

Nervo 80 lecture 8

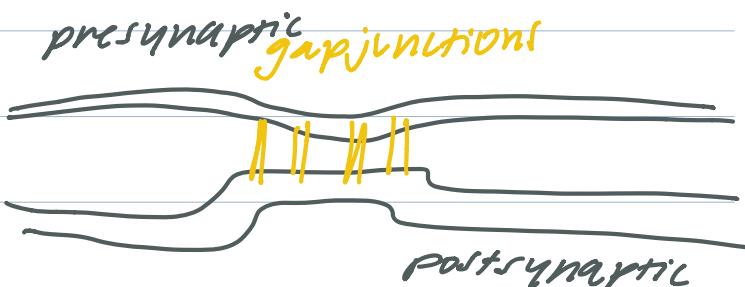
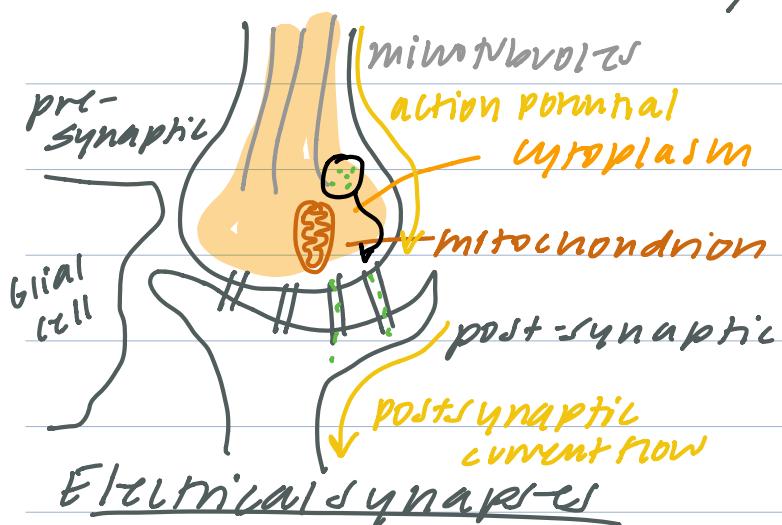
Sept. 30, 2019

Synapse: Presynaptic Mechanisms



- Synapses transmit information from one cell to another
 - into processing (thinking)
 - changes w/ experience (learning)
 - targets of psychoactive drugs

Golgi wasn't (completely) wrong:



Advantages

1. very fast, no channels open
2. bidirectional, and both hyper/po-polarizing
3. Energy efficient
4. Fail-safe
5. Good for rapid synchronization and for circuits where timing is crucial

- Early development
- Brainstem (breathing)
- Thalamus (brain waves)
- "Escape reflex" in many organisms
- Common in glia

Disadvantages:

1. Cannot modulate
2. Difficult to change sign
3. Response is same size / duration or smaller

Chemical synapse

1. Action potential
2. Neurotransmitter release
3. NT diffusion across cleft
4. Post-synaptic receptor cause response

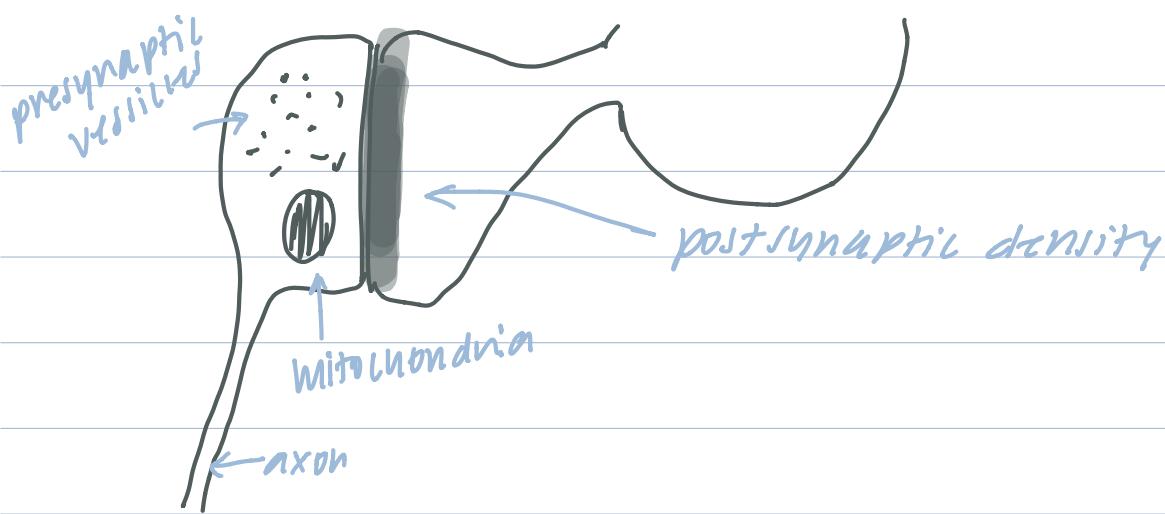
Advantage

- Unidirectional
- Different effects
- Amplification
- Modulation

Disadvantage:

- slow ~1-3 ms
- complex + energetically expensive

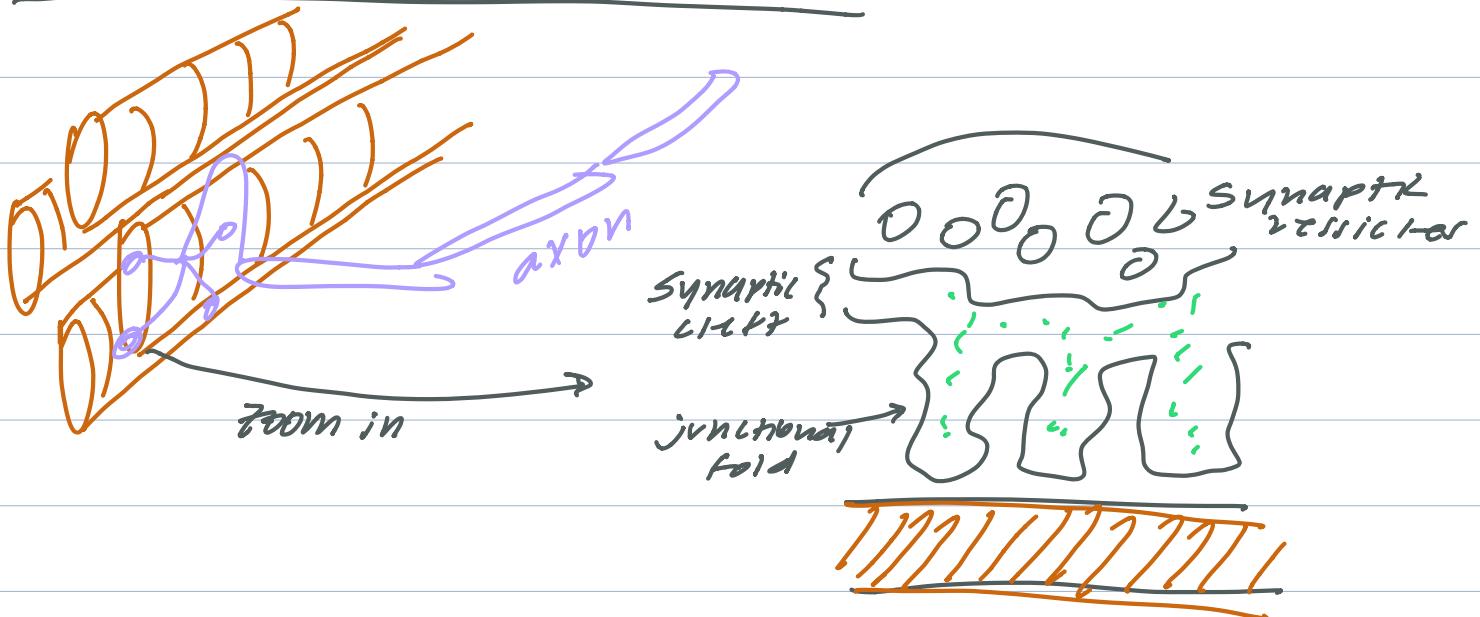
* Pre-synaptic release site has synaptic vesicles



Neurotransmitter median chemical

- Otto Loewi: "vagus substance" acetylcholine
- NT: released from presynaptic terminal \rightarrow depolarization
 - Amino acids: small organic molecules
glutamate, glycine, GABA
 - Amines: small organic molecules
acetylcholine
 - Peptides
- Bernard Katz
- **Quantal:** neurotransmitter released in discrete "quanta"
- **Vesicle:** NT packaged in membrane-bound vesicles
- **Calcium:** voltage-dependent Ca^{2+} channels

Newmusrular Junction (NMJ)



Recording postsynaptic responses

1. Stimulus motor axon

- AP \rightarrow NT released \rightarrow ion channels depolarize

2. Record from muscle fiber

- Large synapse

- block AP

- Synaptic potential

- End-plate Potential (EPP) in muscle

- Excitatory Postsynaptic Potential (EPSP) in CNS

Quantal

- Spontaneous small depolarizations (1-2 mV),

but only near TMJ

- Look like EPPs but much smaller (spontaneous miniature end-plate potential MEPP)

- Postsynaptic made of many MEPPs

- EPP is $\sim 2 \text{ to } 3 \times$ larger than mini

- Amplitude varies in step-like manner (w/ some variation)
- Ω (quantal size) = amplitude of postsynaptic response to one vesicle (mini)
 - how many postsynaptic receptors activated
- M (quantal content/number) = avg. number of vesicles released by 1 presynaptic AP

$$M = \frac{\text{mean EPV amplitude}}{\text{mean Mini amplitude}}$$
- properties in presynaptic terminal

Evidence

1. MEPP amplitude \downarrow w/ dist from NMJ
2. Minis disappear w/o nerve
3. Minis disappear w/ acetylcholine inhibitor

Vesicle Hypothesis

Evidence

1. Many vesicles in nerve terminals
 2. \square figures examined after stimulation
 3. Depletion of vesicles after multiple stimulations
 4. Neurotransmitters have been isolated from vesicles
- One quantal = one vesicle

Calcium Hypothesis

voltage-dependent Ca^{2+} entry \rightarrow quantal release
Mechanism

1. AP from axon \rightarrow presynaptic
2. Depolarization opens voltage-gated Ca^{2+} channels
3. $I_{\text{Ca}} = g_{\text{Ca}}(V_m - E_{\text{Ca}})$ so Ca^{2+} flows in even at peak of depolarization
4. Intramillular Ca stimulates vesicle fusion + neurotransmitter release
 - Vesicles released proportional to $[\text{Ca}^{2+}]^4$

SNARE hypothesis

- intramillular $\text{Ca}^{2+} \leftrightarrow$ vesicle release
 - Nobel Prize in 2013
 - bring membranes together
 - Syntaxin and SNAP-25: on pre-syn plasma membrane
 - Synaptobrevin: on vesicles
 - Synaptotagmin: on vesicles, Ca^{2+} sensor
- Botulinum toxin C1*
1. Syntaxin, SNAP-25, Synaptobrevin: zipper *Botulinum toxins, Tetanus B, D, F, G*
2. Entering Ca^{2+} binds Synaptotagmin
3. Confirmation change of Synaptotagmin causes vesicle fusion (in ~0.2 ms)
- Vesicles are recycled
1. Fusion

2. DOLC

3. Prime

4. Fusion





MCB/Neuro 8o - Neurobiology of Behavior

Today's Topic:

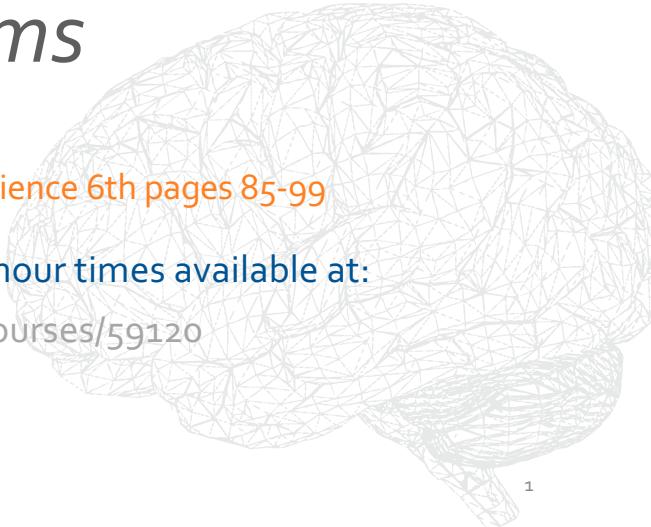
The Synapse: Presynaptic Mechanisms

Lecture 8

Optional reading: Purves et al., Neuroscience 6th pages 85-99

Lecture notes, review questions, office hour times available at:

<https://canvas.harvard.edu/courses/59120>



Mt. Potential

- Snare hypothesis
- Ca⁺⁺ Hypothesis
- Vesicle Hypothesis
- Quantal Hypothesis

Presynaptic properties

- Myelin
- Cable Properties
- Conduction
- VGICs
- Ohm's Law

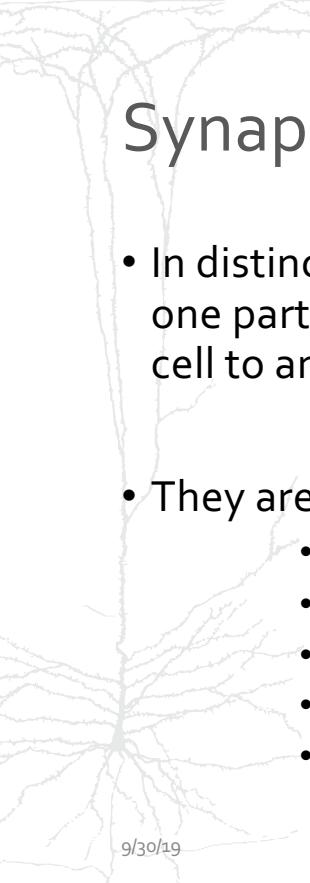
- Na⁺ K⁺ Pump
- GHK Equation
- Nernst Equation
- Impermeable anions & cation-selective channels

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Synaptic Potential

Action Potential

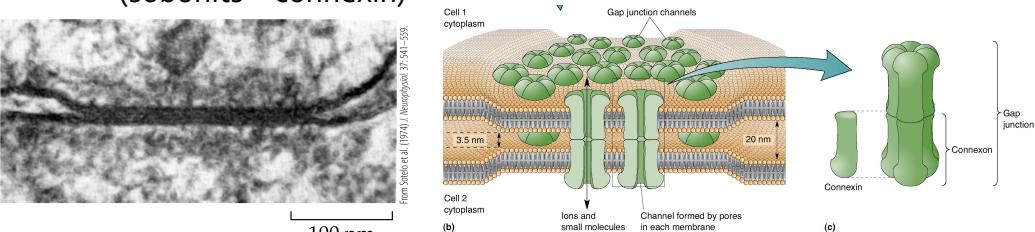
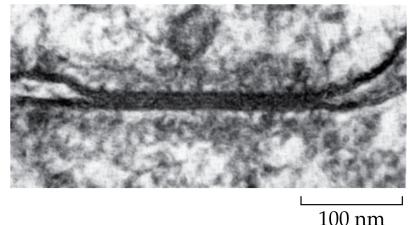
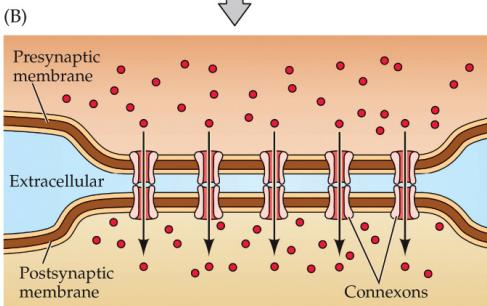
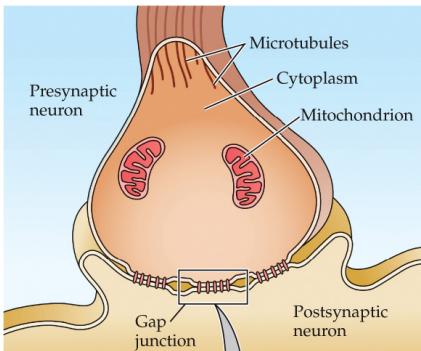
Resting Potential



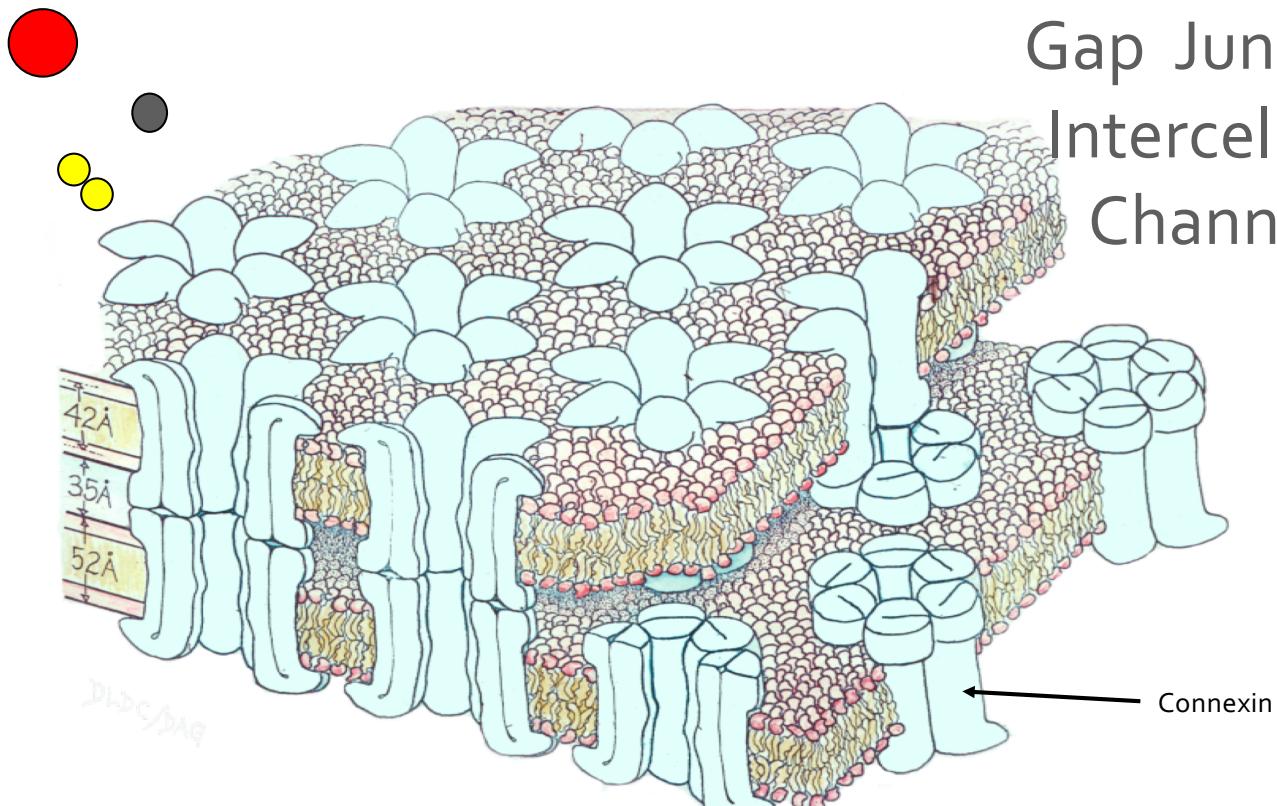
Synapses

- In distinction to action potentials that convey electrical signals from one part of a cell to another, synapses transmit information from one cell to another cell.
- They are implicated in everything the brain does:
 - Information processing (thinking)
 - Changes with experience (learning)
 - Targets of psychoactive drugs
 - Defective in neurological diseases
 - Defective in psychiatric disorders

Golgi's reticular theorem wasn't completely wrong... Electrical Synapses or "Gap Junctions" connect neurons



Gap Junction Intercellular Channels



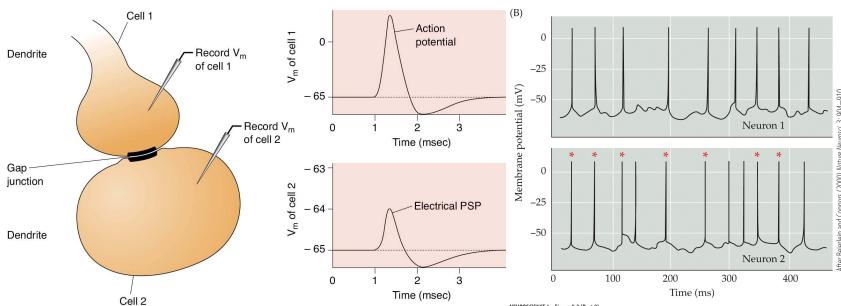
9/30/19 Pass ions and small molecules

Advantages of electrical synapses

- Very fast (< 1ms), no channels to open
- Bi-directional current flow
 - From cell "A" to "B" and from "B" to "A"
 - Passes both depolarizing and hyperpolarizing current
- Energy efficient (don't need many molecules)
- Relatively fail-safe

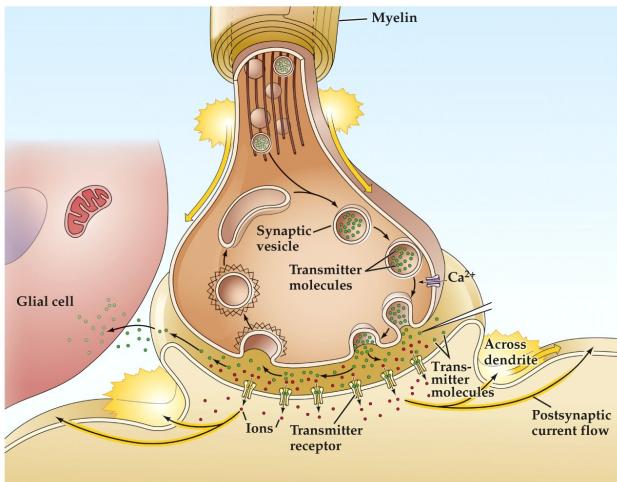
Disadvantages

- Difficult to modulate
- Can't change the "sign"
- Response is same size/duration or smaller (no gain)



- Good for synchronizing neurons (rapid) and for circuits where timing is critical
 - Common in early development (neurons very synchronized)
 - Brainstem (cells synchronize breathing)
 - Thalamus (generate brain waves)
 - 'Escape reflex' in many organisms
 - Also common in glia

Chemical synapse



Major steps in chemical synaptic transmission

1. Action potential reaches the terminal
2. AP triggers release of neurotransmitter
3. Neurotransmitter diffuses across the synaptic cleft, binding to receptors
4. Post synaptic receptors cause a response (synaptic potential)

• Advantages

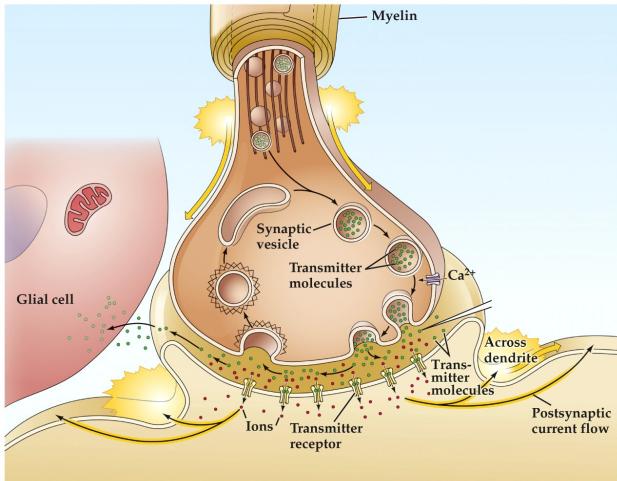
- Unidirectional
- Different effects (excitation, inhibition)
- Amplification
- Modulation (can increase or decrease)

- Disadvantages
 - Relatively slow ~1-3 ms
 - Complex and energetically expensive

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Chemical synapse



Major steps in chemical synaptic transmission

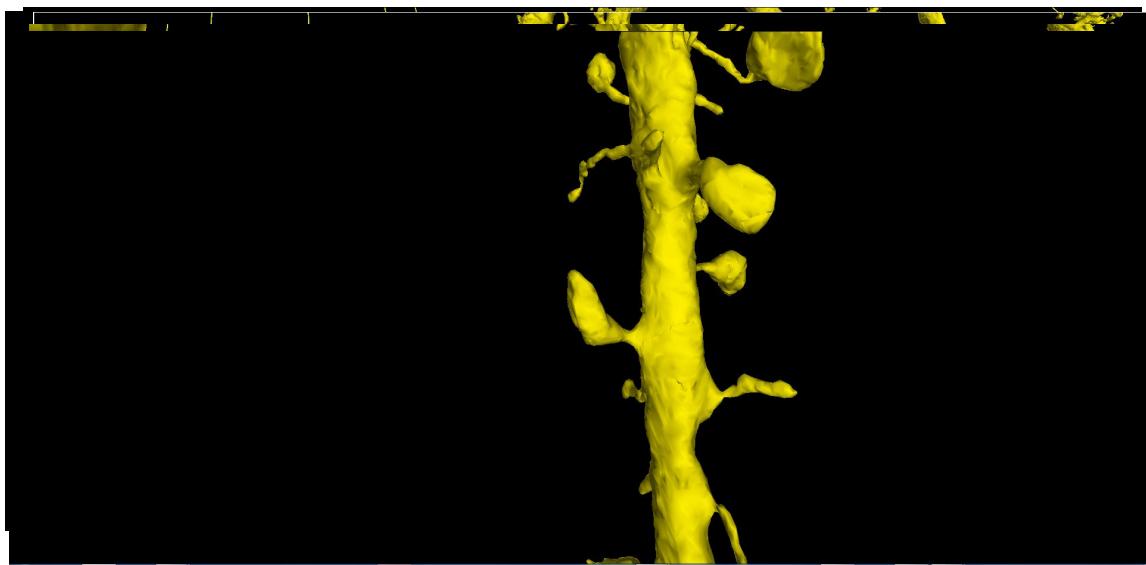
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• Advantages

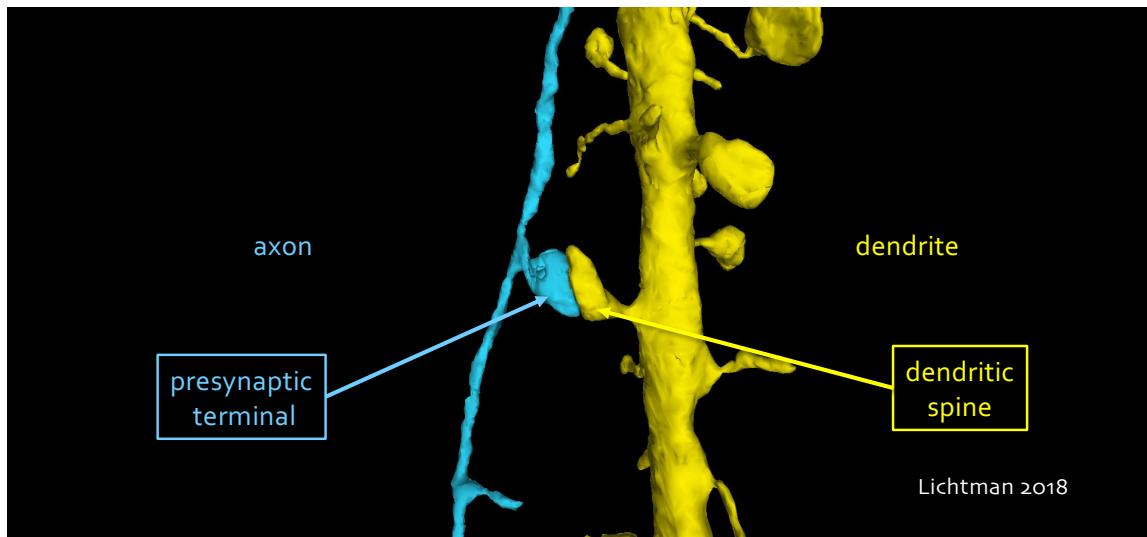
- Unidirectional
- Different effects (excitation, inhibition)
- Amplification
- Modulation (can increase or decrease)

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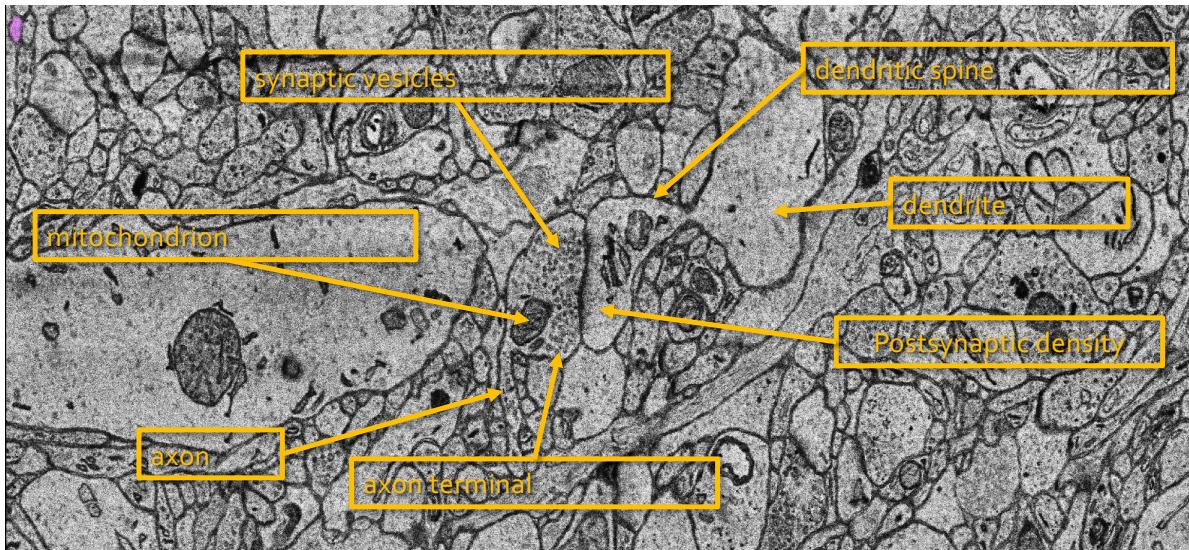
Let's find a chemical synapse



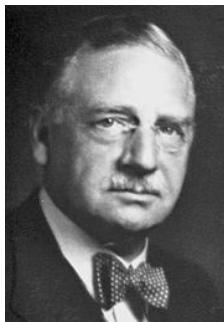
Most common synapse in your brain



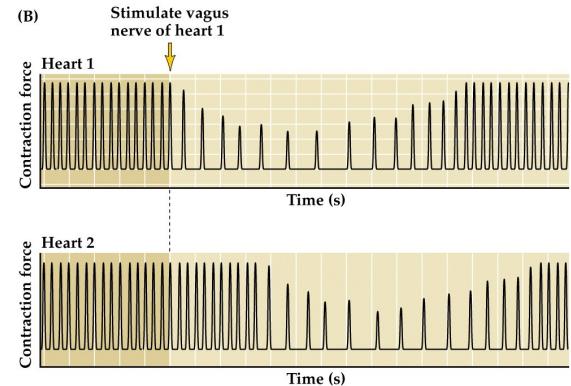
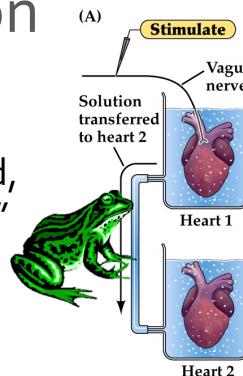
Components of a chemical synapse on an electromicrograph



Neurotransmitters (NTs) mediate chemical synaptic transmission



- Otto Lowei found proof for dissolved, "Vagus substance" (acetylcholine)
- Nobel Prize 1936



Neurotransmitter (NT):

- Released from the presynaptic terminal in response to presynaptic depolarization
 - Specific receptors on the postsynaptic cell cause a change in the electric properties
- Types:
 - Amino acids: small organic molecules
 - Examples: glutamate, glycine, GABA
 - Amines: small organic molecules
 - Examples: acetylcholine, dopamine, histamine
 - Peptides: short amino acid chains (proteins)
 - Examples: dynorphin, enkephalins

What the world knows about synapses owes a lot to

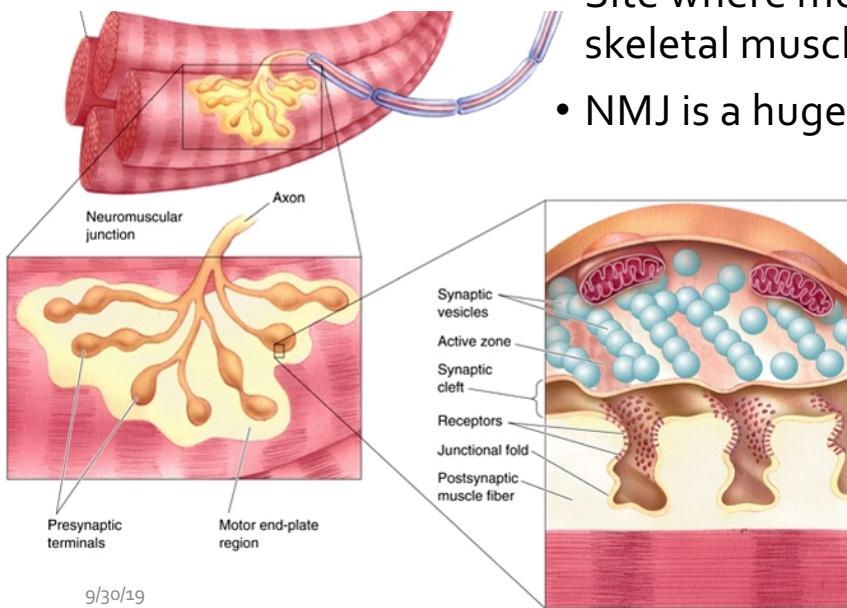
Bernard Katz (1911-2003) Nobel 1970



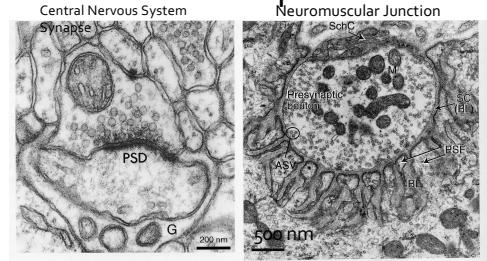
- **Quantal** – neurotransmitter is released in discrete “packets” or “quanta”
- **Vesicle** – NT is packaged into and released from membrane bound vesicles
- **Calcium** – voltage-dependent calcium entry couples AP to release
- **SNARE** – interactions between SNARE proteins on the vesicle and presynaptic membrane, modulated by calcium, lead to vesicle fusion

Katz proposed and provided the first evidence for the **quantal**, **vesicle** and **calcium** hypotheses (which we will describe now)... setting the stage for the **SNARE** hypothesis, which is the molecular basis of the other three.

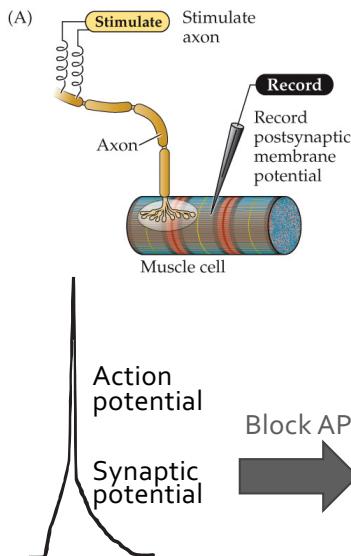
The neuromuscular junction (NMJ) as a model synapse



- Site where motor neuron synapses onto a skeletal muscle
- NMJ is a huge, reliable synapse
 - ~30 microns (size of a soma)
 - Located in the periphery
 - Convoluted folds on post-synaptic side increase the number of receptors



Recording postsynaptic responses



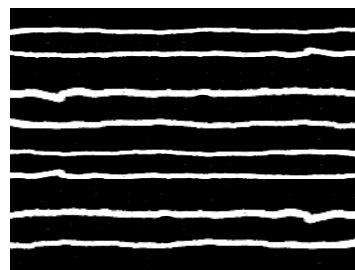
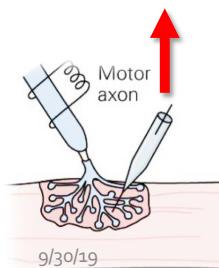
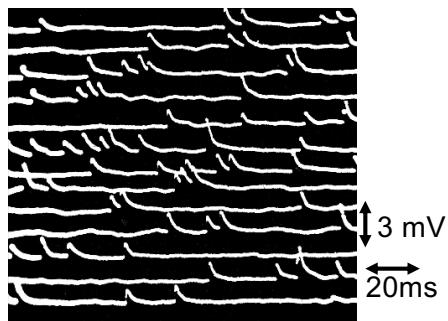
- Stimulate motor axon

- AP triggers release of NT (acetylcholine into the cleft)
- Receptors are ion-channels that depolarize the muscle

- Record from muscle fiber

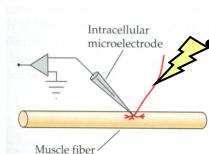
- Large synapse, causes action potential (so block)
- Synaptic potential
 - Nomenclature:
 - End Plate Potential (EPP) in muscle
 - Excitatory postsynaptic potential (EPSP) at CNS

Neurotransmitter is released in “packets” containing many molecules



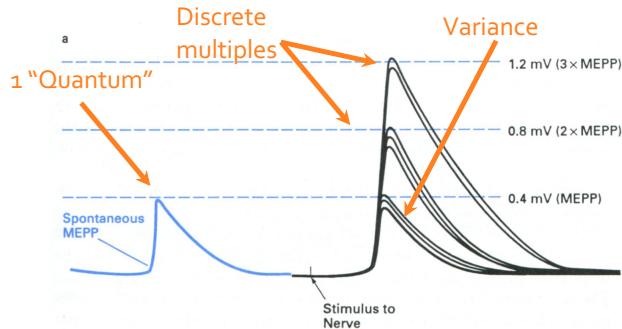
- Spontaneous small depolarizations (1-2mVs) in muscle fiber membrane potential traces, but only near NMJ
 - Look like EPPs but much smaller
 - A spontaneous miniature endplate potential (MEPP) or “mini”
- EPP > 30 mV MEPP 0.5-1 mV

Postsynaptic potentials built of multiple minis

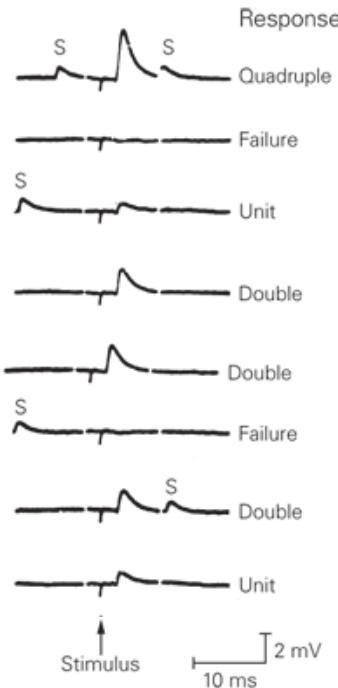


Stimulate motor axon under conditions to make EPP smaller (low Ca^{++}).

- EPP only ~2-3 times larger than a mini
- Amplitude varies in step like manner

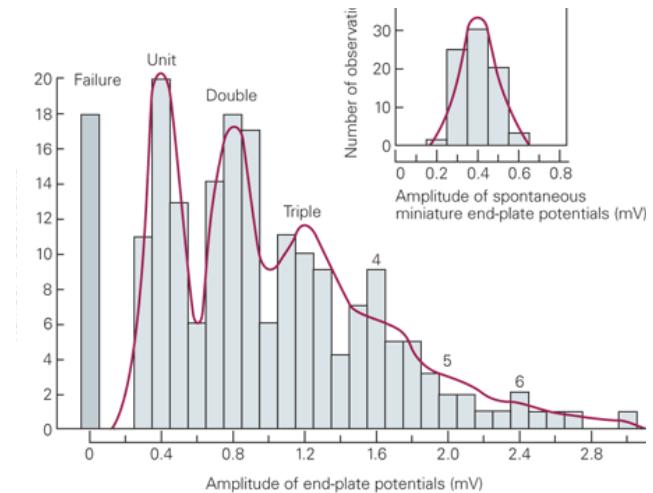
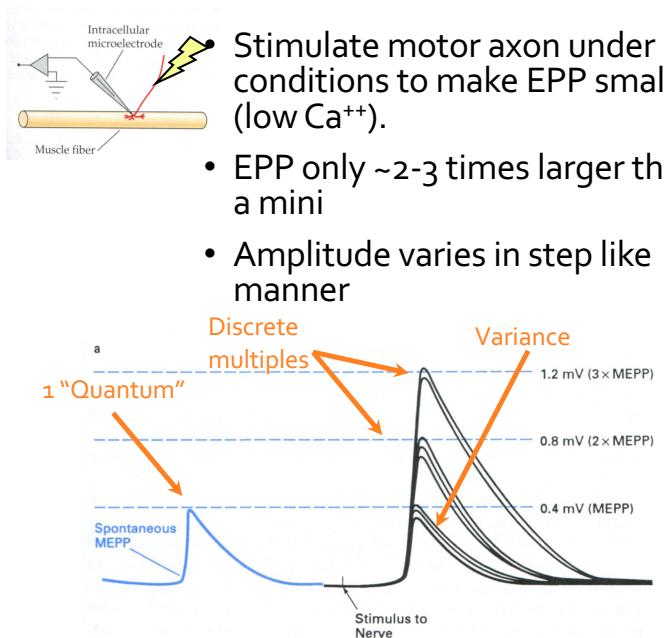


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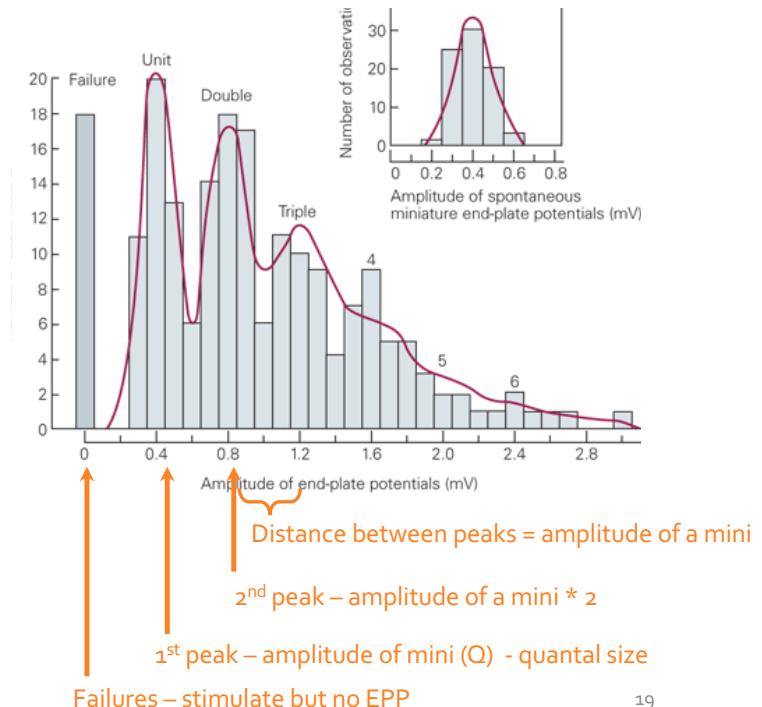
Postsynaptic potentials built of multiple minis



Distribution matches a Poisson distribution (low probability discrete event)

Postsynaptic potentials built of multiple minis

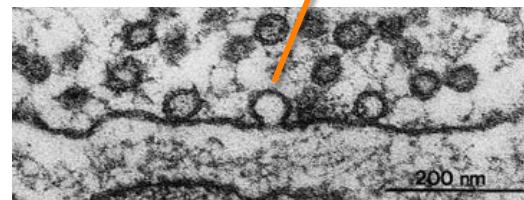
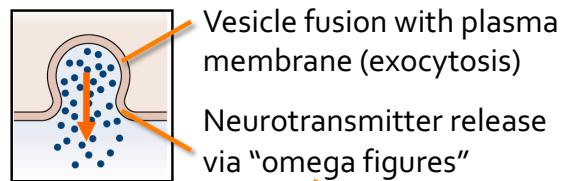
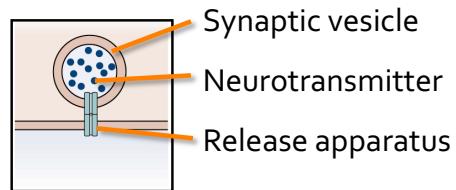
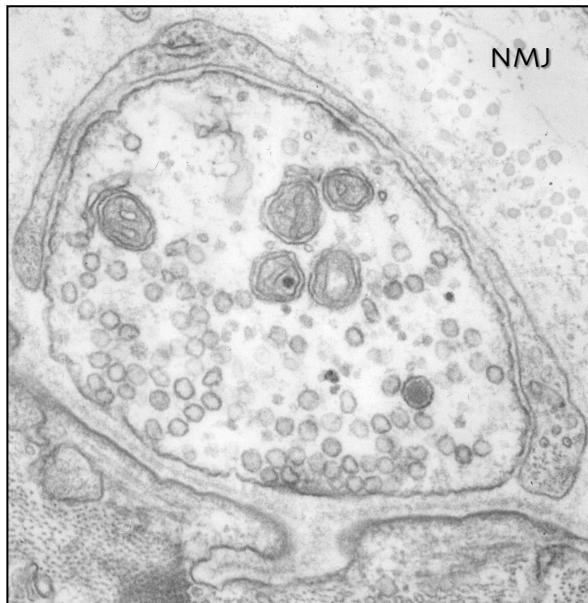
- Q (quantal size): Amplitude of the postsynaptic response to one vesicle (mini)
 - Tells you about how many postsynaptic receptors were activated by NT
- M (Quantal content/number) : average number of vesicles released by 1 presynaptic action potential
 - Tells you about properties in the presynaptic terminal
 - $M = \frac{\text{Mean EPP amplitude}}{\text{Mean mini amplitude}}$



Evidence for the quantal hypothesis

1. MEPP (or “mini”) amplitude declines with distance from neuromuscular junction
⇒ Minis arise at the synapse
2. Minis disappear when nerve is removed
⇒ originate in the nerve
3. Minis disappear when inhibitors of acetylcholine action are applied
⇒ Minis are caused by ACh neurotransmitter
4. Minis are mimicked by puffing ~5,000 molecules of acetylcholine at the neuromuscular junction
⇒ Minis are packets of neurotransmitter molecules (i.e. “quanta”) spontaneously released by nerve at the NMJ
5. Nerve evoked release looks like a very large mEPP. Smaller EPPs, are produced in multiples of a mEPP.
Synaptic potentials are due to the synchronous release of many quanta, each of which is a multimolecular packet of neurotransmitter

Electron microscopy showed axon terminals are filled with spherical vesicles



Evidence supporting the vesicle hypothesis

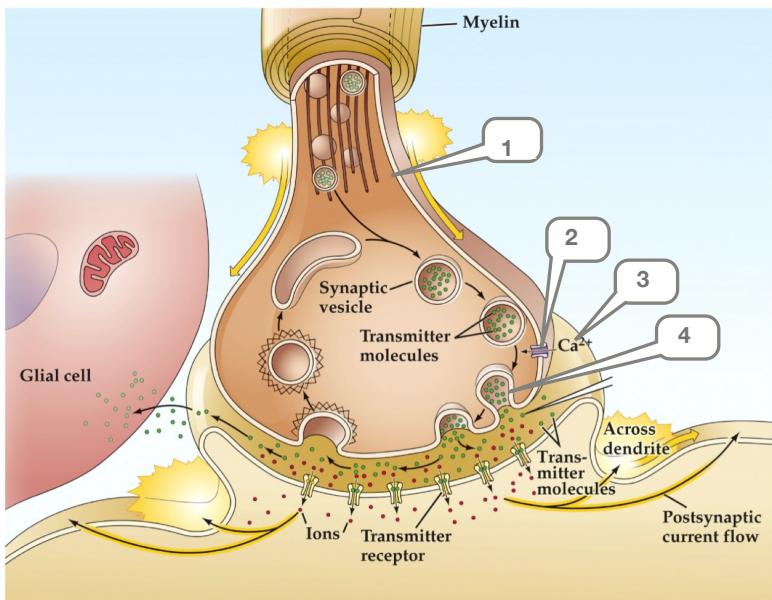
1. Numerous vesicles in nerve terminals (presynaptic part of synapses)
2. Omega figures (Ω) seen if the nerve is examined shortly (milliseconds) after it is stimulated
3. Prolonged high frequency stimulation leads to depletion of vesicles
4. Neurotransmitter has been isolated from vesicles. Each vesicle contains thousands
 - Therefore

Evidence supporting the vesicle hypothesis

1. Numerous vesicles in nerve terminals (presynaptic part of synapses)
 2. Omega figures (Ω) seen if the nerve is examined shortly (milliseconds) after it is stimulated
 3. Prolonged high frequency stimulation leads to depletion of vesicles
 4. Neurotransmitter has been isolated from vesicles. Each vesicle contains thousands
- Therefore... synaptic vesicles are the anatomical substrate for quanta

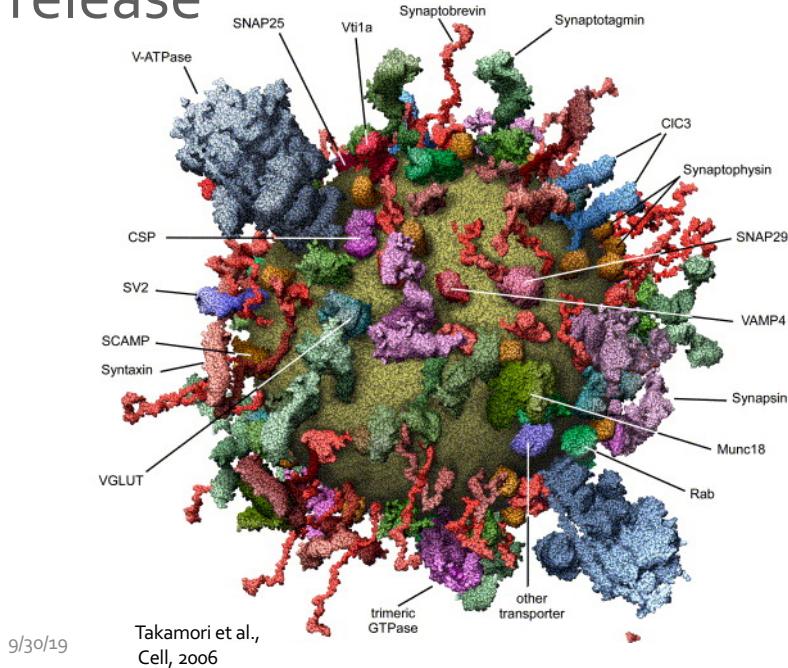
1 quantum = 1 vesicle

Voltage-dependent calcium entry couples presynaptic action potentials to quantal release of NT



1. Action potential conducted along axon and into presynaptic nerve terminal
 2. The ensuing depolarization opens voltage-gated calcium channels
 3. $I_{\text{Ca}} = g_{\text{Ca}} (V_m - E_{\text{Ca}})$ so Ca flows in even at peak pf depolarization ($E_{\text{Ca}} = 120\text{mV}$)
 4. Intracellular Ca triggers vesicle fusion and neurotransmitter release
- Vesicles released proportional to $[\text{Ca}^{++}]^4$ so 2x more extracellular Ca leads to 16x more quanta released

The SNARE hypothesis is the molecular mechanism linking intracellular $[Ca^{++}]$ to vesicle release



The synaptic vesicle is probably the most studied organelle. Essentially ALL of its proteins have been identified and studied

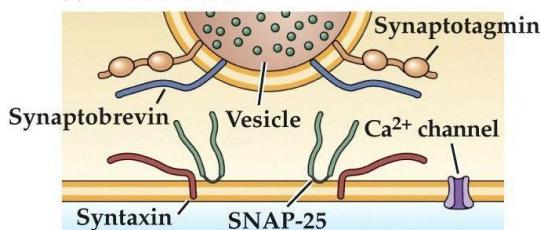
Interactions between SNARE proteins on the vesicle and the presynaptic plasma membrane, modulated by calcium, lead to vesicle fusion.

Nobel Prize to
Tom Sudhof and
James Rothman, 2013

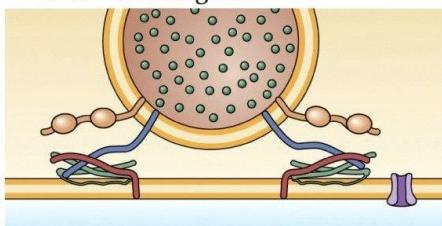


Vesicle fusion is catalyzed by SNARE proteins twisting together

(1) Vesicle docks



(2) SNARE complexes form to pull membranes together



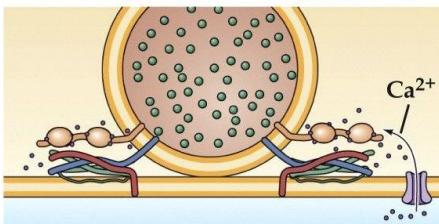
NEUROSCIENCE 5e, Figure 5.14 (Part 2)

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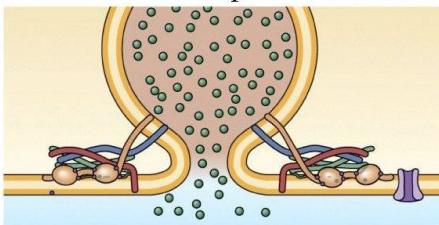
- Proteins that bring membranes together:
 - **Syntaxin** and **SNAP-25**: found on presynaptic plasma membrane
 - **Synaptobrevin**: found on synaptic vesicles
 - **Synaptotagmin**: found on vesicles, acts as Ca⁺⁺ sensor
- **Syntaxin**, **SNAP-25**, **Synaptobrevin** and ATP “Zipper” down into a tight, high energy complex (nanometers from fusion)

Vesicle fusion is catalyzed by SNARE proteins twisting together

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

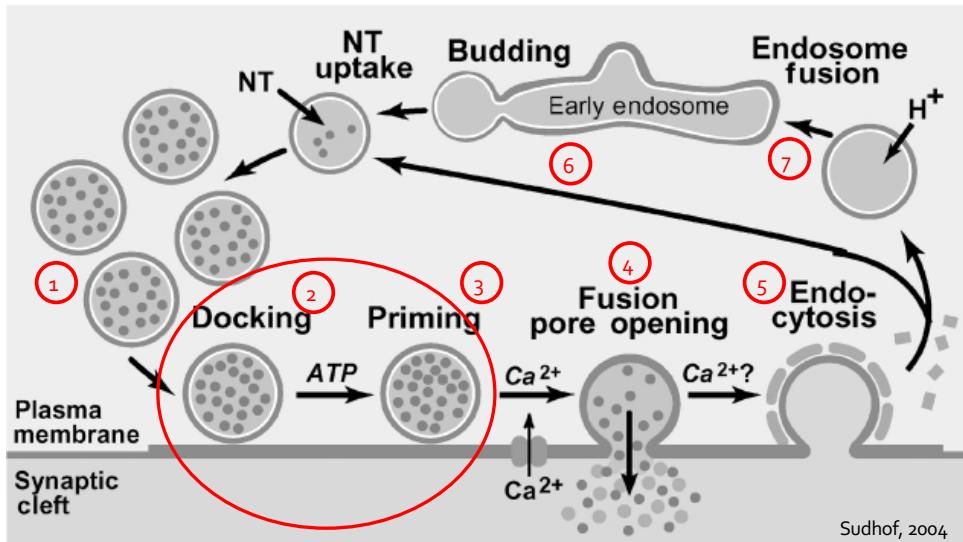


(4) Ca^{2+} -bound synaptotagmin catalyzes membrane fusion by binding to SNAREs and the plasma membrane



- Proteins that bring membranes together:
 - **Syntaxin** and **SNAP-25**: found on presynaptic plasma membrane
 - **Synaptobrevin**: found on synaptic vesicles
 - **Synaptotagmin**: found on vesicles, acts as Ca^{++} sensor
- **Syntaxin**, **SNAP-25**, **Synaptobrevin** and ATP “Zipper” down into a tight, high energy complex (nanometers from fusion)
- Entering Ca^{++} binds to **Synaptotagmin**
- Confirmation change of **Synaptotagmin** causes vesicle fusion (very fast – 0.2 ms)

Vesicle lifecycle

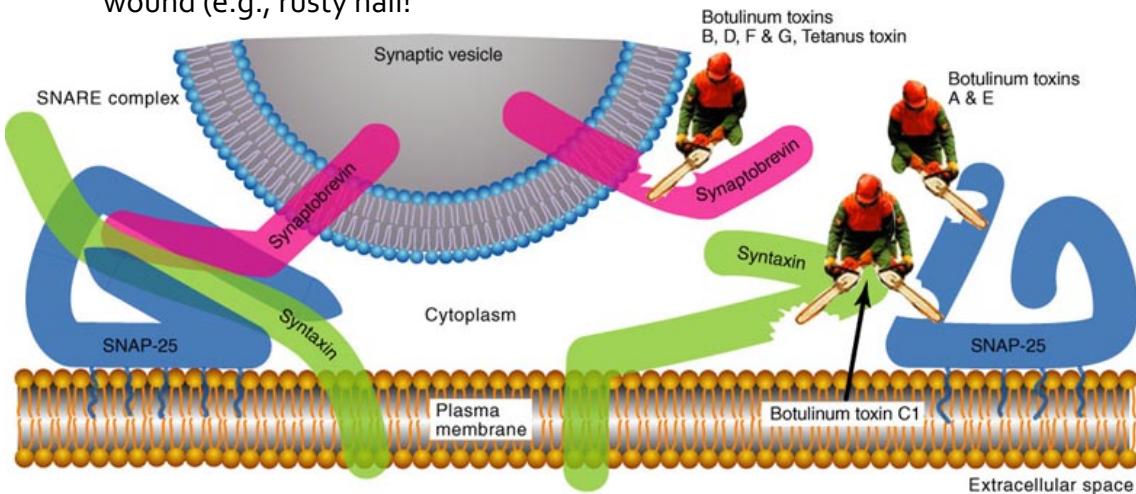


- Vesicles cluster at the active zone (1), dock (2), and are primed (3) to ready them for Ca^{2+} -triggered fusion (4).
- After NT is released, vesicles are endocytosed (5) and recycled.
- Vesicles are refilled with NT directly (6) or after passing through an endosomal intermediate (7).
- ATP is

There is a subset of vesicles docked and primed
(Readily-Releasable Pool)

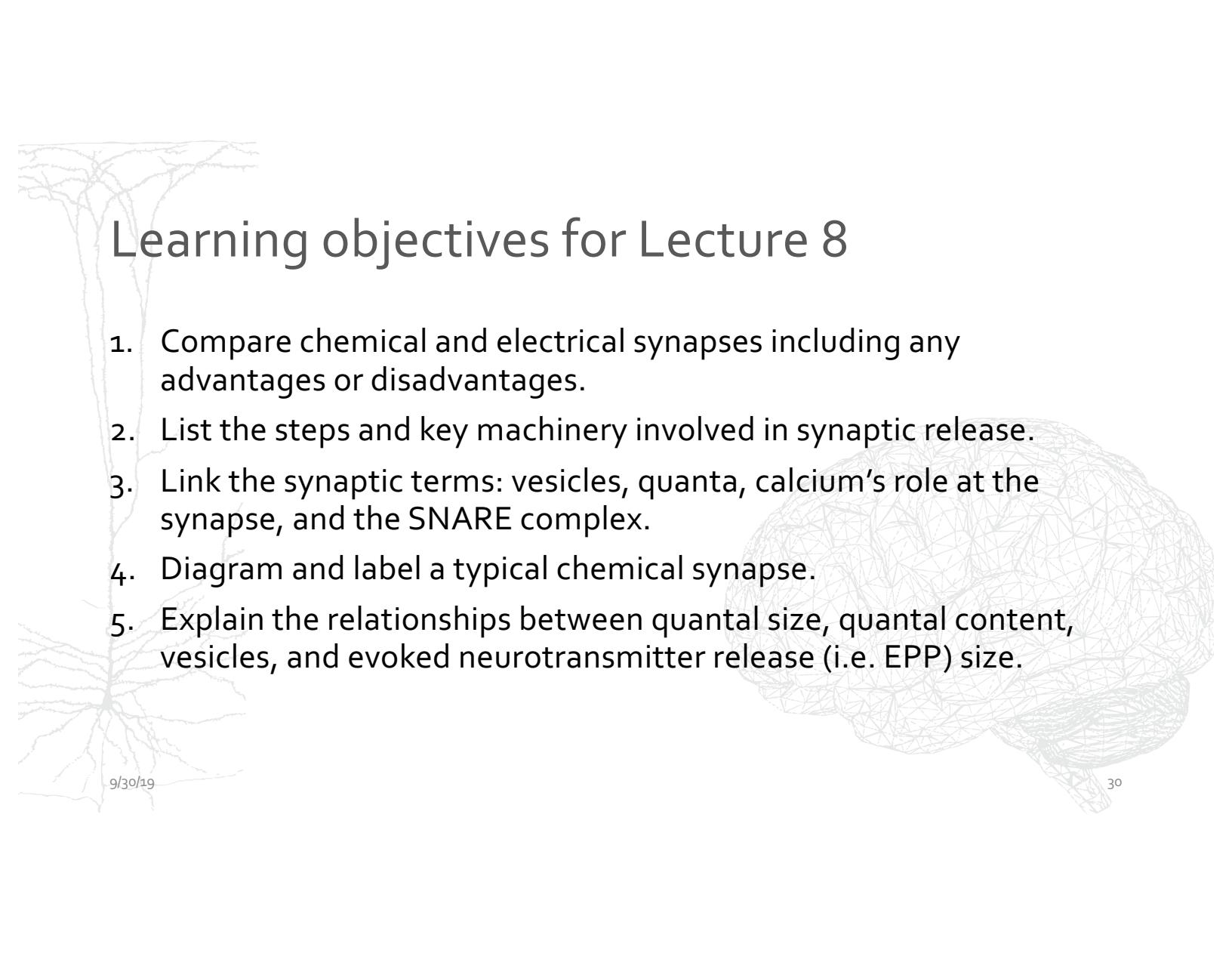
Many toxins affect SNARE proteins

- Botulism: paralytic disease caused by a toxin produced by a species of anaerobic *Clostridium* bacteria. Bacteria can infect the gut, or toxin can be present in food. Leads to muscle weakness and sometimes lethal respiratory paralysis.
- Tetanus (lockjaw): disease involving uncontrollable muscle spasms caused by a different toxin produced by a different *Clostridium* bacteria. Bacteria usually enter the body through a wound (e.g., rusty nail!)



Botulism Toxin - Low doses of toxin are injected into facial muscles to prevent acetylcholine release and relax muscles





Learning objectives for Lecture 8

1. Compare chemical and electrical synapses including any advantages or disadvantages.
2. List the steps and key machinery involved in synaptic release.
3. Link the synaptic terms: vesicles, quanta, calcium's role at the synapse, and the SNARE complex.
4. Diagram and label a typical chemical synapse.
5. Explain the relationships between quantal size, quantal content, vesicles, and evoked neurotransmitter release (i.e. EPP) size.

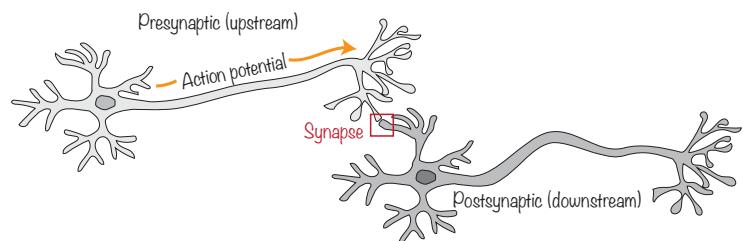
Lecture 8 - Synapses: structure and function

Pre-class notes for September 30, 2019

Reading: *Neuroscience* by Purves et al., pages 85-99

Synapses are where much of the information processing of the nervous system comes into play. Synapses are where signals can be changed from excitatory or inhibitory and as information passes from one neuron to the next it is where the information is integrated and dispersed through the nervous system. As we are discussing the connections between two neurons, we need specific terms to identify the neuron that is sending vs receiving the synaptic message:

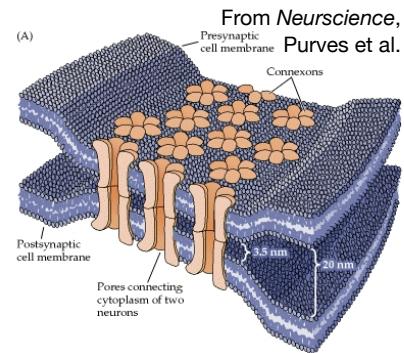
Presynaptic - the neuron that releases the neurotransmitter or sends a signal, usually caused by a presynaptic action potential. Known as the “upstream” neuron.



Postsynaptic - the neuron that receives a signal from the presynaptic neuron. Known as the “downstream” neuron.

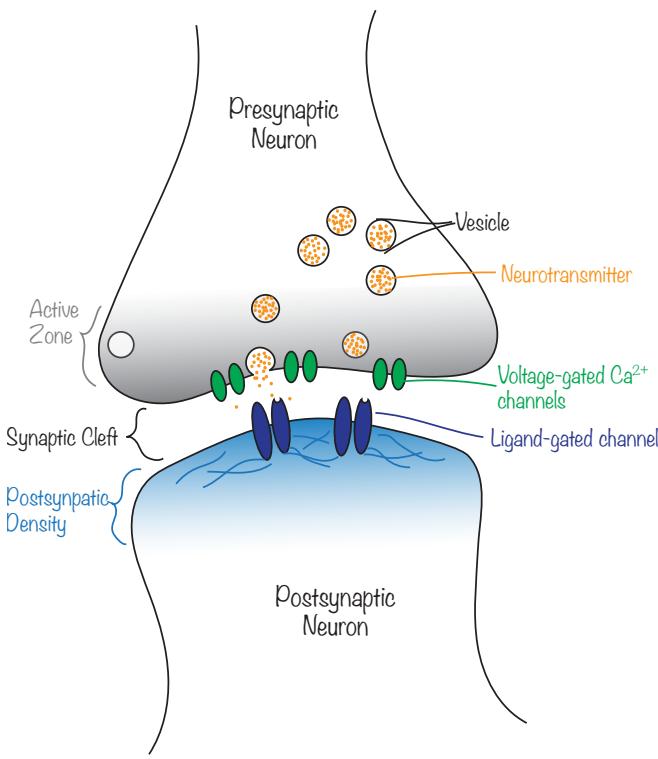
Although Golgi's reticular theory has largely been refuted, there are some places in the nervous system where there is continuity between neurons and electric signals can pass directly from one neuron to the next. **Electrical synapses**, are where **gap junctions** connect the cytoplasm between two cells and allow the direct passage of electrical current (ions). They allow for the fast and often bidirectional transfer of electrical information between cells. **Connexon** is the specialized membrane protein (channel) that connect two cells at gap junctions. Composed of *connexin* subunits on each side, an electrical synapse will have several *connexon* channels to allow the flow of ions between the neurons.

Because there is direct electric connection, electrical synapses are incredibly fast with the postsynaptic response occurring less than 1 ms after the change in the presynaptic membrane potential. Electrical synapses are especially useful for synchronizing populations of neurons or when the response needs to be very reliable and quick.



The vast majority of synapses in the nervous system are **chemical synapses** - Which are functional connections between two cells where information can be transferred from one cell to the next without being physically connected. At chemical synapses, an action potential in the presynaptic cell triggers the release of neurotransmitter molecules which bind to receptors on the postsynaptic cell membrane and lead to a response in the postsynaptic cell. Chemical synapses may be **excitatory** (where the release of neurotransmitter increases the likelihood that the *postsynaptic* neuron fires an action potential), or **inhibitory** (where the release of neurotransmitter decreases the likelihood that the *postsynaptic* neuron fires an action potential) they may be strong or weak as well as fast or slow .

The **Neuromuscular Junction (NMJ)** is a chemical synapse between a motor neuron axon and a muscle fiber. Typically it is a large, powerful, fast synapse with multiple vesicles released per presynaptic action potential. Because the NMJ is easily accessible and large, it was the focus of much of the pioneering research. Some of the important features of a synapse are:



Active zone - area on the presynaptic neuron where vesicles fuse with the cell membrane to release neurotransmitter.

Postsynaptic density - structures on the post-synaptic neuron that contains neurotransmitter receptors and associated cyto-skeletal elements.

Synaptic cleft - space between the pre- and postsynaptic neurons at a chemical synapse. Approximately 20 nm.

Vesicle - membrane bound organelle that contains neurotransmitter molecules in the presynaptic terminal.

Neurotransmitter - molecule that is packaged in vesicles in the presynaptic neuron and released to bind to receptors.

Voltage-gated Calcium channel - calcium channels clustered near the active zone of a presynaptic terminal that are voltage gated and open when the presynaptic terminal is depolarized by an action potential.

Our understanding of neurotransmitters and how they are released is based on 4 successive hypothesis that each build on each other:

Quantal hypothesis - neurotransmitter is released in discrete “packets” or “quanta”

Vesicle hypothesis - neurotransmitter is packaged into and released from membrane bound vesicles

Calcium hypothesis - voltage-dependent calcium entry couples presynaptic action potential to neurotransmitter release

SNARE hypothesis - interactions between SNARE proteins on the vesicle and presynaptic membrane, modulated by calcium, lead to vesicle fusion

Electrophysiological analysis of the synaptic potentials at the NMJ by the scientist Bernard Katz provided the initial support for these hypothesis.

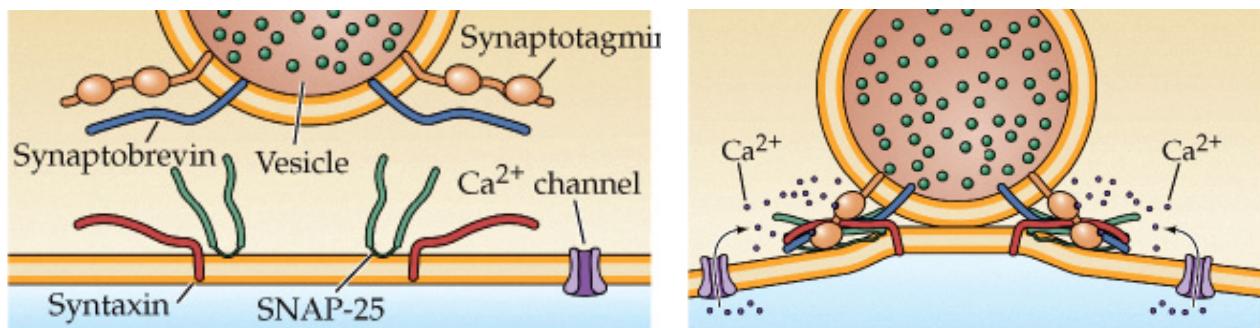
End plate potential (EPP) - change in membrane potential of a muscle fiber (the area near the synapse is also called the *end plate*) as a result of a presynaptic action potential which evokes the release of vesicles at the neuromuscular junction. Also known as an evoked response.

Miniature end plate potential (mini or mEPP) - change in membrane potential of a muscle fiber after a vesicle in the *presynaptic terminal* spontaneously fuses with the presynaptic cell membrane.

Quantal Content - the average number of quanta (vesicles) that are released after a single presynaptic action potential.

Quantal Size - the average size of the *postsynaptic* membrane depolarization (in mV) after a single quantal event (i.e. fusion of one vesicle with the presynaptic terminal cell membrane).

SNARE complex - a group of proteins that cause vesicle fusion and release of neurotransmitter in response to an elevation in Ca^{2+} levels in the presynaptic terminal. Consists of proteins on both the presynaptic cell membrane at the active zone (*Syntaxin* and *SNAP-25*) as well as proteins on the vesicle membrane (*Synaptobrevin* and *Synaptotagmin*). *Synaptotagmin* is a calcium sensor, it binds calcium and triggers vesicle fusion.



Learning Objectives: (By the end of Lecture 8 you should be able answer the following)

1. Compare chemical and electrical synapses including any advantages or disadvantages.
2. List the steps and key machinery involved in synaptic release.
3. Link the synaptic terms: vesicles, quanta, calcium's role at the synapse, and the SNARE complex.
4. Diagram and label a typical chemical synapse.
5. Explain the relationships between quantal size, quantal content, vesicles, and evoked neurotransmitter release (i.e. EPP) size.