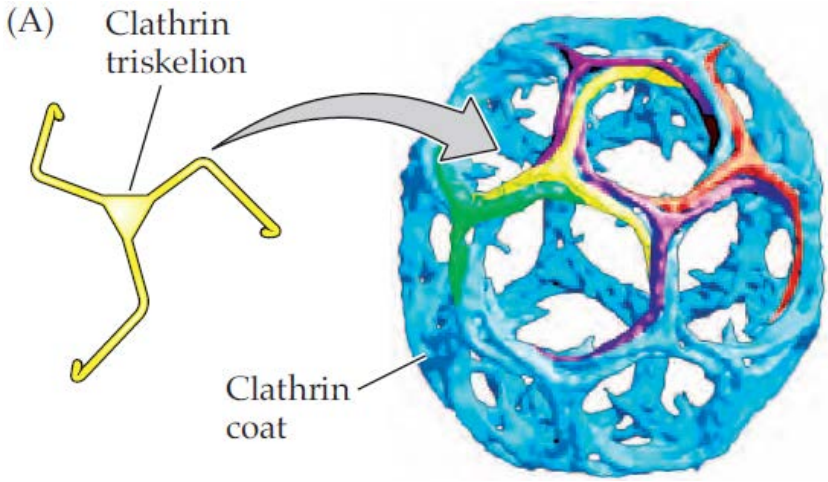
(4) Ca^{2+}-bound synaptotagmin catalyzes membrane fusion by binding to SNAREs and the plasma membrane



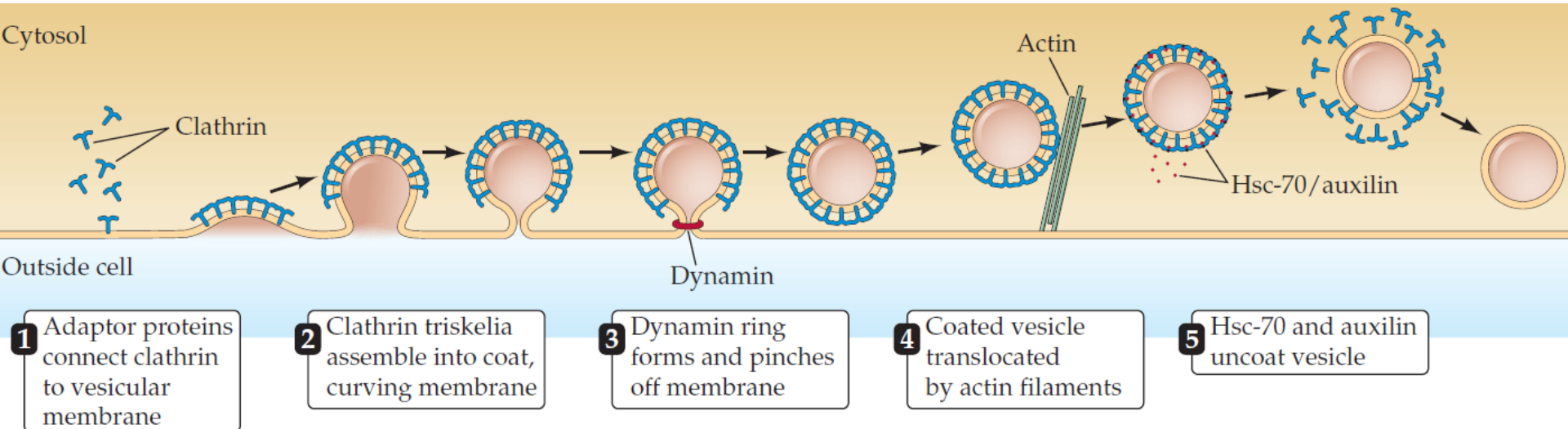
- <u>A model for Ca^{2+}-triggered vesicle fusion</u>. SNARE proteins on the synaptic vesicle and plasma membranes form a complex that brings together the two membranes. Ca^{2+} then binds to synaptotagmin, causing the cytoplasmic region of this protein to catalyze membrane fusion by binding to SNAREs and inserting into the plasma membrane.

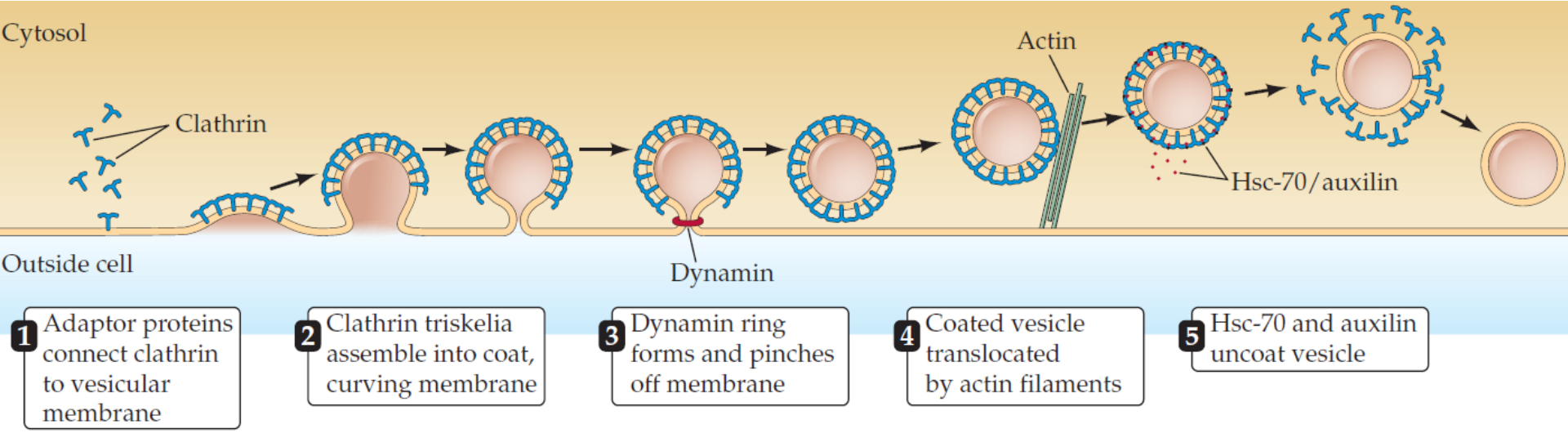


- The most important protein involved in endocytotic <u>budding</u> of vesicles from the plasma membrane is **clathrin**.
  - Clathrin has a unique structure that is called a triskelion because of its three-legged appearance.

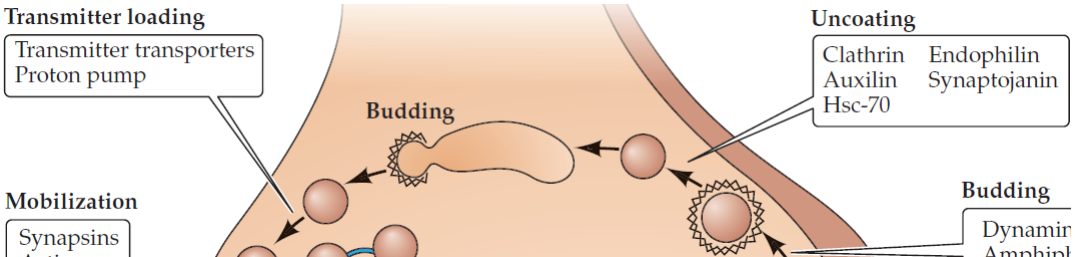


- During endocytosis, clathrin triskelia attach to the vesicular membrane that is to be retrieved.
- A number of adaptor proteins, such as **AP-2** and **AP180**, connect clathrin to the proteins and lipids of this membrane.



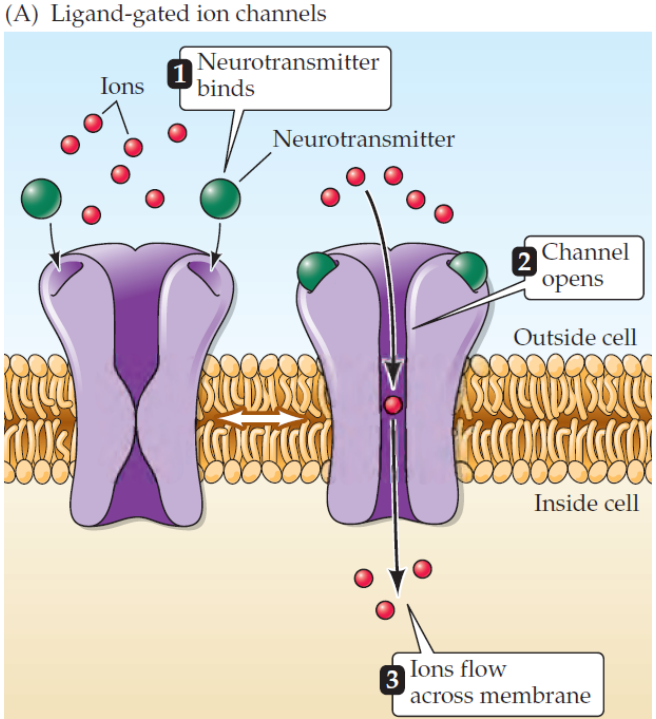


- Such dome-like structures form coated pits that initiate membrane budding, increasing the curvature of the budding membrane until it forms a coated vesicle-like structure.
- Another protein, called **dynamin**, causes the final pinching-off of membrane that completes the production of coated vesicles.
- The clathrin coats then are removed by an ATPase, **Hsc70**, with another protein, **auxilin**, serving as a co-factor that recruits Hsc70 to the coated vesicle. Other proteins, such as **synaptojanin**, are also important for vesicle uncoating.
- Uncoated vesicles can then continue their journey through the recycling process, eventually becoming refilled with neurotransmitter due to the actions of neurotransmitter transporters in the vesicle membrane.



Neurotransmitter receptors

- **Neurotransmitter receptors** are proteins that are embedded in the plasma membrane of postsynaptic cells and have an extracellular neurotransmitter binding site that detects the presence of neurotransmitters in the synaptic cleft.
- There are two broad families of receptor proteins that differ in their mechanism of transducing transmitter binding into postsynaptic responses.
  1. The receptors containing a membrane-spanning domain that forms an ion channel.

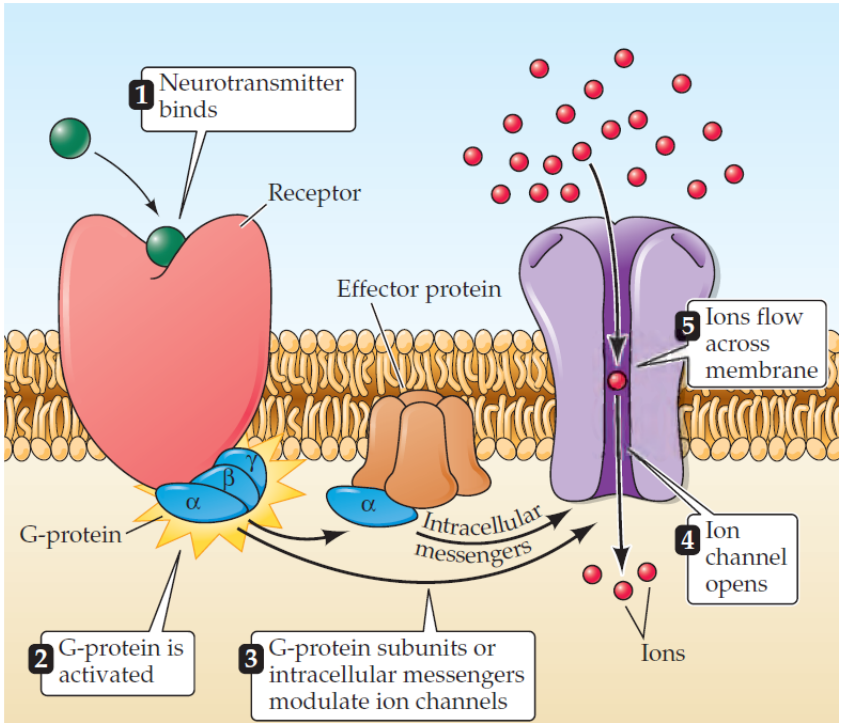


- These receptors combine transmitter-binding and channel functions into a single molecular entity and thus are called **ionotropic receptors** (the Greek *tropos* means to move in response to a stimulus) or **ligand-gated ion channels**.
- -tropic?.

Neurotransmitter receptors

2. **Metabotropic receptors**: the eventual movement of ions through a channel depends on intervening metabolic steps.
   - These receptors do not have ion channels as part of their structure; instead, they have an intracellular domain that indirectly affects channels though the activation of intermediate molecules called **G-proteins**.
   - Neurotransmitter binding to these receptors activates G-proteins, which then dissociate from the receptor and interact directly with ion channels or bind to other effector proteins, such as enzymes, that make intracellular messengers that open or close ion channels.

(B) G-protein-coupled receptors



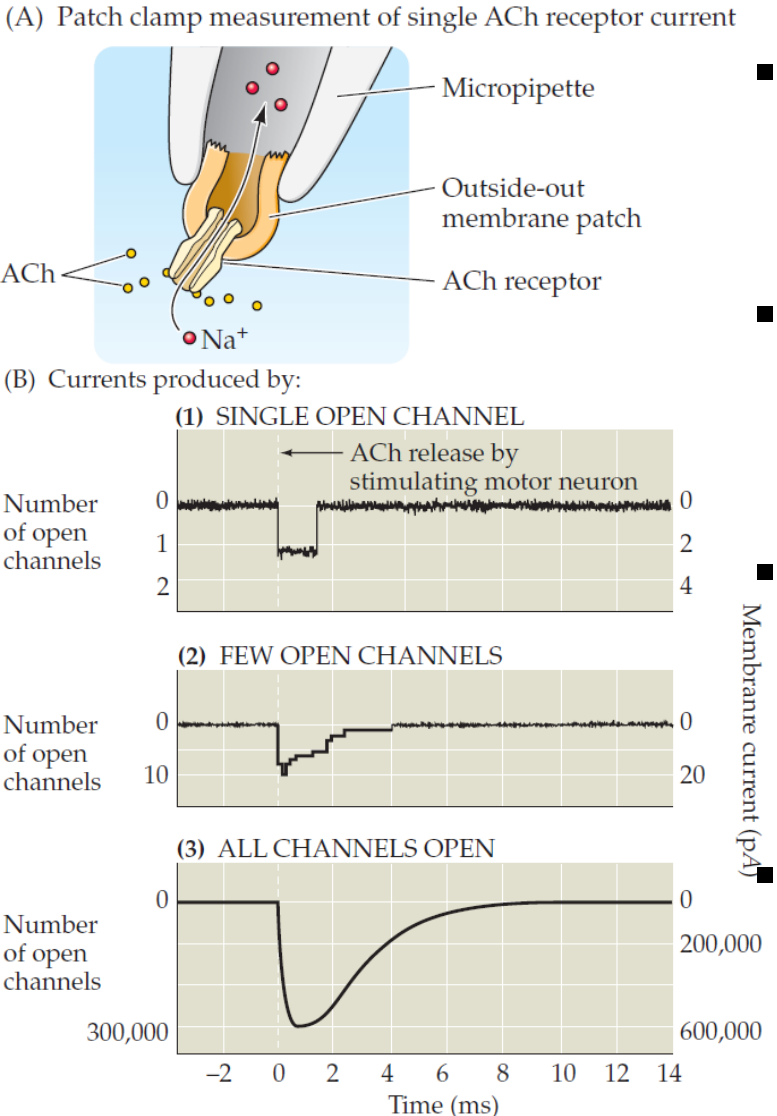
- G-proteins can be thought of as transducers that couple neurotransmitter binding to the regulation of postsynaptic ion channels. For this reason, metabotropic receptors are also called **G-protein-coupled receptors**.

Neurotransmitter receptors

- Ionotropic receptors generally mediate rapid postsynaptic effects.
- The activation of metabotropic receptors typically produces much slower responses, ranging from hundreds of milliseconds to minutes or even longer.
  - ?
- A given transmitter may activate both ionotropic and metabotropic receptors to produce both fast and slow **postsynaptic potential** (PSP) at the same synapse.

End plate current

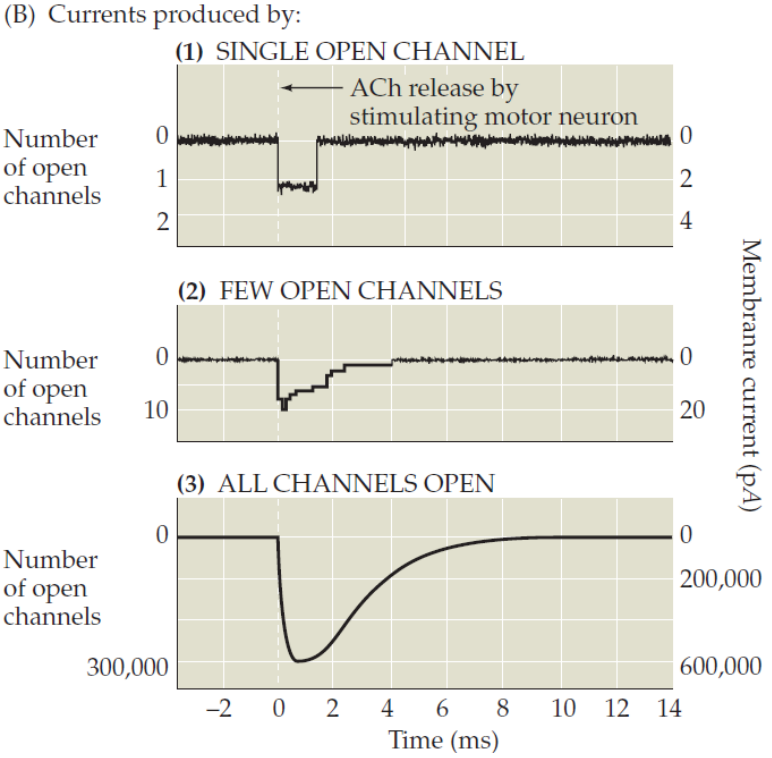
❖ Neuromuscular synapses are valuable for understanding the mechanisms that allow neurotransmitter receptors to generate postsynaptic signals..



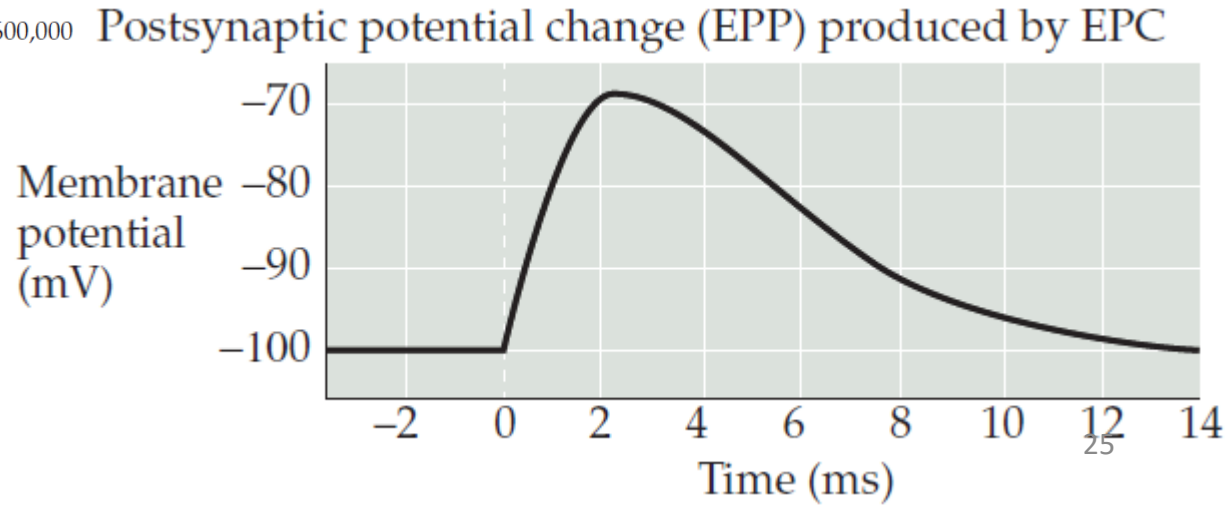
- The binding of ACh to postsynaptic receptors opens ion channels in the muscle fiber membrane, which generate the minute postsynaptic currents.
- Exposure of the extracellular surface of a patch of postsynaptic membrane to ACh causes single-channel currents to flow for a few milliseconds.
- The electrical actions of ACh are greatly multiplied when an action potential in a presynaptic motor neuron causes the release of millions of molecules of ACh into the synaptic cleft.
- Although individual ACh receptors only open briefly, the opening of a large number of channels is synchronized by the brief duration during which ACh is secreted from presynaptic terminals.

End plate potential

- The macroscopic current resulting from the summed opening of many ion channels is called the **end plate current**, or **EPC**.

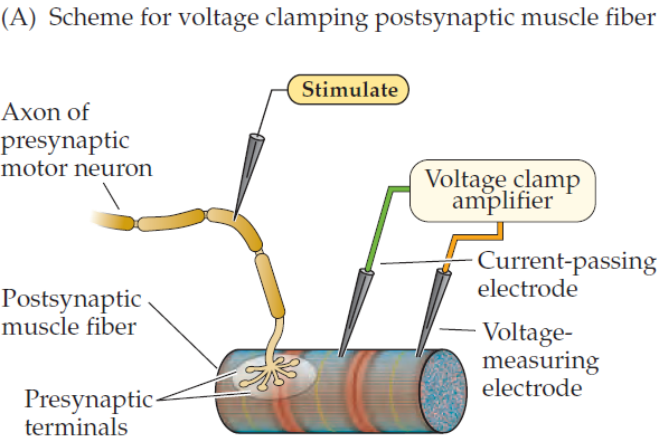


- Because the current flowing during the EPC is normally inward, it causes the postsynaptic membrane potential to depolarize. This depolarizing change in potential is the **EPP**, which typically triggers a postsynaptic action potential by opening voltage-gated Na^+ and K^+ channels.

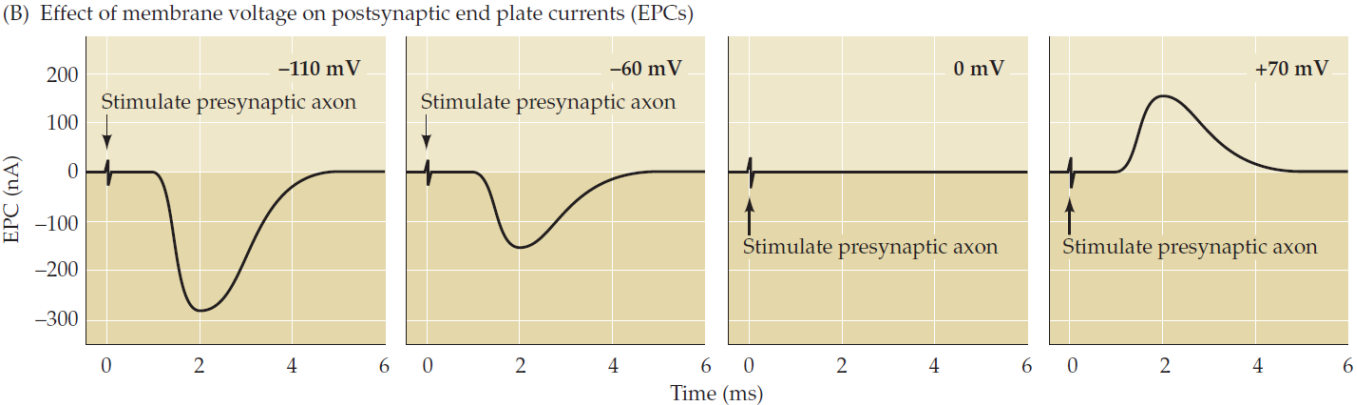


Reversal potentials

- When the potential of the postsynaptic muscle cell is controlled by the voltage clamp method, the magnitude of the membrane potential clearly affects the amplitude and polarity of EPCs.
  - When the postsynaptic membrane potential is made more negative than the resting potential, the amplitude of the EPC becomes larger, whereas this current is reduced when the membrane potential is made more positive.



- At approximately 0 mV, no EPC is detected, and at even more positive potentials, the current reverses its polarity, becoming outward rather than inward.
- The potential where the EPC reverses, about 0 mV in the case of the neuromuscular junction, is called the **reversal potential**.



Postsynaptic current and potential

- Although this discussion has focused on the neuromuscular junction, similar mechanisms generate postsynaptic responses at all chemical synapses.
- The general principle is that transmitter binding to postsynaptic receptors produces a postsynaptic conductance change as ion channels are opened (or sometimes closed).
- The postsynaptic conductance is increased if—as at the neuromuscular junction— channels are opened, and decreased if channels are closed.
- This conductance change typically generates an electrical current, the **postsynaptic current** (**PSC**), which in turn changes the postsynaptic membrane potential to produce a **postsynaptic potential** (**PSP**).
- The conductance changes and the PSPs that typically accompany them are the ultimate outcome of most chemical synaptic transmission, concluding a sequence of electrical and chemical events that begins with the invasion of an action potential into the terminals of a presynaptic neuron.

Excitatory and inhibitory postsynaptic potentials

- In many ways, the events that produce PSPs at synapses are similar to those that generate action potentials in axons; in both cases, conductance changes produced by ion channels lead to ionic current flow that changes the membrane potential.

- PSPs are called **excitatory** (or **EPSPs**) if they increase the likelihood of a postsynaptic action potential occurring, and **inhibitory** (or **IPSPs**) if they decrease this likelihood.

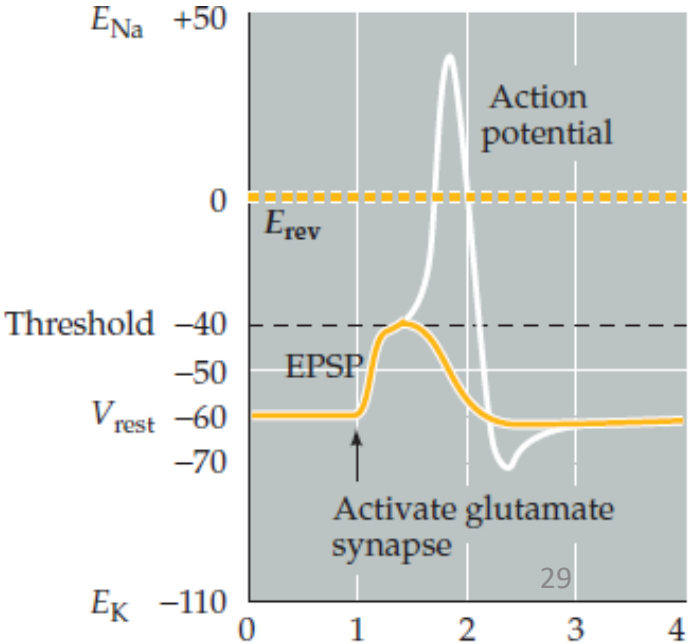
- In both cases, neurotransmitters binding to receptors open or close ion channels in the postsynaptic cell.

- Whether a postsynaptic response is an EPSP or an IPSP depends on the type of channel that is coupled to the receptor, and on the concentration of permeant ions inside and outside the cell.

- In fact, the only distinction between postsynaptic excitation and inhibition is the reversal potential of the PSP in relation to the threshold voltage for generating action potentials in the postsynaptic cell.

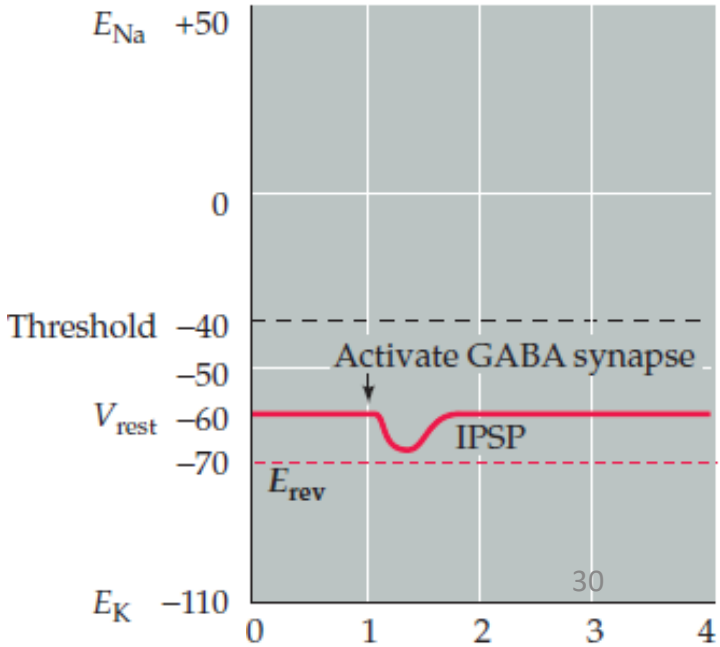
Excitatory postsynaptic potentials

- As an example of excitatory postsynaptic action, consider a neuronal synapse that uses glutamate as the transmitter.
- Many such synapses have receptors that, like the ACh receptors at neuromuscular synapses, open ion channels that are nonselectively permeable to cations.
- When these glutamate receptors are activated, both Na^+ and K^+ flow across the postsynaptic membrane, yielding an E_{rev} of approximately 0 mV for the resulting postsynaptic current.
- If the resting potential of the postsynaptic neuron is –40 mV, the resulting EPSP will depolarize by bringing the postsynaptic membrane potential toward 0 mV.
- Thus, a glutamate-induced EPSP will increase the probability that this neuron produces an action potential, defining the synapse as excitatory.

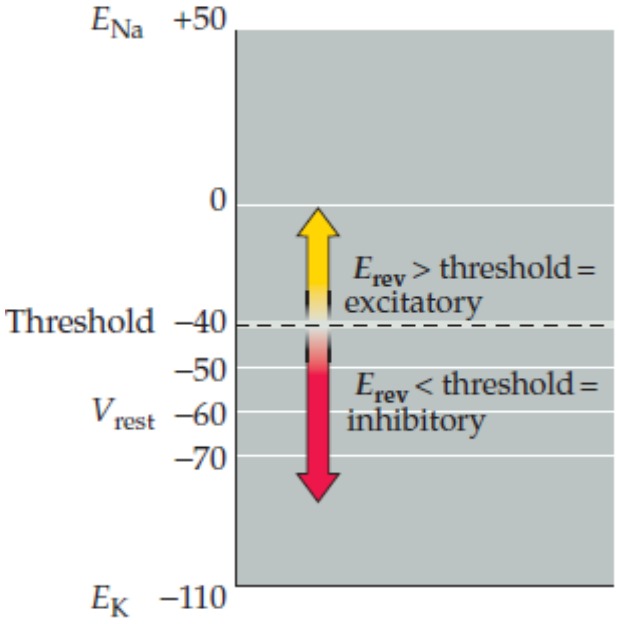


Inhibitory postsynaptic potentials

- As an example of inhibitory postsynaptic action, consider a neuronal synapse that uses GABA as its transmitter.
- At such synapses, the GABA receptors typically open channels that are selectively permeable to Cl^-, and the action of GABA causes Cl^- to flow across the postsynaptic membrane into the cell and produce a hyperpolarizing IPSP.
- This hyperpolarizing IPSP will take the postsynaptic membrane away from the action potential threshold of –40 mV, clearly inhibiting the postsynaptic cell.



Excitatory and inhibitory postsynaptic potentials



- A simple rule distinguishes postsynaptic excitation from inhibition: An EPSP has a reversal potential more positive than the action potential threshold, whereas an IPSP has a reversal potential more negative than threshold.

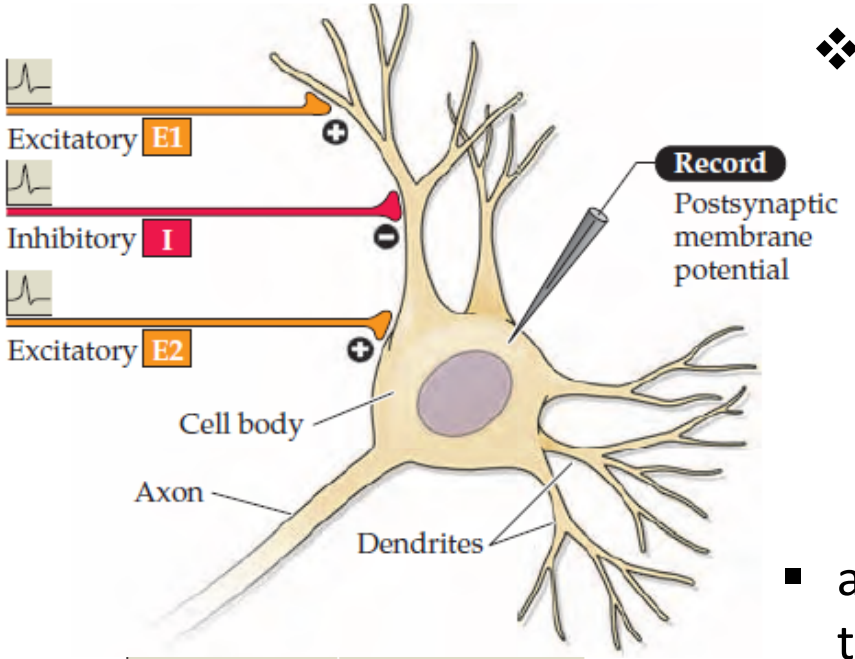
Summation of Synaptic Potentials

- The PSPs produced at most synapses in the brain are much smaller than those at the neuromuscular junction; indeed, EPSPs produced by individual excitatory synapses may be only a fraction of a millivolt and are usually well below the threshold for generating postsynaptic action potentials.

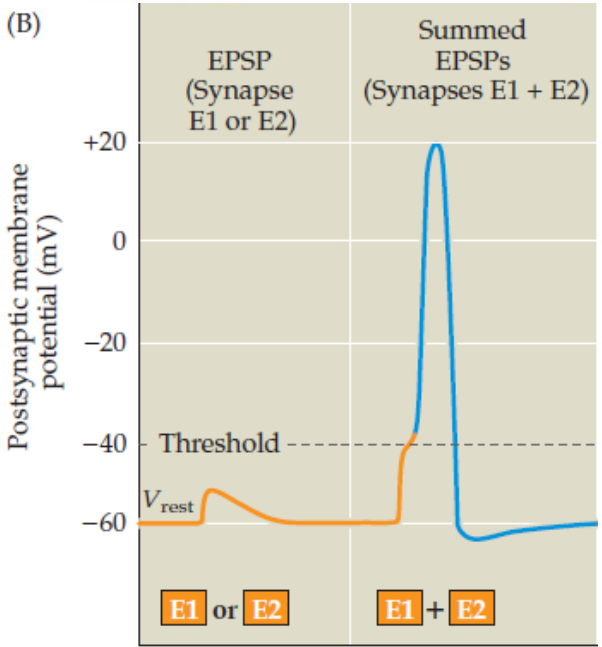
- How then, can such synapses transmit information if their PSPs are subthreshold?

- The answer is that neurons in the central nervous system are typically innervated by thousands of synapses, and the PSPs produced by each active synapse can *sum together*—in space and in time—to determine the behavior of the postsynaptic neuron.

Summation of Synaptic Potentials

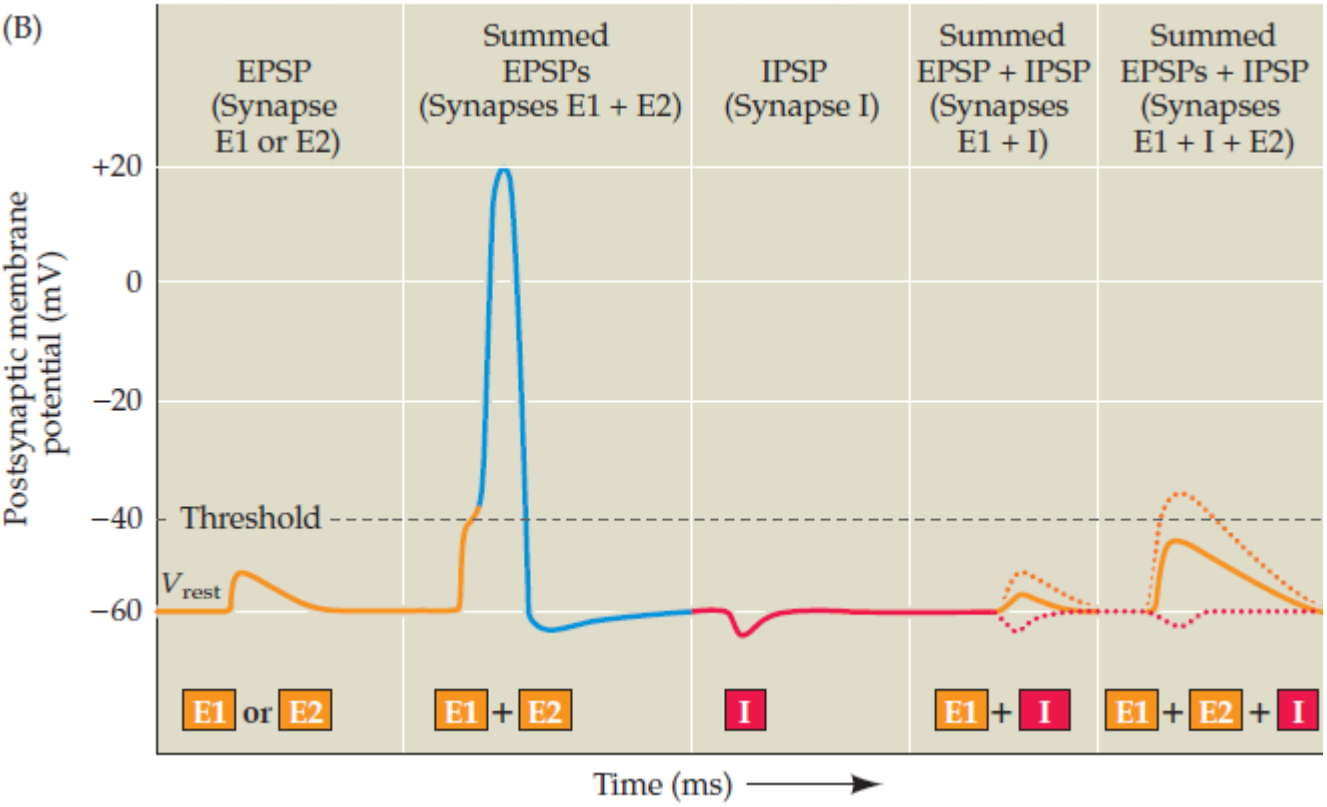


- A microelectrode records the postsynaptic potentials produced by the activity of two excitatory synapses (E1 and E2) and an inhibitory synapse (I).
  - activation of either one of the excitatory synapses alone (E1 or E2) produces a subthreshold EPSP.
  - activation of both excitatory synapses at about the same time causes the two EPSPs to sum together.



- If the sum of the two EPSPs (E1 + E2) depolarizes the postsynaptic neuron sufficiently to reach the threshold potential, a postsynaptic action potential results.
- Summation thus allows subthreshold EPSPs to influence action potential production.

Summation of Synaptic Potentials



- Likewise, an IPSP generated by an inhibitory synapse (I) can sum (algebraically speaking) with a subthreshold EPSP to reduce its amplitude (E1 + I) or can sum with suprathreshold EPSPs to prevent the postsynaptic neuron from reaching threshold (E1 + I + E2).

Summation of Synaptic Potentials

- In short, the summation of EPSPs and IPSPs by a postsynaptic neuron permits a neuron to integrate the electrical information provided by all the inhibitory and excitatory synapses acting on it at any moment.

- Whether the sum of active synaptic inputs results in the production of an action potential depends on the balance between excitation and inhibition.

- Normally, the balance between EPSPs and IPSPs changes continually over time, depending on the number of excitatory and inhibitory synapses active at a given moment and the magnitude of the current at each active synapse.