

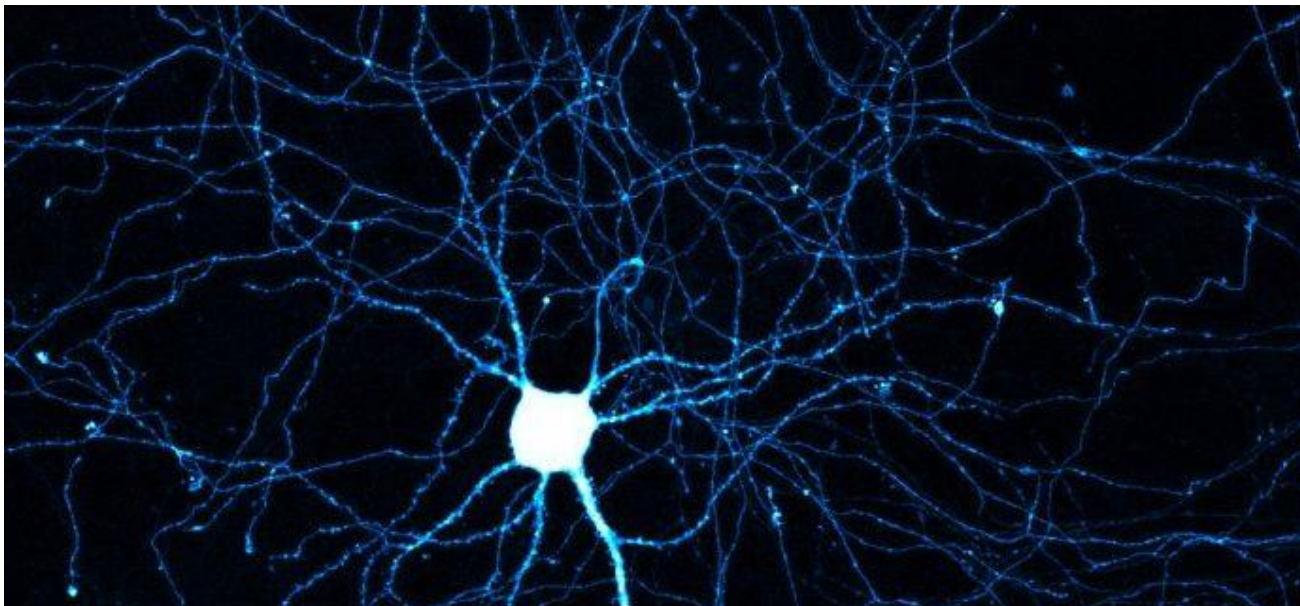
A crash course in Neuronal Biophysics

Neurons, action potentials and synapses

Julie Semmelhack

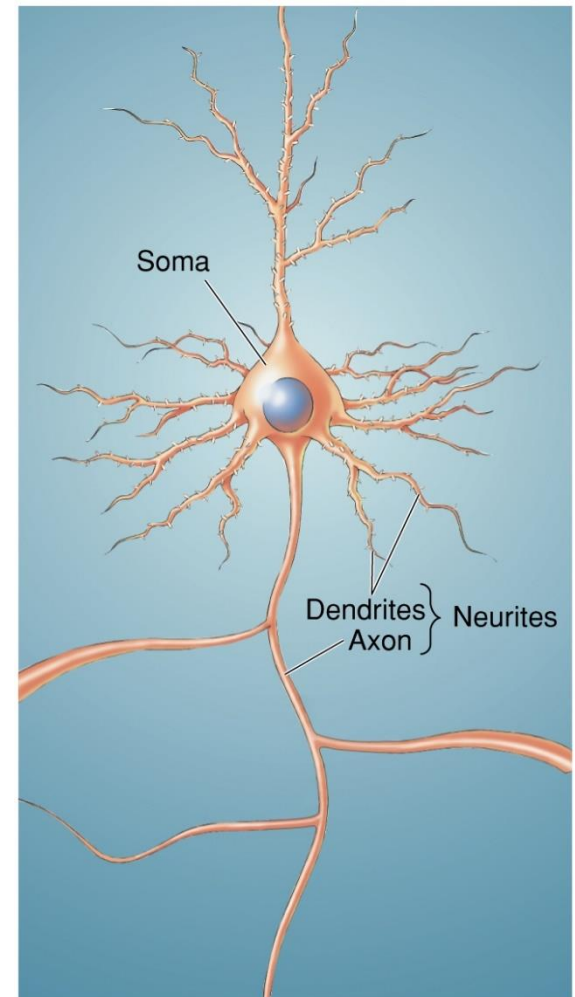
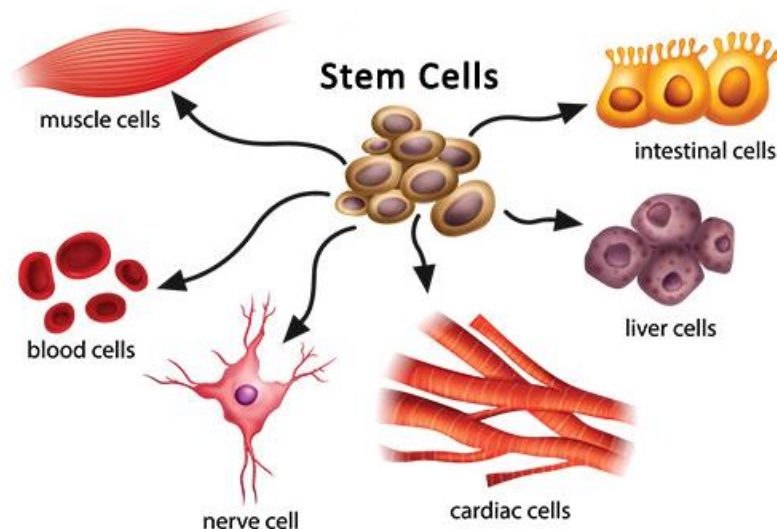
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Questions are welcome!



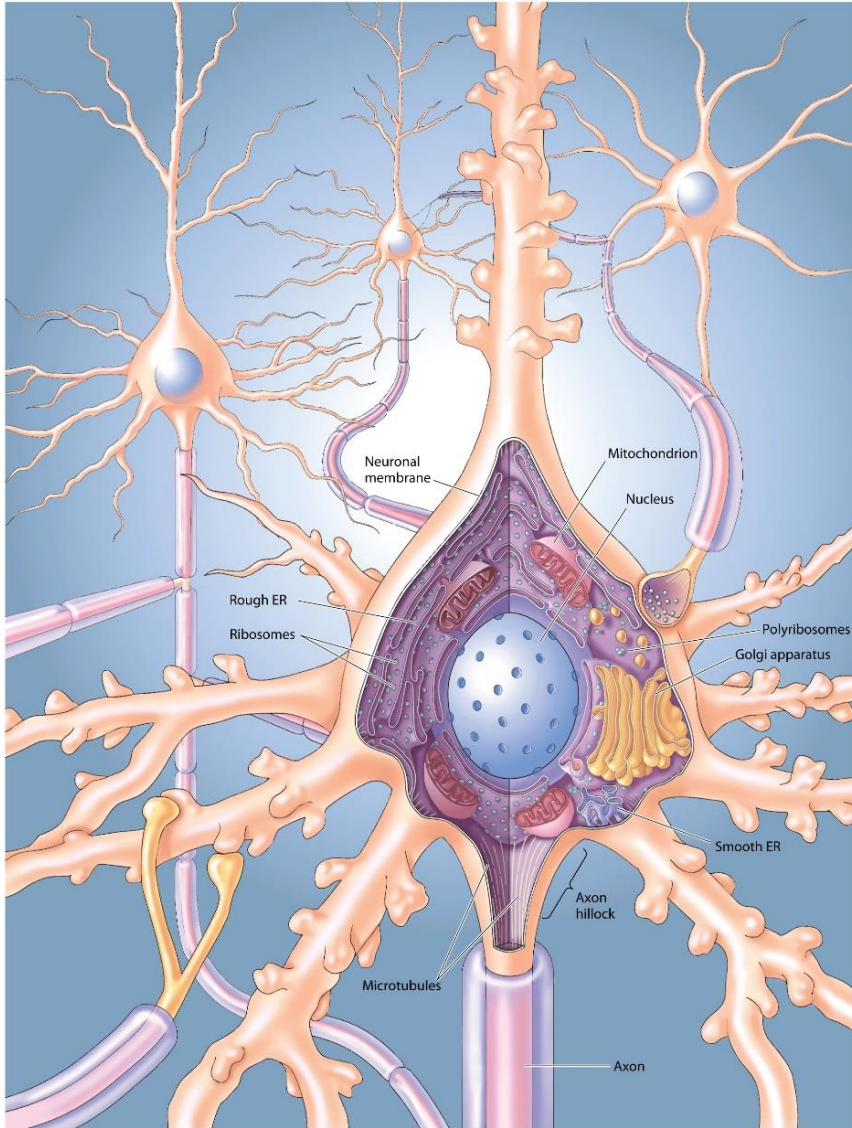
Neurons are highly specialized cells

- Neurons are one possible outcome of cell differentiation
- Expression of neuron-specific genes gives them specialized structure and functional properties
- The key features of neurons; they are excitable (fire APs [action potentials]) and communicate with other neurons, or muscles



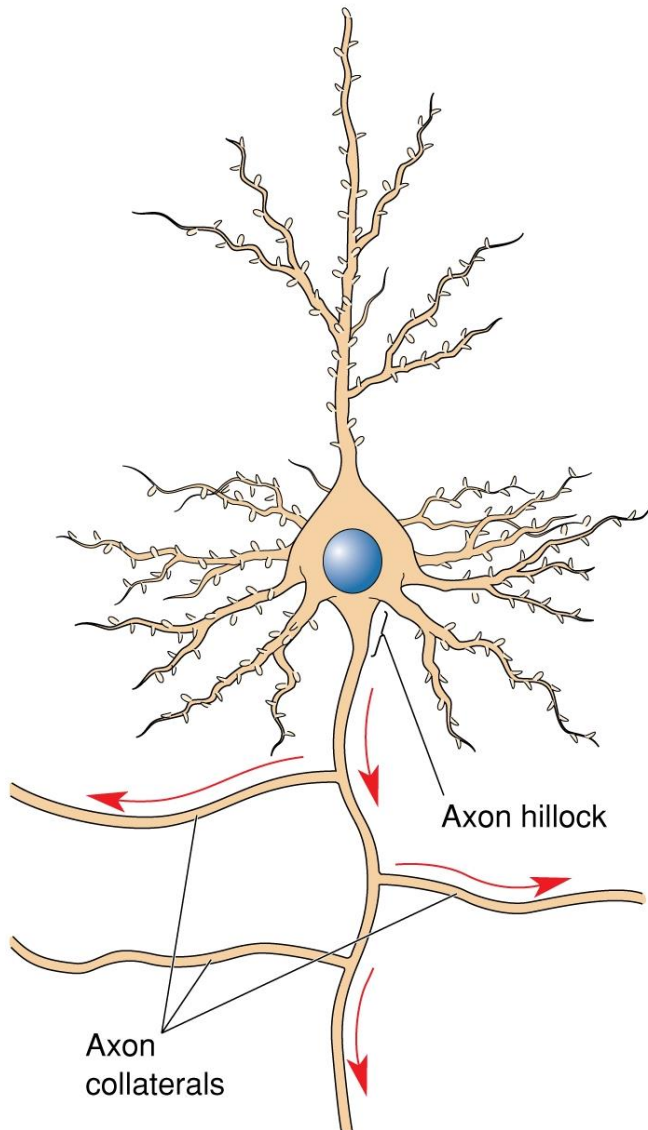
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Close-up of a Neuron



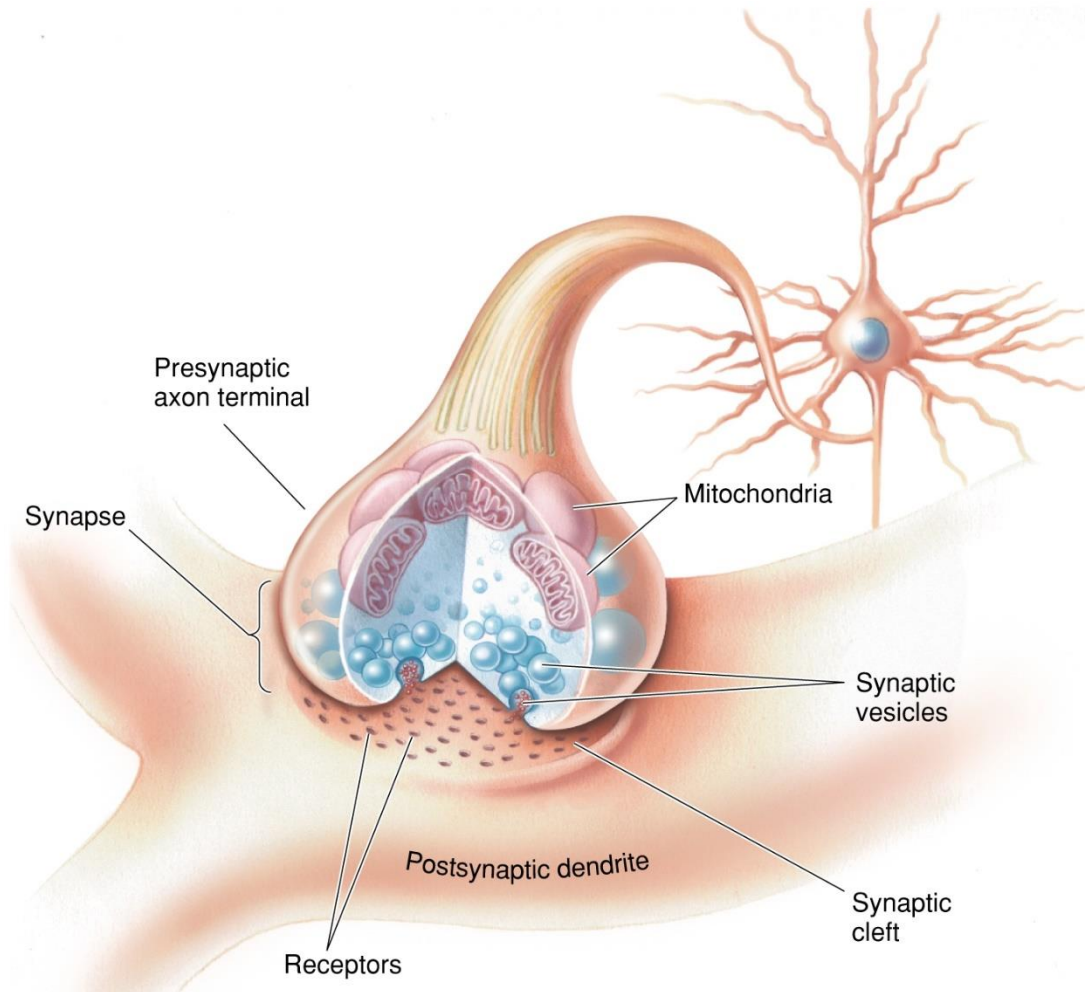
- Neurons have many features that are also present in other cell types
- Cell body has essential components for metabolism, making proteins, etc.
- Processes have synapses
- The cell membrane separates cytoplasm from the surroundings

Axons



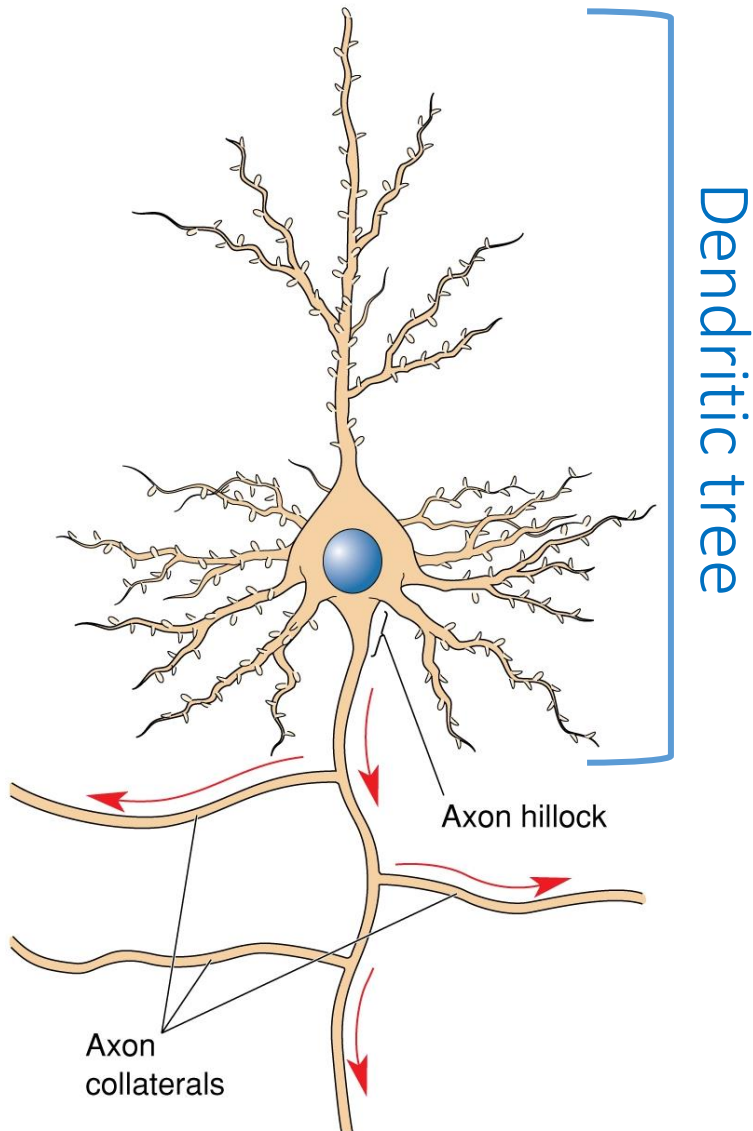
- The axon is a long fiber that conveys the output of the neuron
- Can be branched

Axon terminal



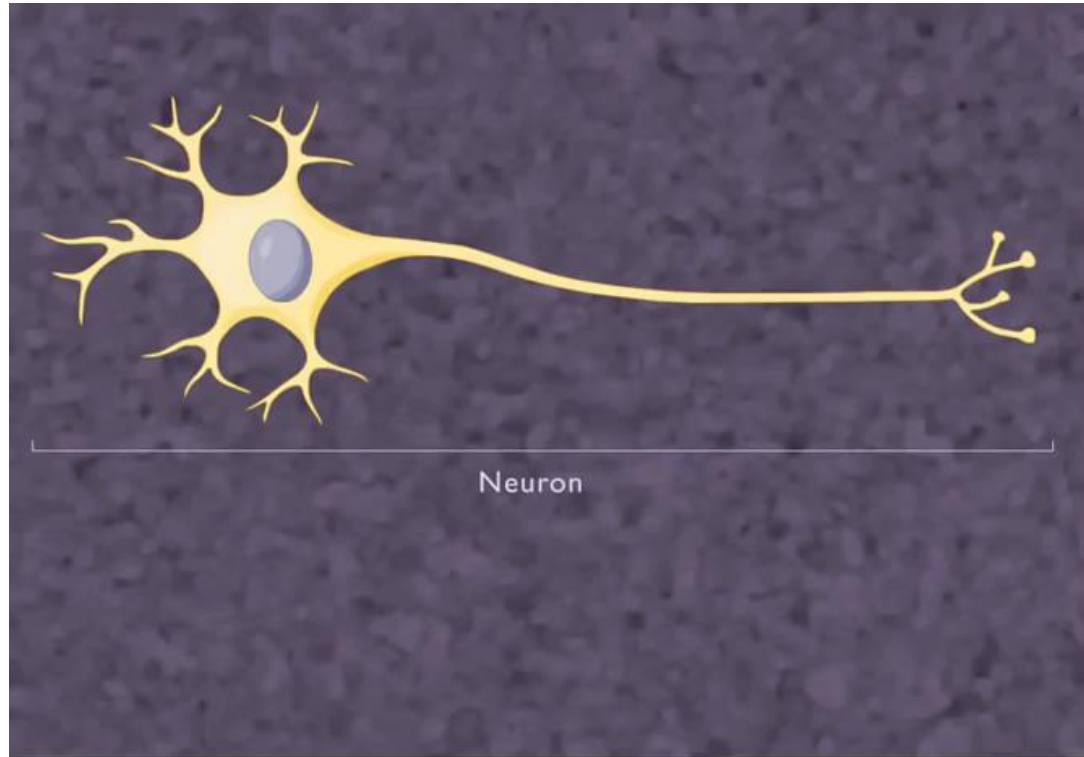
- Axons typically end in a swollen region called a bouton.
- This is where a synapse is formed for communication with other neurons
- More on this in Lecture 4!

Dendrites



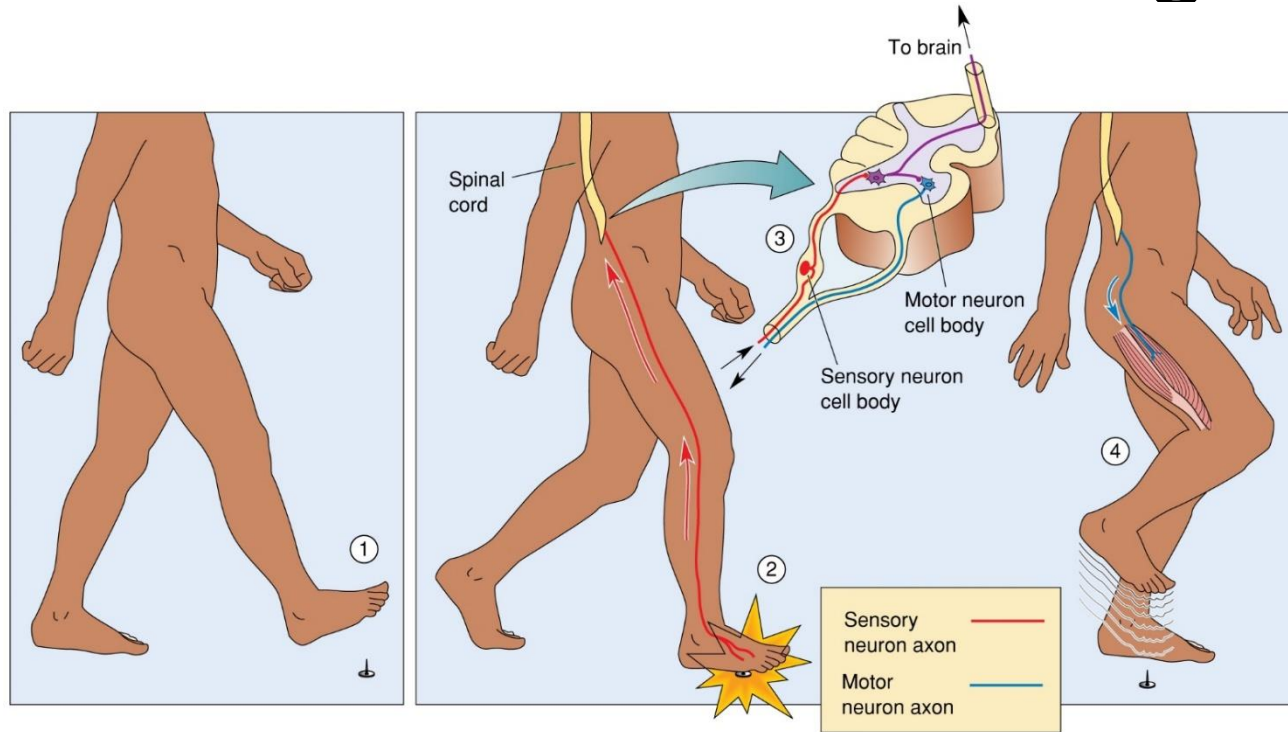
- The part of the neuron that receives information
- Often highly branched like a tree

Neurons have excitable cell membranes and can produce action potentials



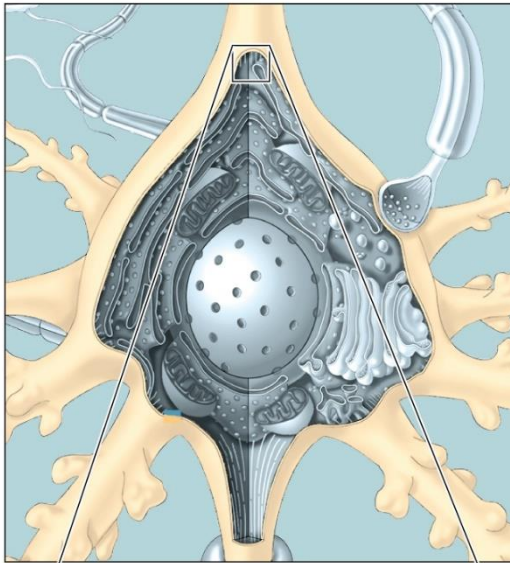
- The action potential is a fast change in voltage that travels along the neuronal membrane.
- Q: Why do neurons need this?

Action Potentials allow fast signalling

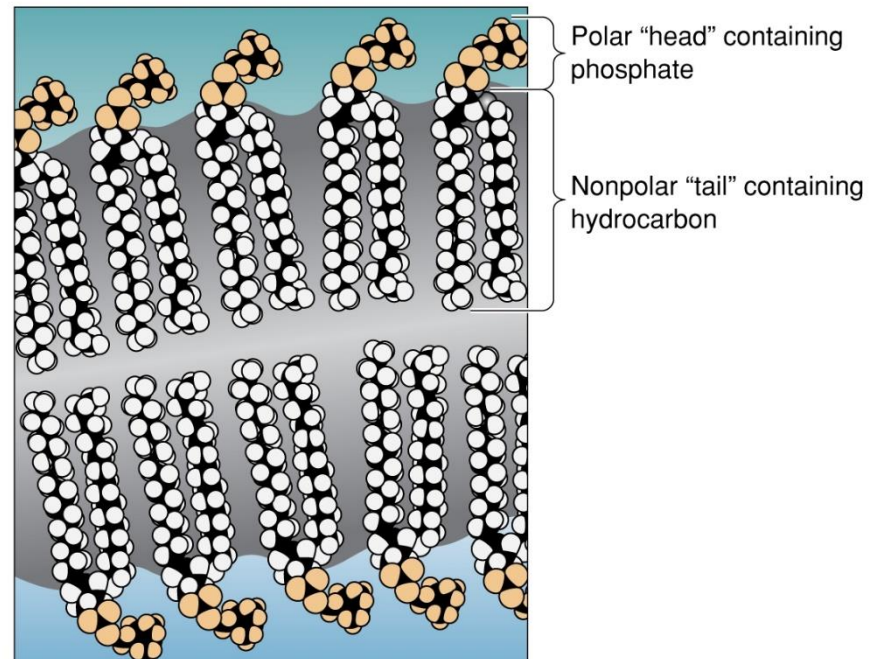
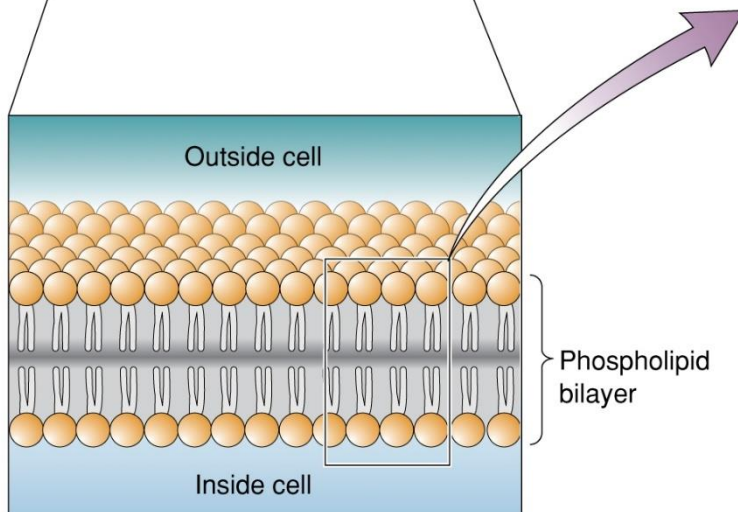


- Most cellular signalling mechanisms are limited by diffusion
- Neurons have to be faster to transmit sensory information into a response with a short reaction time
- Example; pain from stepping on a sharp object leads to withdrawing the foot
- Action potentials can exceed 100 m/s
- Firing action potentials requires certain special features of the neuronal membrane

The Cell Membrane

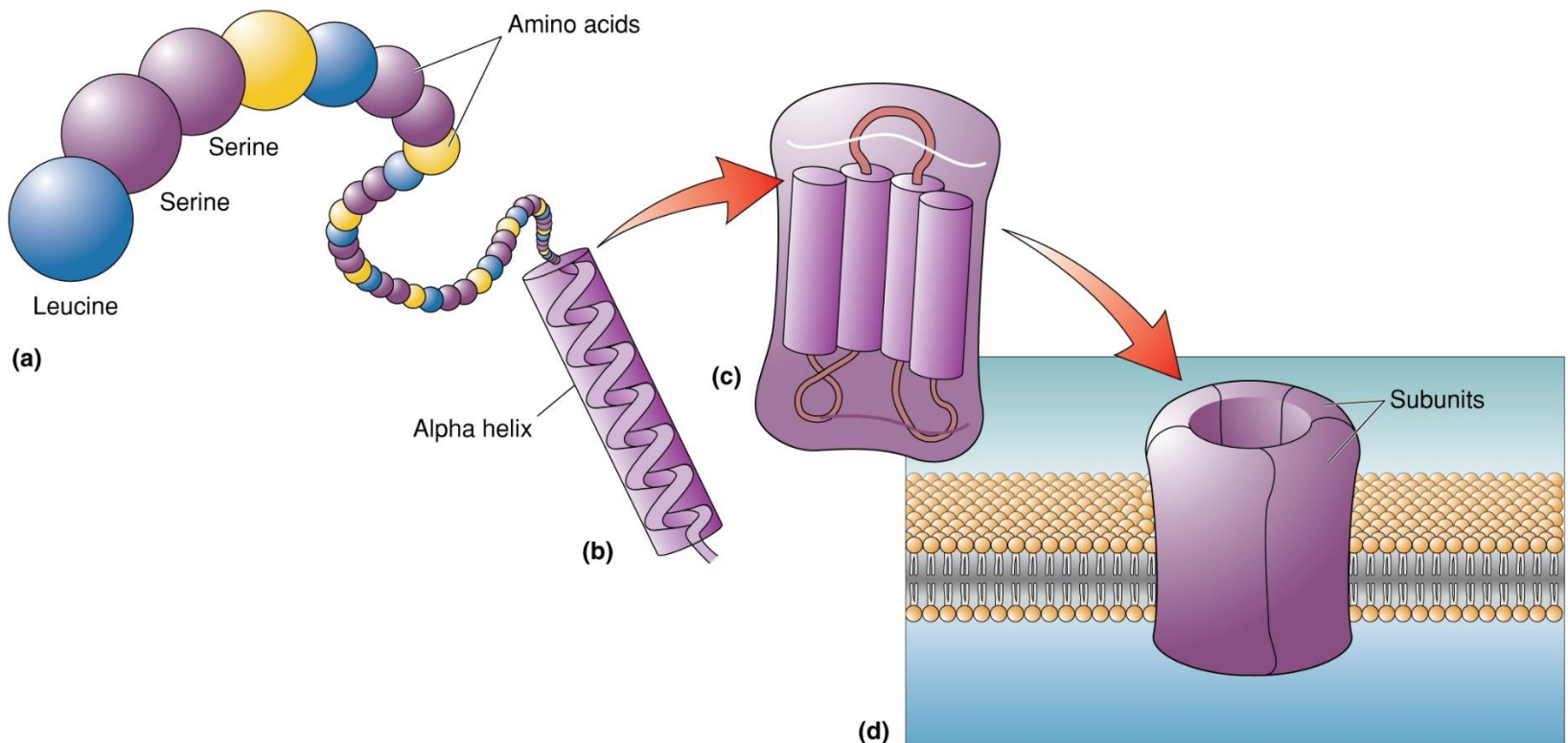


- Basic structure of neuronal membrane is similar to other cells
- Phospholipid bilayer forms a barrier to water-soluble ions
- Separates cytosol (intracellular fluid) from extracellular fluid.



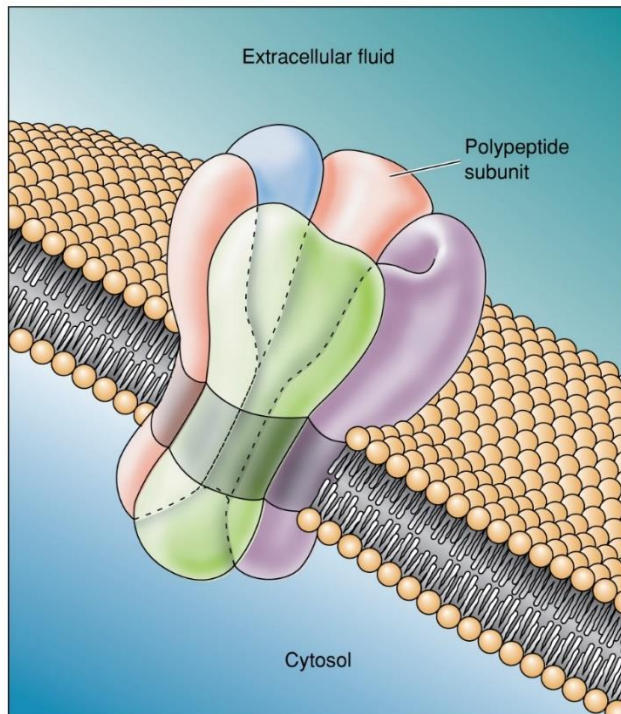
Proteins in the membrane

- Proteins are chains of amino acids.
- They have secondary structures such as alpha helices
- Proteins further fold to form a 3D tertiary structure
- Multiple subunits can come together to form a larger structure

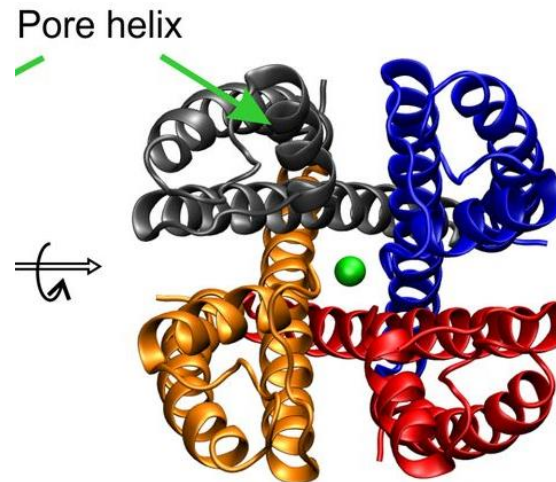


Ions can pass through the membrane via membrane-spanning proteins

- Ion Channels are membrane-spanning proteins that form a pore.
- Often made up of multiple subunits
- Most are selective for certain ions, e.g. K^+
- They are gated (opened by certain events), e.g. ligand binding
- They passively allow ions to cross the membrane

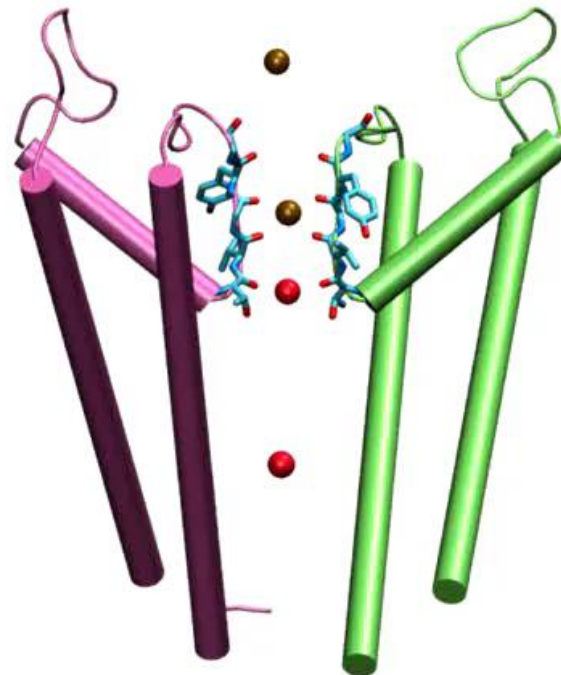
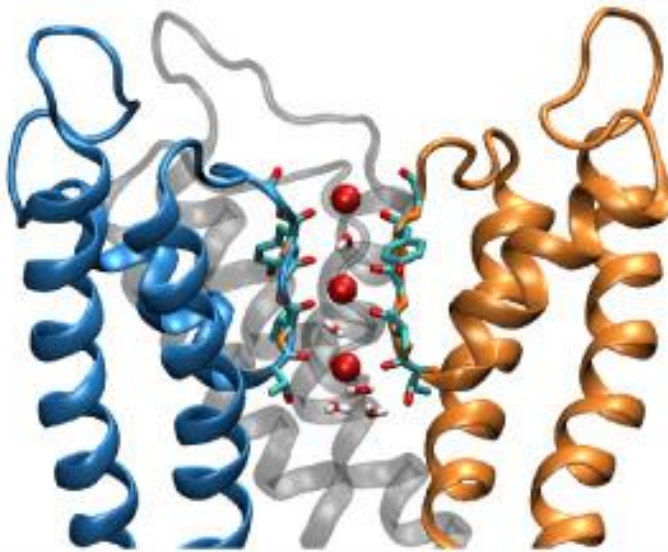


Potassium Channel



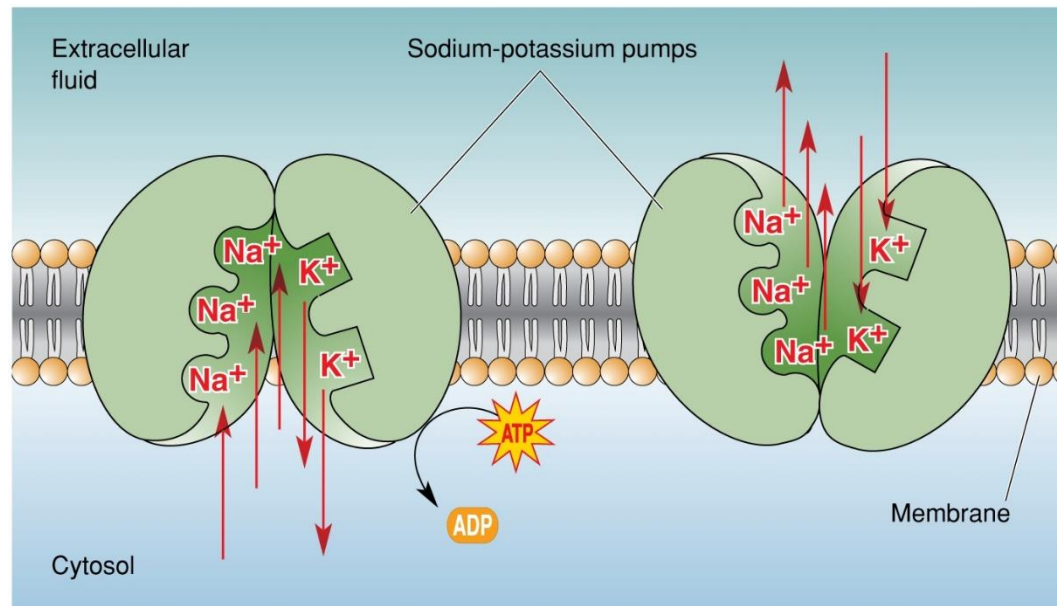
Ion Channel selectivity

- Example; *Shaker* potassium channel
- The pore loop has a sequence of 5 amino acids, TVGYG
- These are highly conserved among Potassium channels
- This region is lined with Oxygen atoms which interact with K⁺ ions
- Other types of ions cannot pass through

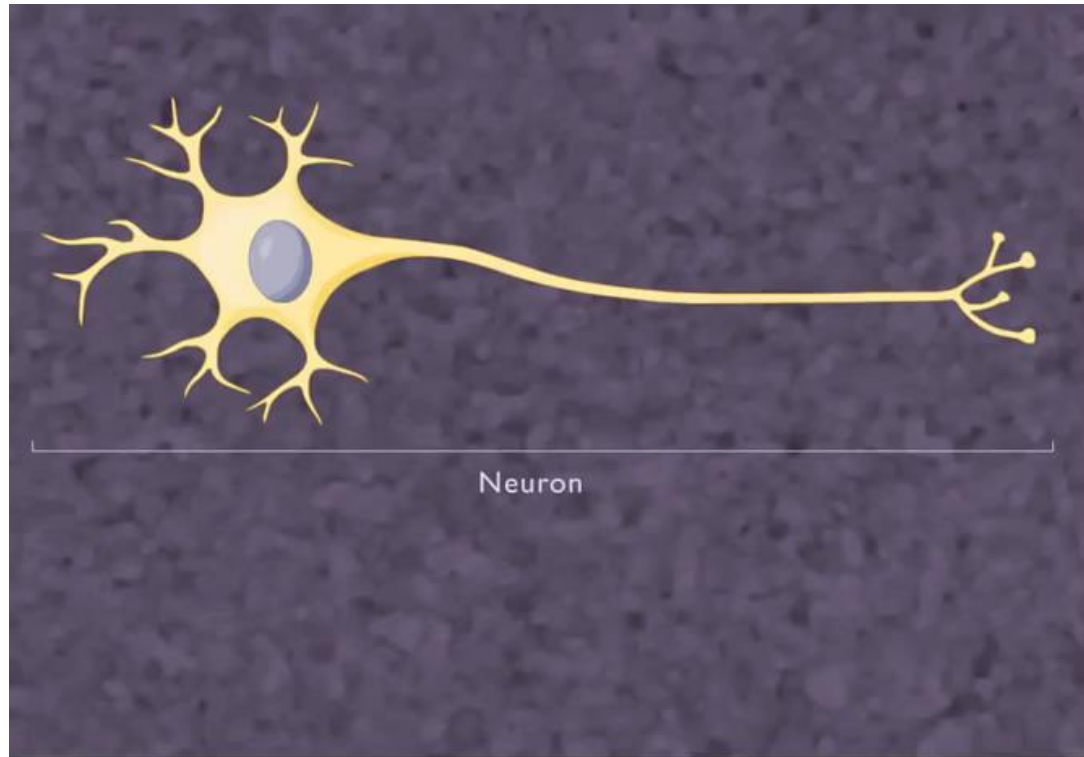


Ions can pass through the membrane via membrane-spanning proteins

- Ion pumps can also transport ions across the cell membrane.
- Operation requires ATP; therefore active
- Pumps play a key role in maintaining balance of ions required to generate action potentials

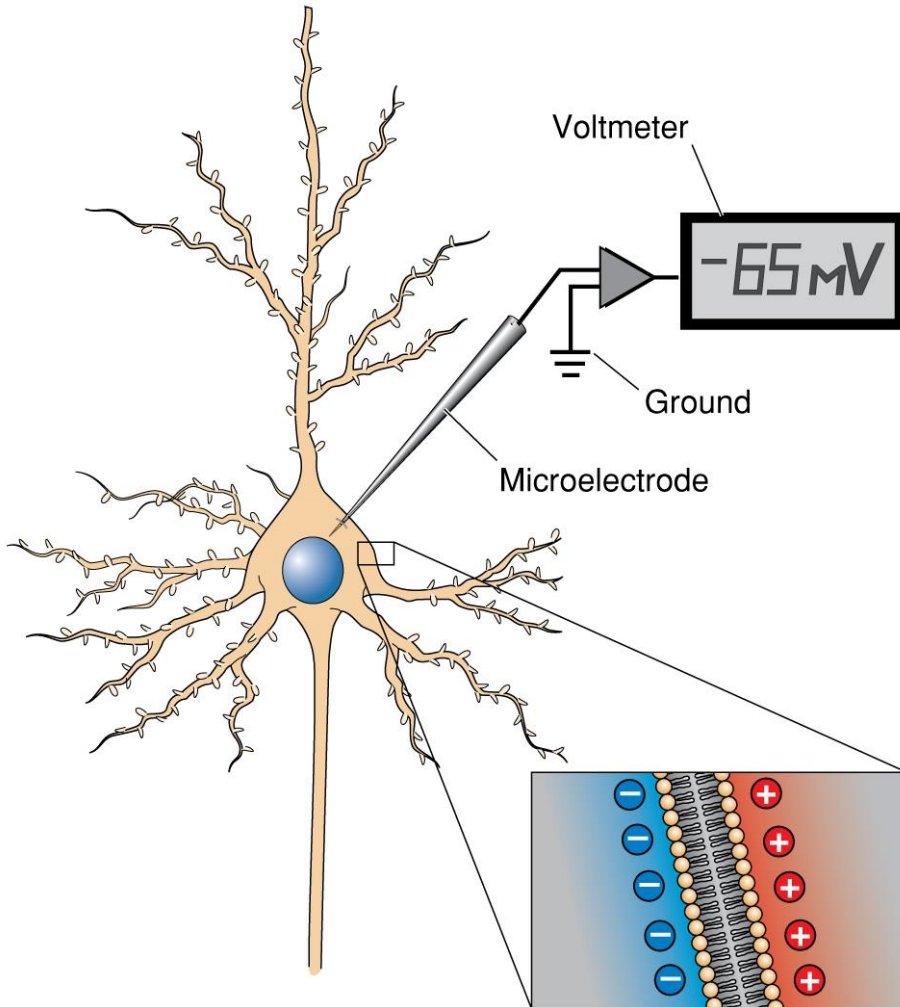


The action potential is a reversal of the resting membrane potential



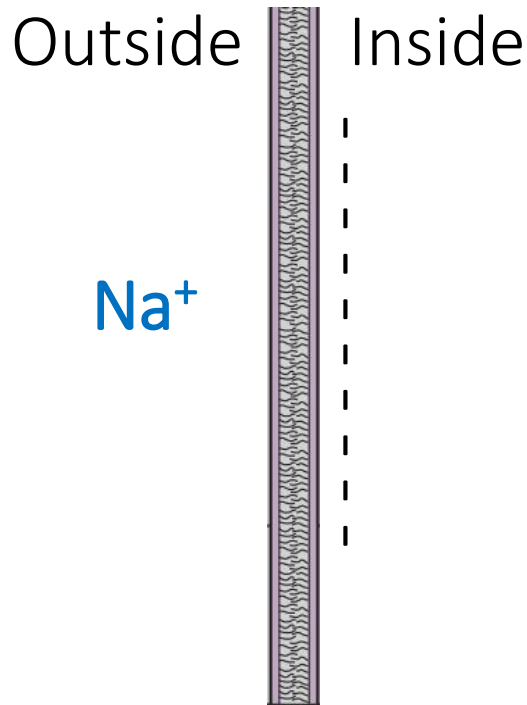
- This reversal travels like a wave down the axon
- So... what is the resting membrane potential?

The Resting Potential



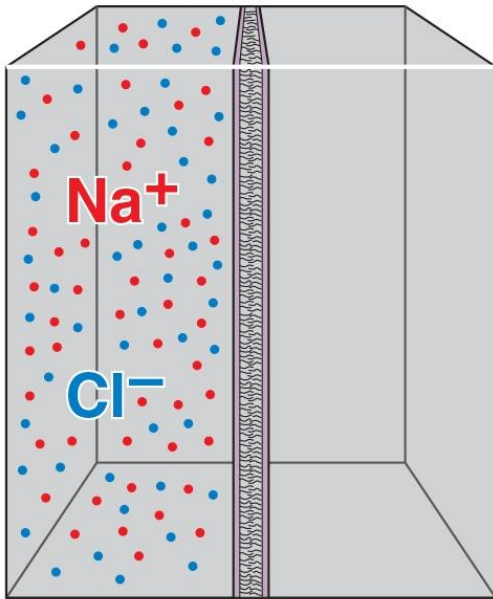
- Healthy neurons have a difference in electrical charge distribution across the cell membrane; this is the resting potential.
- The inside of the cell has more negatively charged ions
- This separation of charges can be measured as Voltage
- Conventionally the outside of the cell is zero, so the inside has a negative voltage, usually -60 to -70 mV.
- This potential can be measured with a microelectrode inside the cell

Electrical forces acting on ions; Cation and a cell membrane



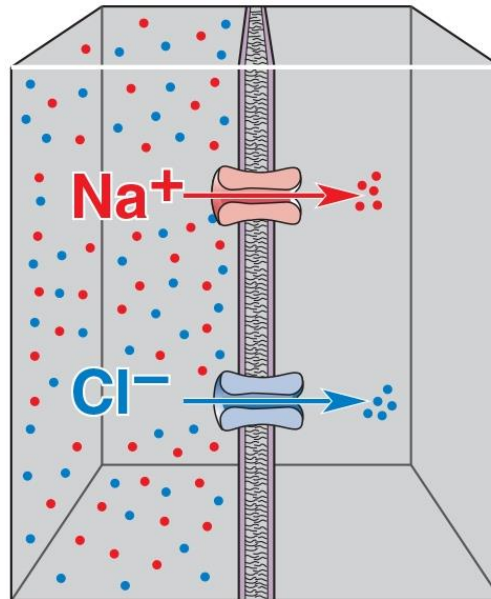
- Opposite charges attract and like charges repel
- Key cations in neurons: Potassium (K^+), Sodium (Na^+), Calcium (Ca^{2+}).
- Key anion: Chloride (Cl^-).
- Here we have a net negative charge inside the cell.
- If we open Sodium Channels, which direction will sodium ions flow?

Concentration gradients also affect ions



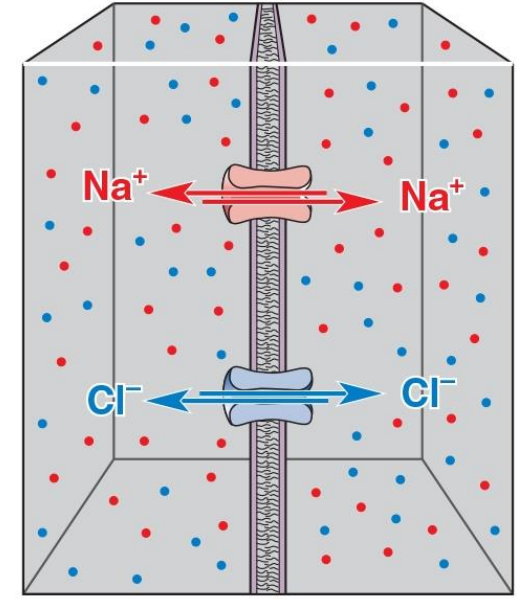
(a)

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(b)

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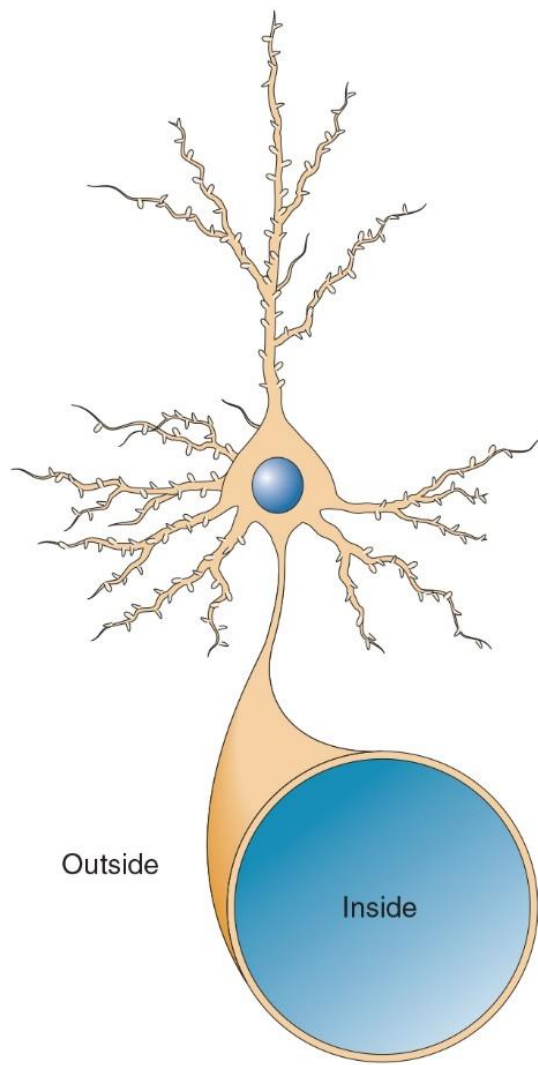


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- a) If the membrane is impermeable (as lipid bilayer is to Sodium and Chloride ions) no ions can pass
- b) When there are open channels the ions flow down their concentration gradient (from high concentration to low)
- c) After enough ions flow, and there are equal concentrations on each side, equilibrium is reached.

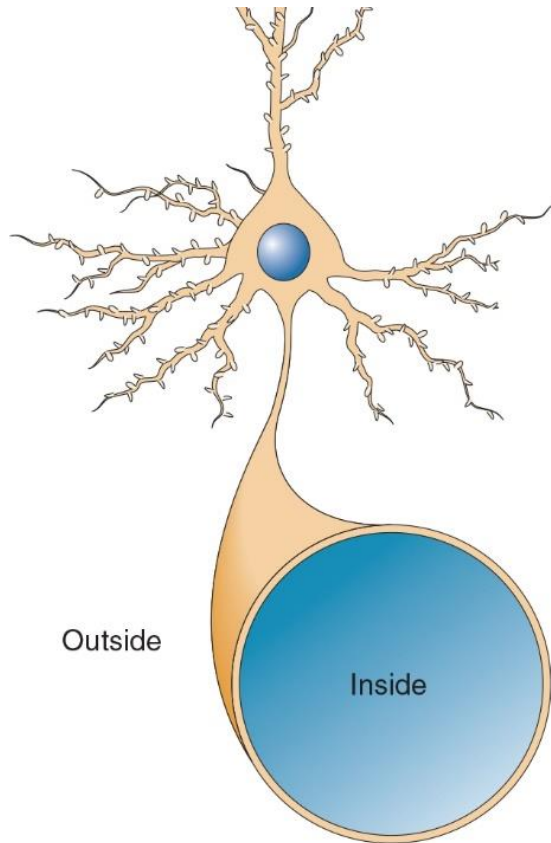
The distribution of ions across the membrane determines membrane potential



- In neurons, Potassium is more concentrated on the inside, Sodium and calcium are more concentrated on the outside.
- These gradients are established by pumps, e.g. the sodium-potassium pump.

Ion	Concentration outside (in mM)	Concentration inside (in mM)	Ratio Out : In	E_{ion} (at 37°C)
K^+	5	100	1 : 20	-80 mV
Na^+	150	15	10 : 1	62 mV
Ca^{2+}	2	0.0002	10,000 : 1	123 mV
Cl^-	150	13	11.5 : 1	-65 mV

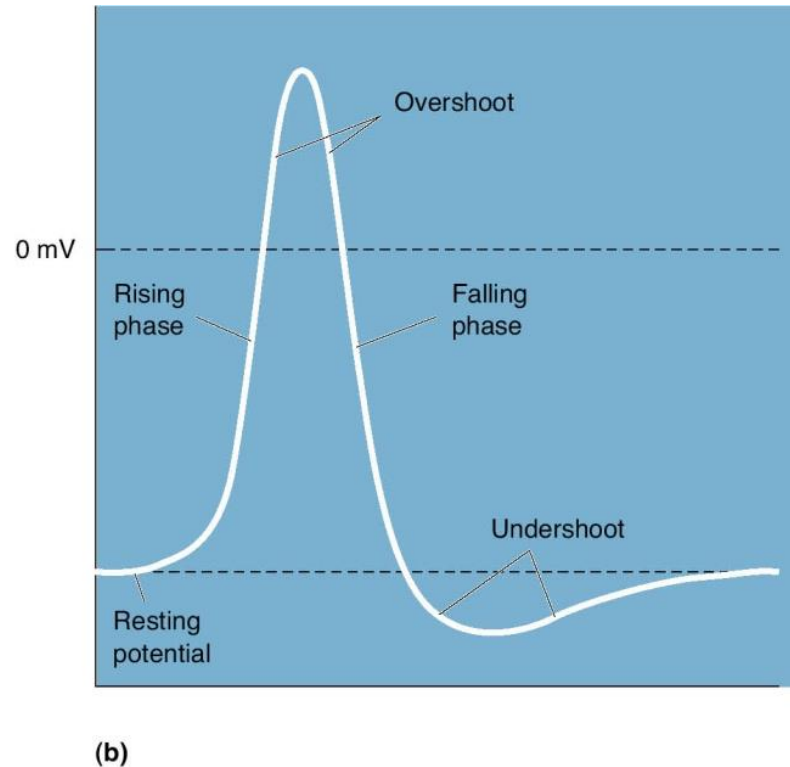
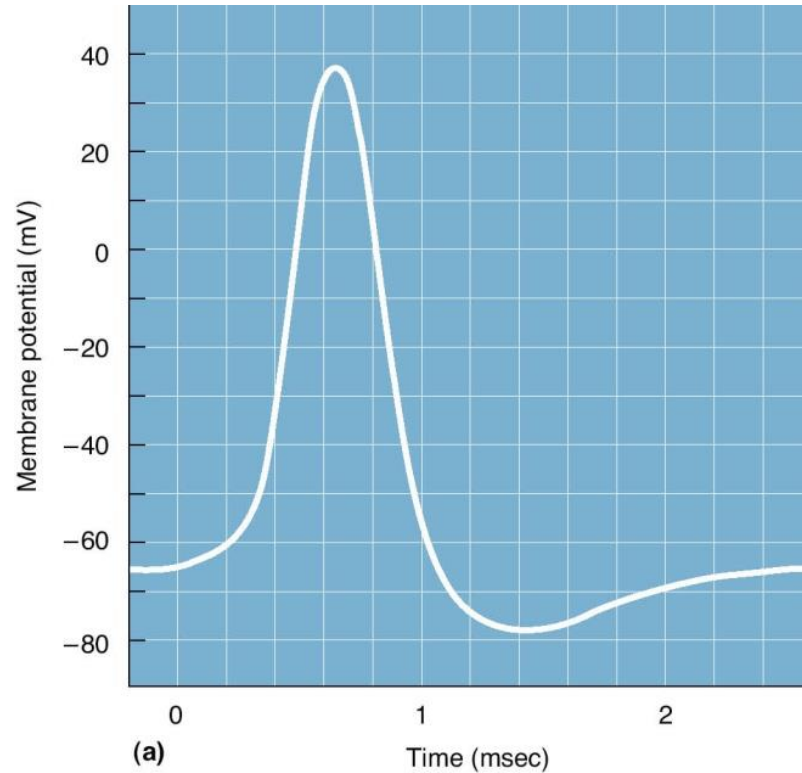
What happens when Sodium channels open?



- Equilibrium potential for sodium is 62 mV
- If we open sodium channels, sodium flows into the neuron, making the membrane potential more positive (depolarizing it) until mb potential gets to 62 mV
- At that point, the net flow of sodium ions will be zero

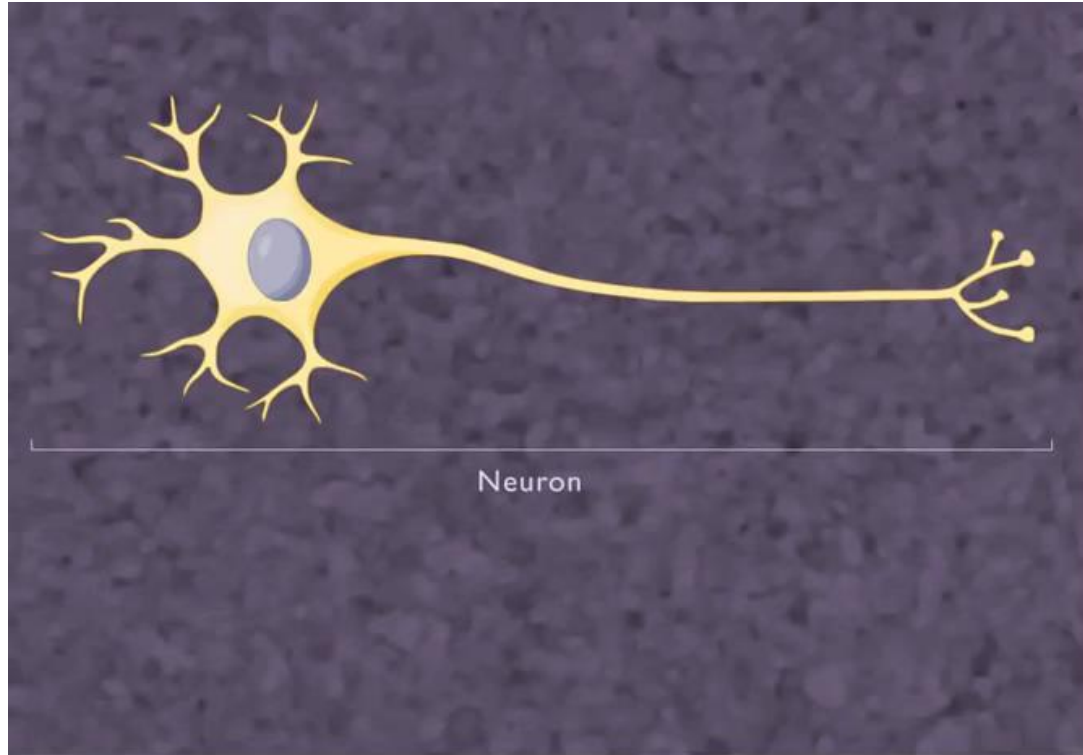
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Phases of the AP



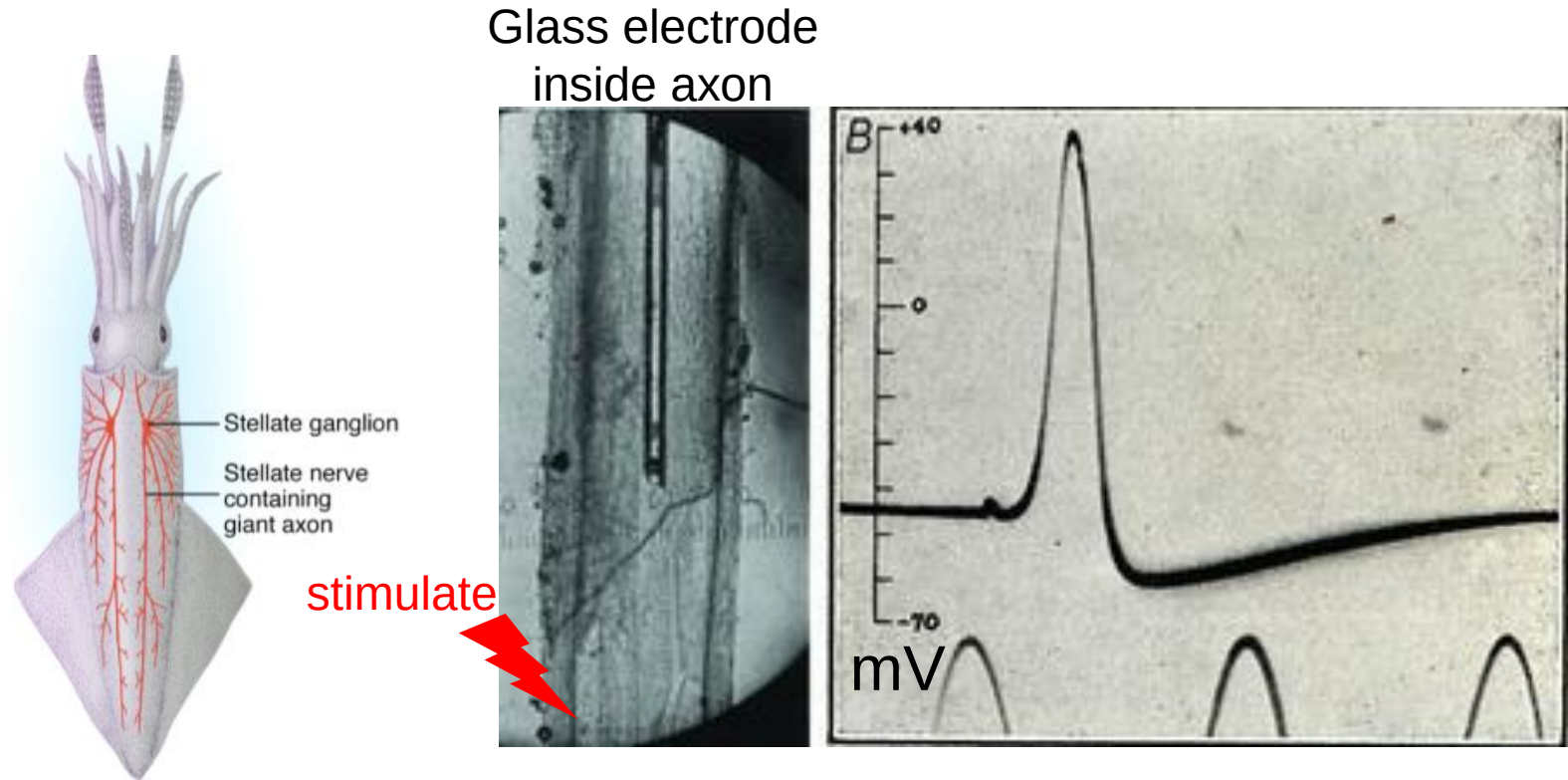
- Membrane potential goes from about -65 mV to positive value, then quickly back down.

Key features of APs



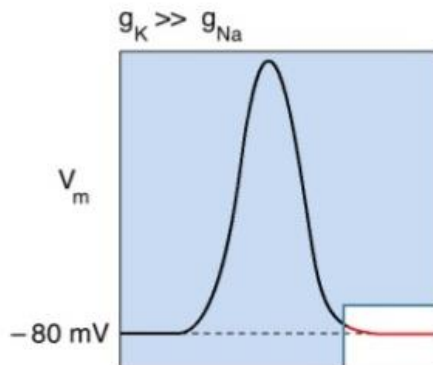
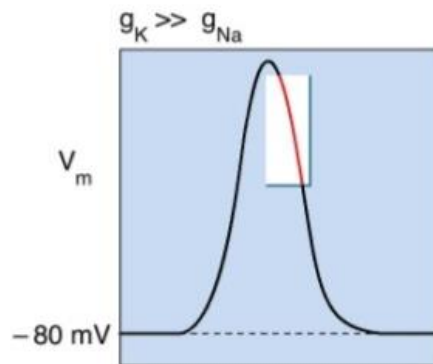
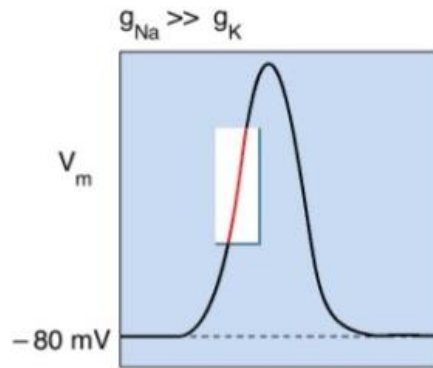
- All or nothing; meaning that there is no in between
- Does not decay as it travels down the neuron
- Only propagates in one direction down the axon

First recording of AP by Hodgkin and Huxley



- Used squid giant axon; large diameter so could insert electrode
- Stimulate further down axon, recorded a fast depolarization and repolarization of the membrane (an AP!)
- Depends on Sodium and Potassium ions

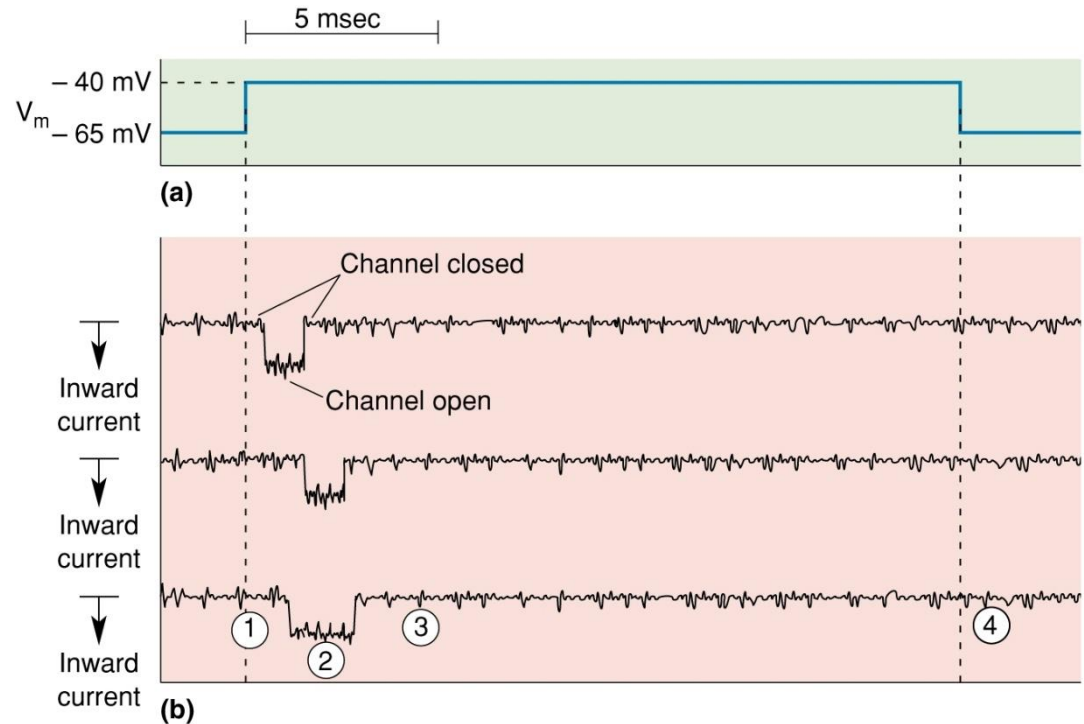
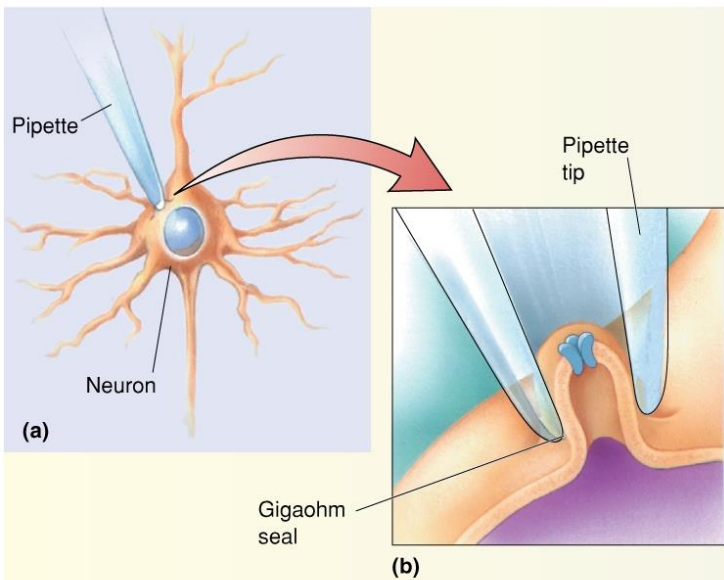
Phases of an AP



Time →

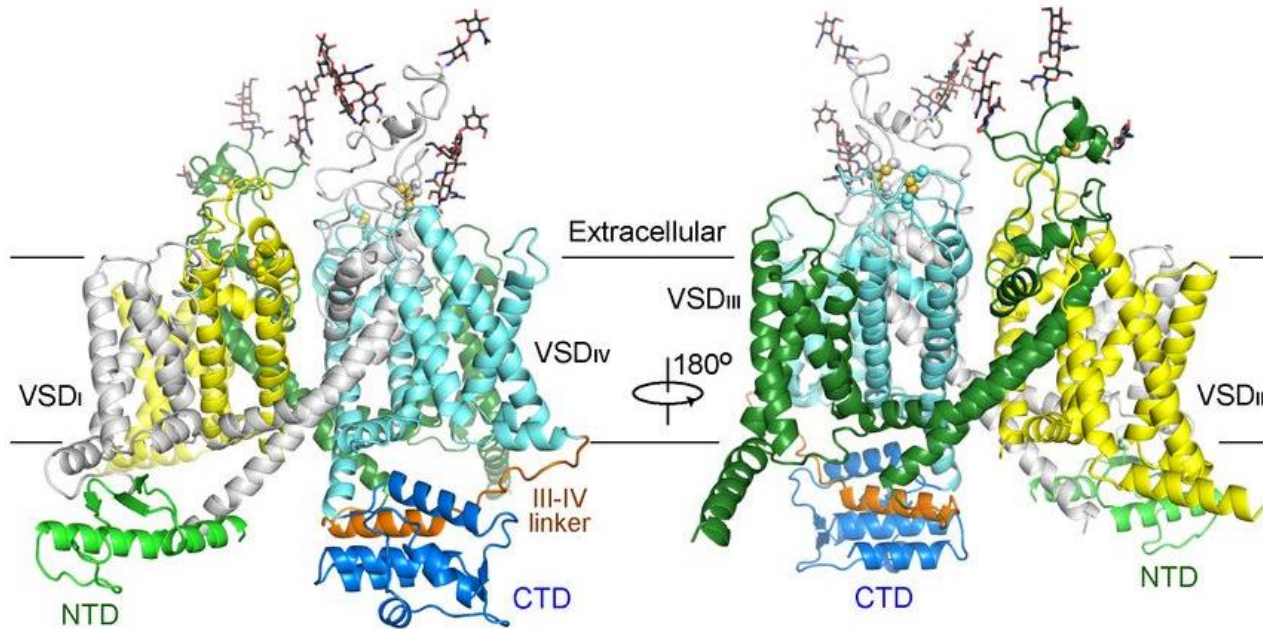
- Rising phase; fast sodium current comes on first
- Falling phase; sodium channels inactivate, slower potassium channels come online, hyperpolarizing the neuron
- Neuron returns to baseline, potassium channels close
- Hodgkin and Huxley proposed that these currents were mediated by voltage gated channels
- However, no direct evidence, until...

Voltage gated sodium channel recording



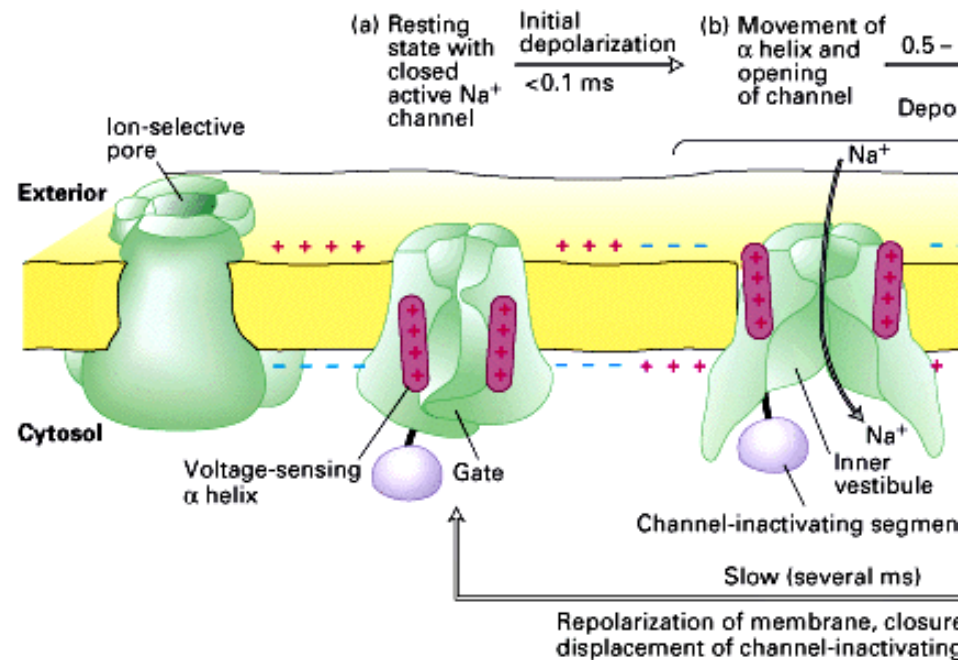
- Patch clamp recording of 3 different channels
- When membrane is depolarized to -40 mV, channel opens briefly, stays open less than 1 ms
- If the membrane is still depolarized, it stays closed; this is called inactivation

What is the structural basis for these properties?



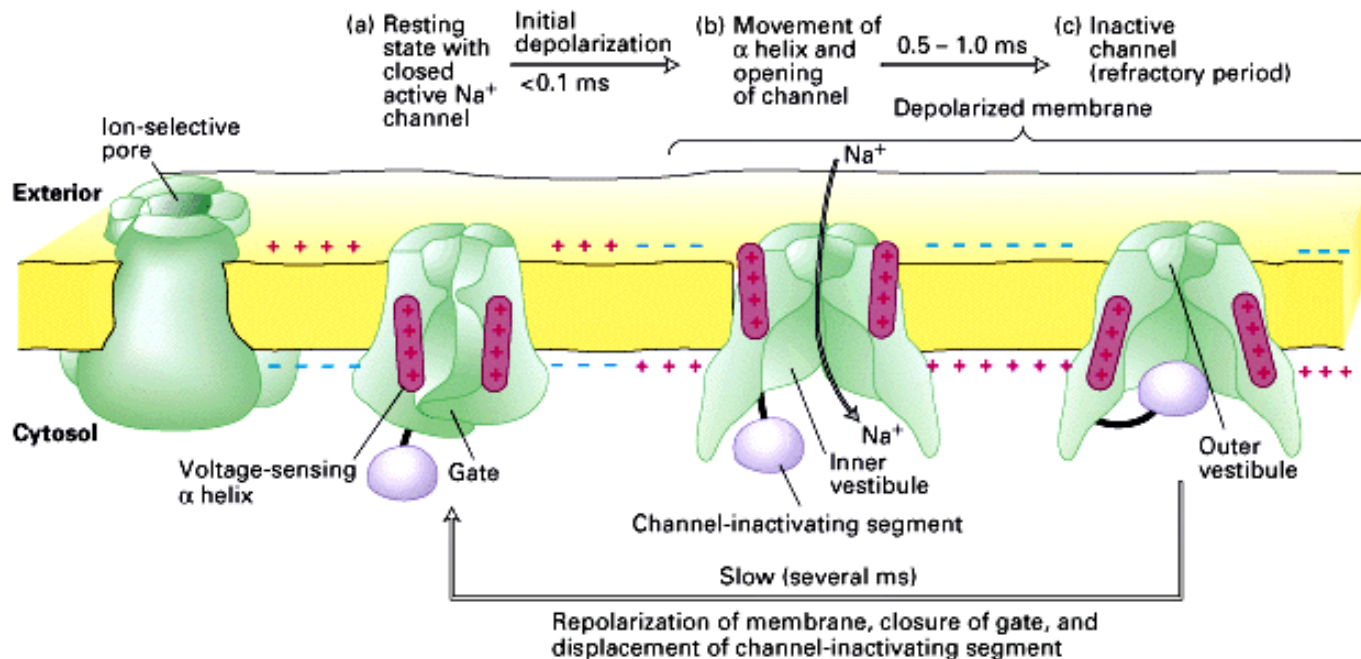
- Genetic and structural studies have led to models of how the channel senses voltage

What is the structural basis of voltage gating?



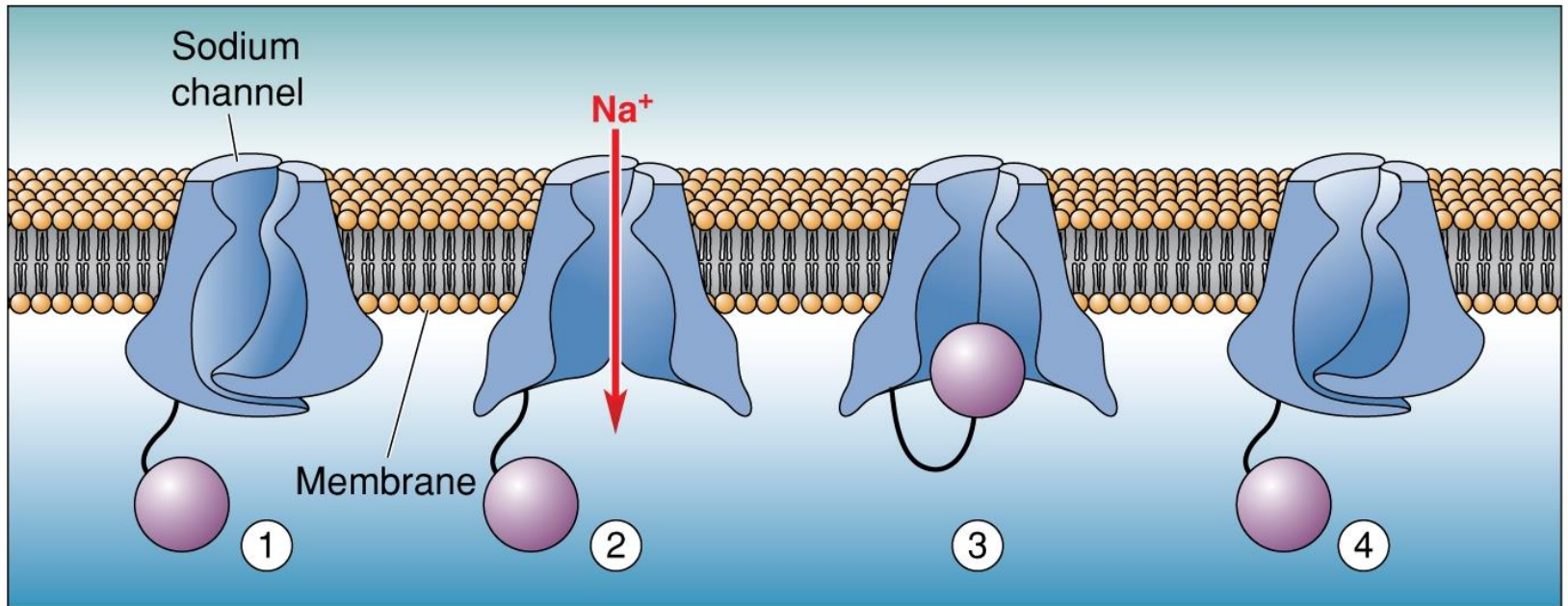
- At resting potential, positively charged amino acids (the voltage sensor) are attracted to the negative charge inside the cell
- When the outside of the cell becomes more negative, the voltage sensor moves, changing the conformation and opening the pore

Structural basis for inactivation



- The inactivation domain swings closed shortly after the channel has opened
- The block is removed only by closure of the channel when the membrane returns to resting potential

Structural basis for inactivation

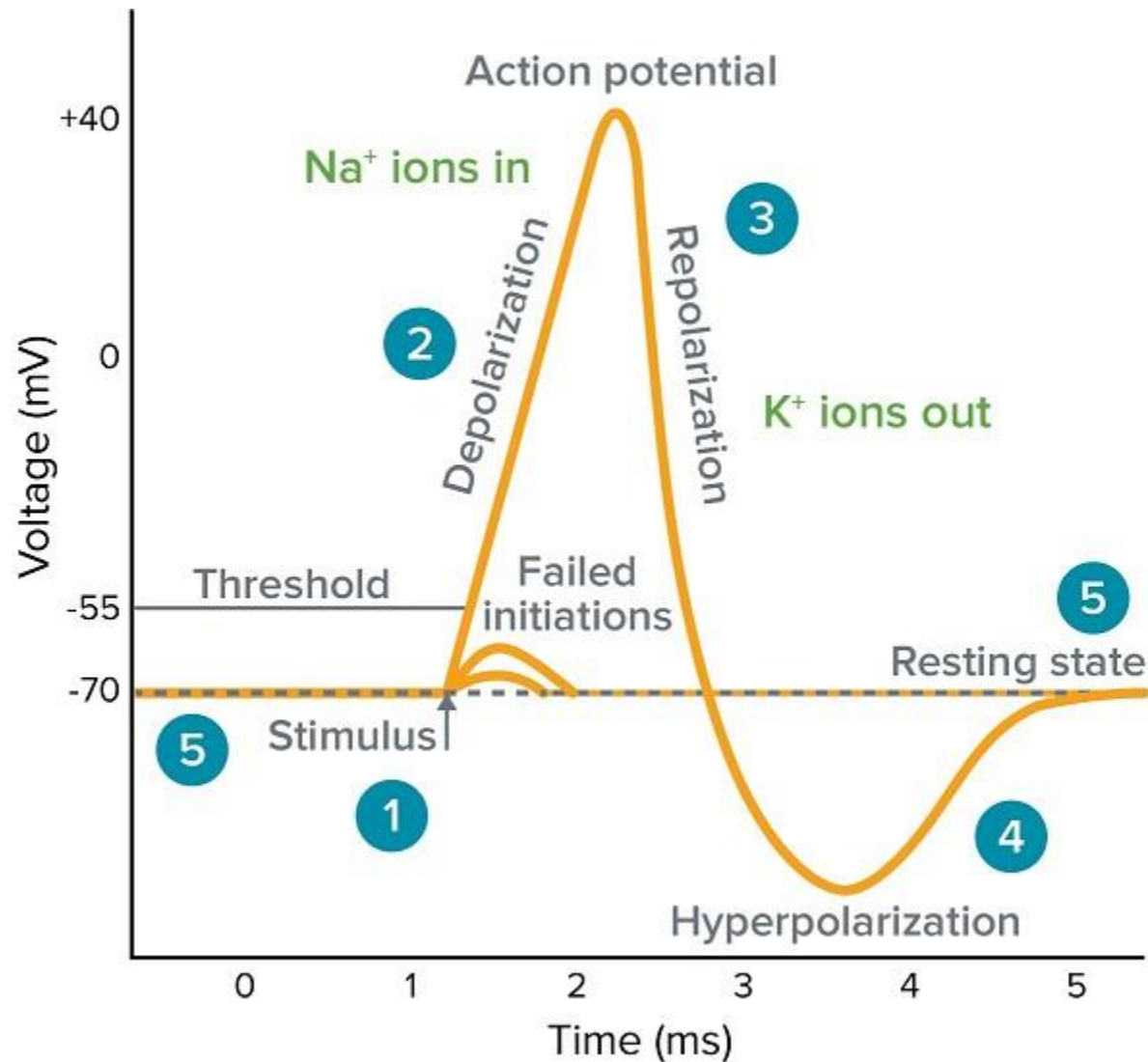


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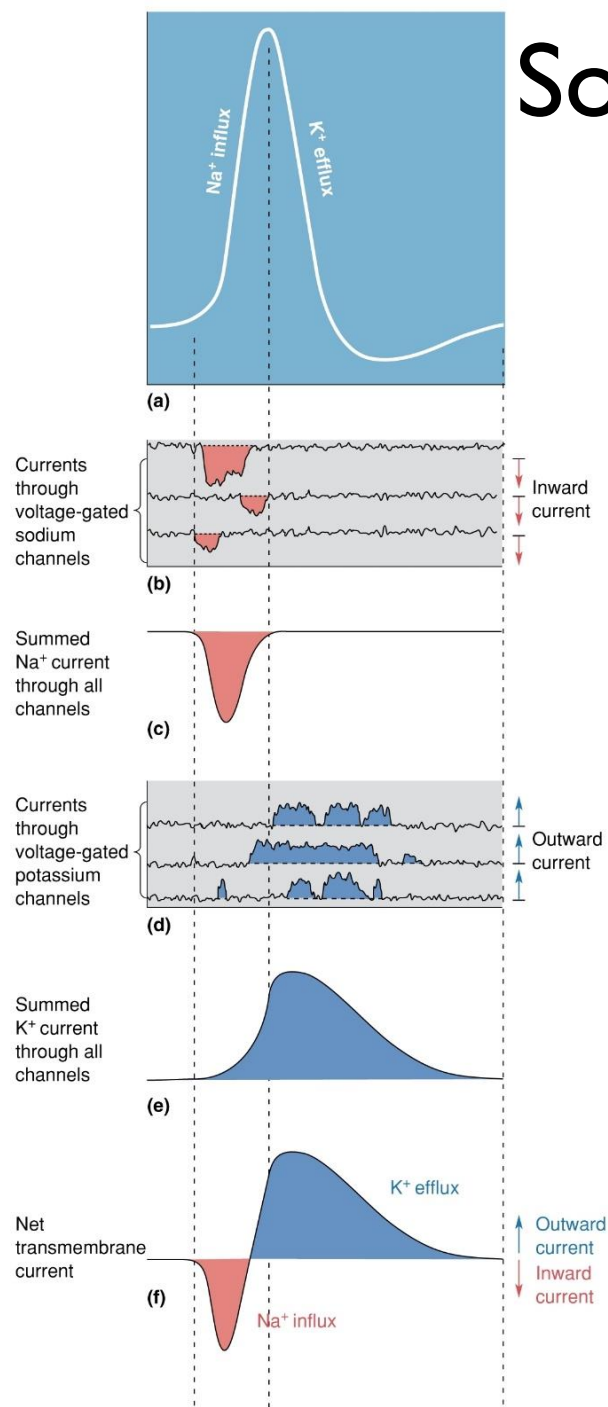
- The inactivation domain swings closed shortly after the channel has opened
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Phases of the AP



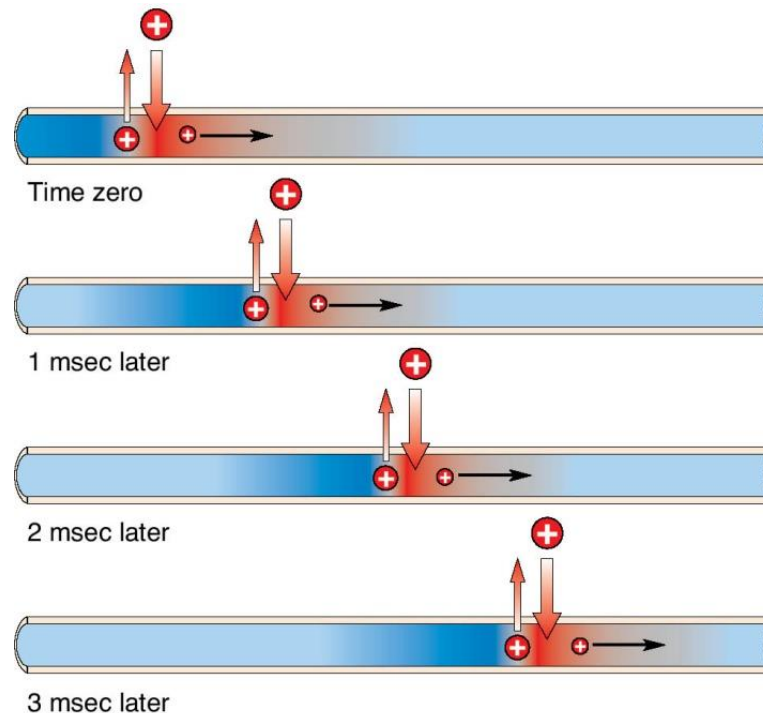
- Glass electrode inserted into giant axon

Sodium and potassium channels in different phases



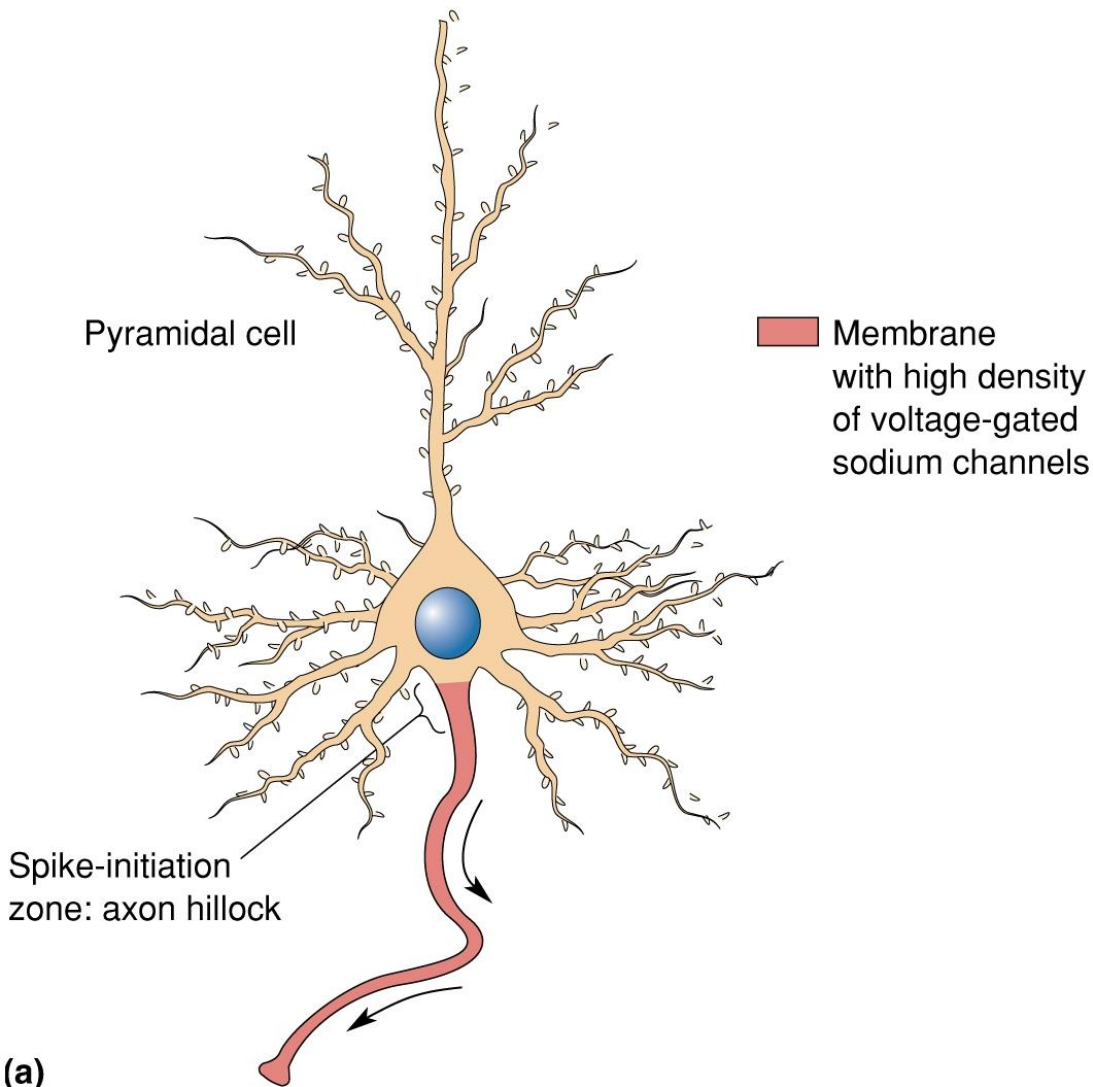
1. Rising phase; Sodium channels are quickly opened by depolarization of membrane
2. Positively charged sodium ions flow into the cell, depolarizing it.
3. Sodium channel inactivation domain blocks pore; channel will not open again until repolarization
4. Voltage gated potassium channels, which are slower to open, now online
5. Potassium flows out of cell, hyperpolarizing it
6. Sodium channel returns to closed state, dislodging inactivation domain. Ready for next AP!

Action potential propagation



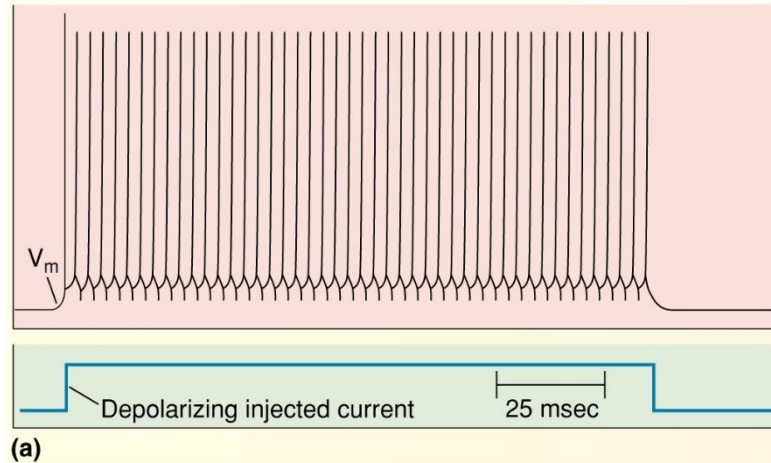
- Depolarization past threshold opens Voltage-gated Sodium channels.
- Sodium ions flow through the channel and down the axon, depolarizing the next segment of membrane past threshold
- Voltage gated Sodium channels in that segment of membrane open, letting in more sodium.

The spike initiation zone

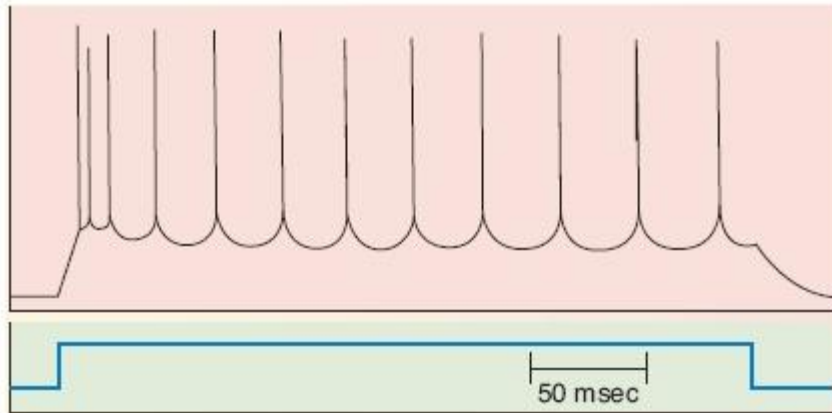


- Spikes are initiated in a specialized part of the neuron
- Spike initiation zone has higher density of V gated sodium channels

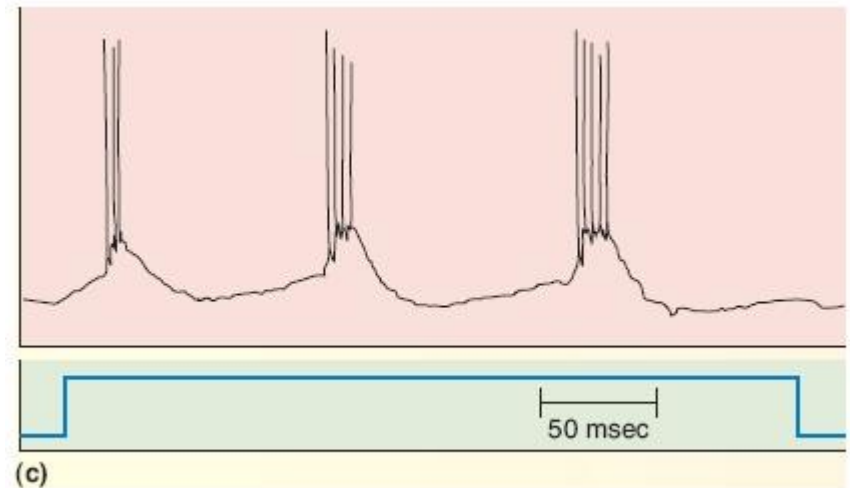
Neurons can have diverse firing patterns



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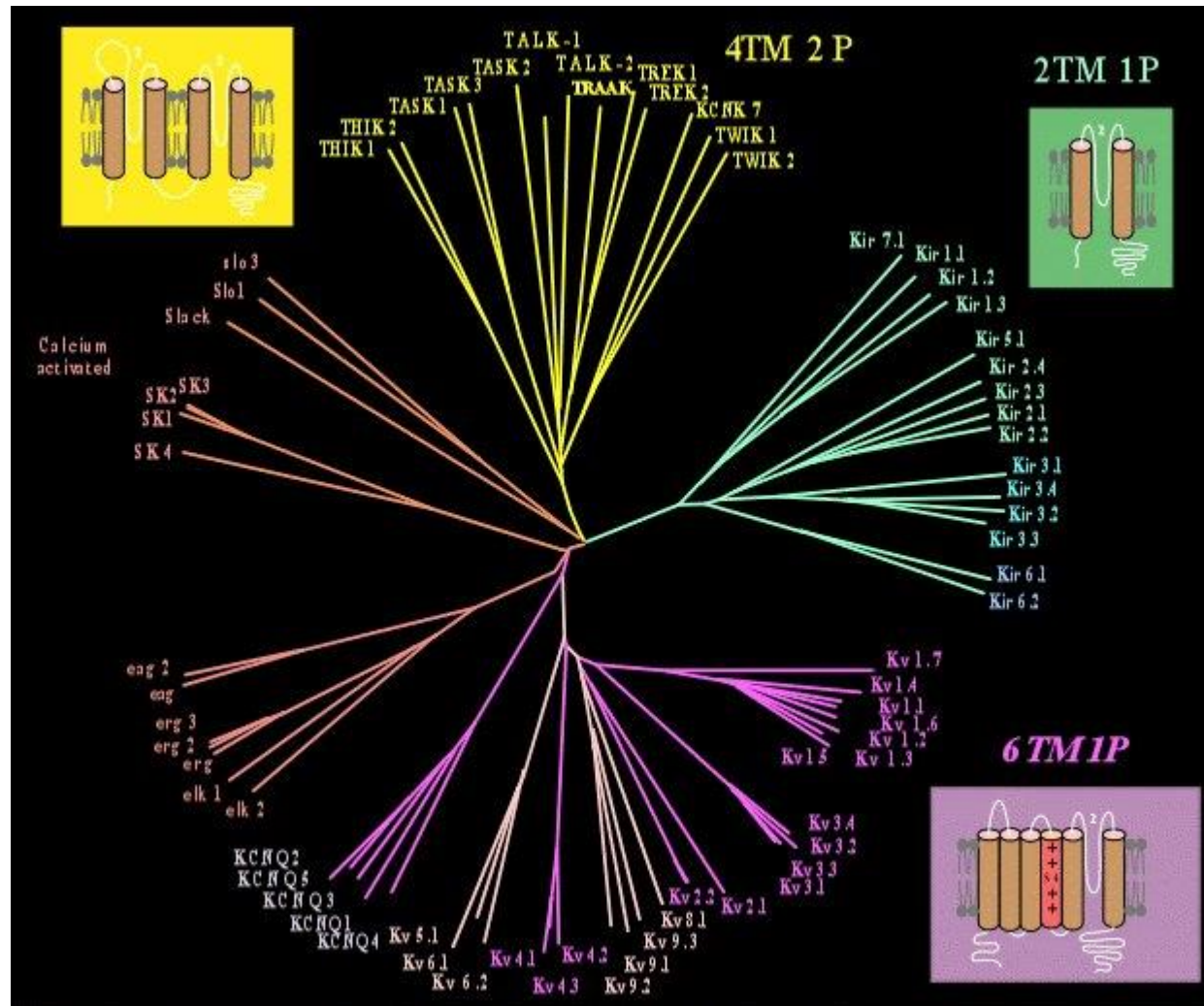
adaptation



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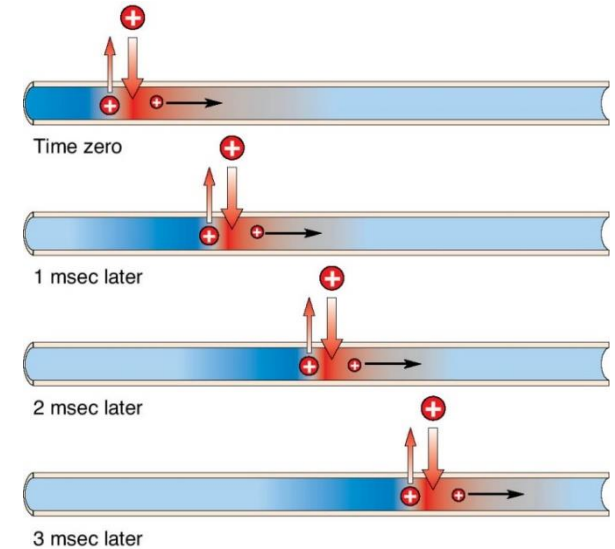
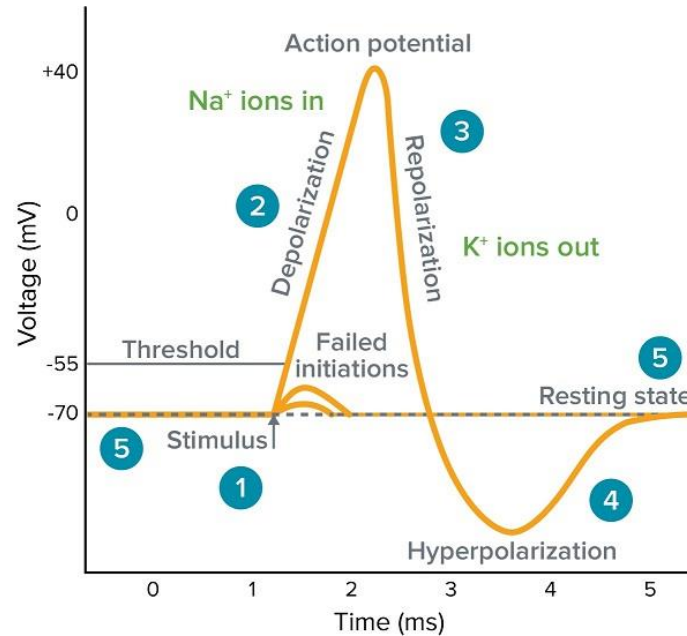
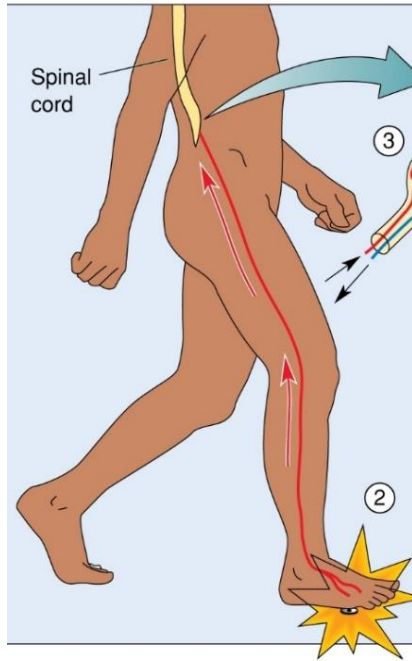
Bursting

Why neurons have different firing patterns



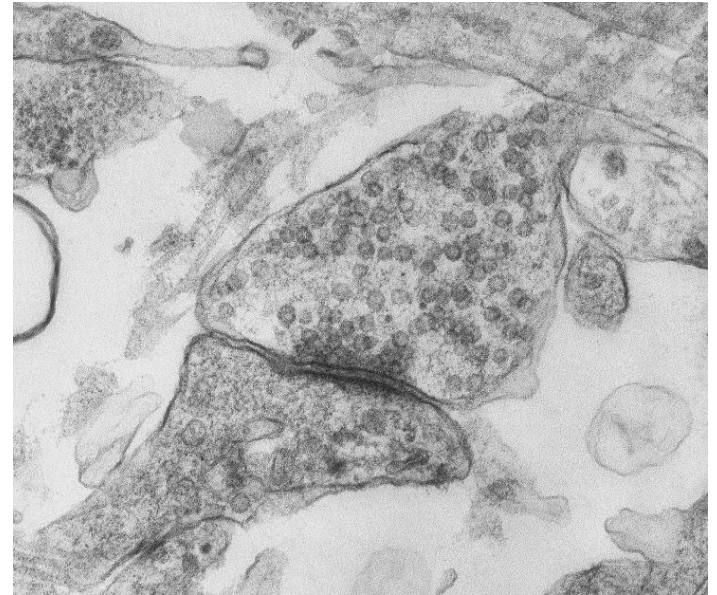
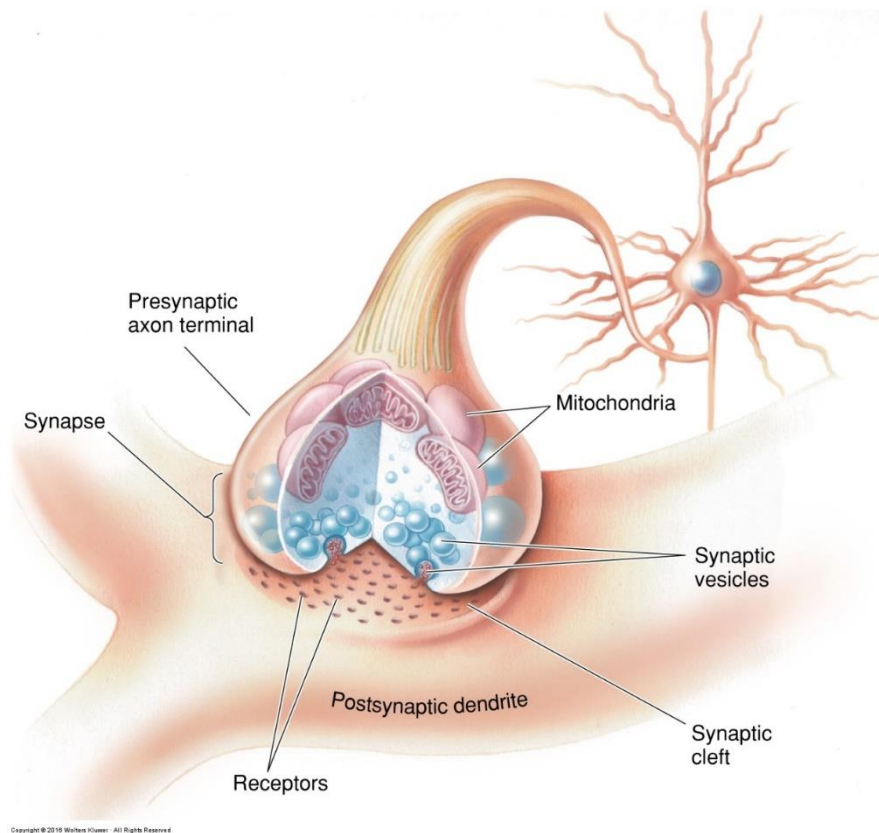
- There is more than one type of voltage gated potassium channel

The full pathway; what happens when you step on a thumbtack?



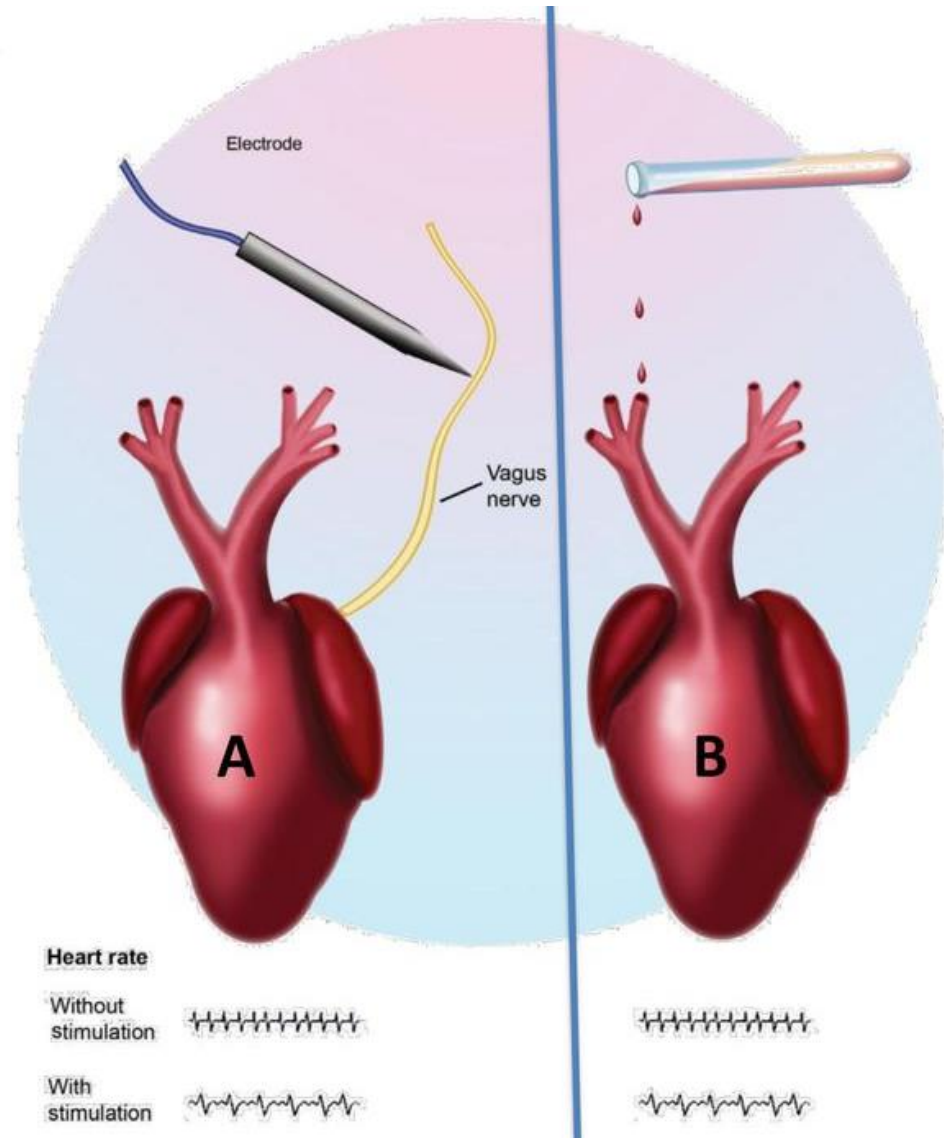
- Sensory neurons in your foot are depolarized
- Action potential is initiated if depolarization is strong enough to activate V gated sodium channels
- AP propagates up to the spinal cord

Synapses and Neurotransmitters

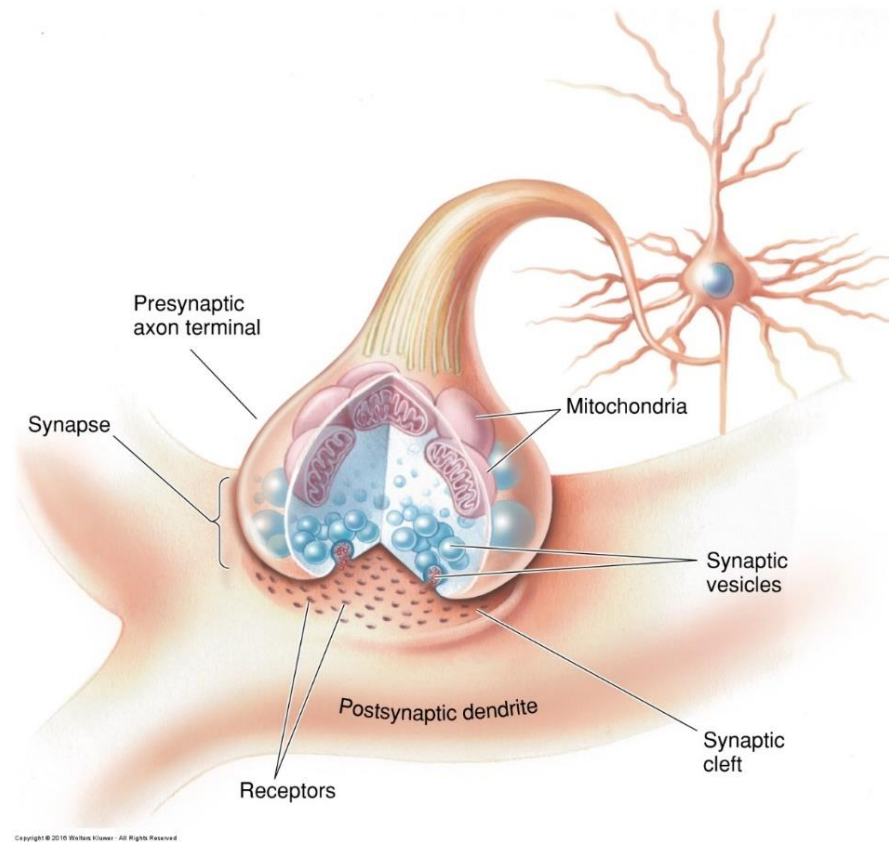


Evidence for chemical transmission; Otto Loewi's experiment

- Stimulated vagus nerve → slowed heart A
- Injected solution from heart A into heart B → Heart B rate slowed
- Stimulated sympathetic nerve → sped up heart A.
- Injected solution from heart A into heart B → Heart B rate sped up
- Loewi's conclusion
 - Heart uses chemical messengers, not electrical signal to control heart rate.
 - Loewi got a Nobel prize in 1936

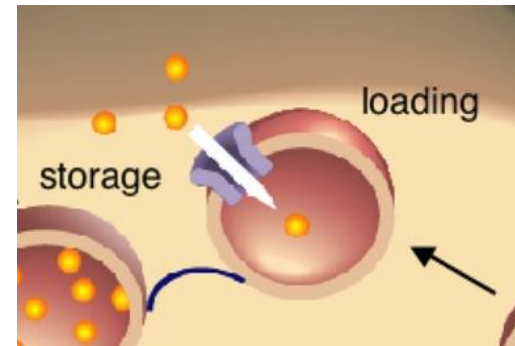
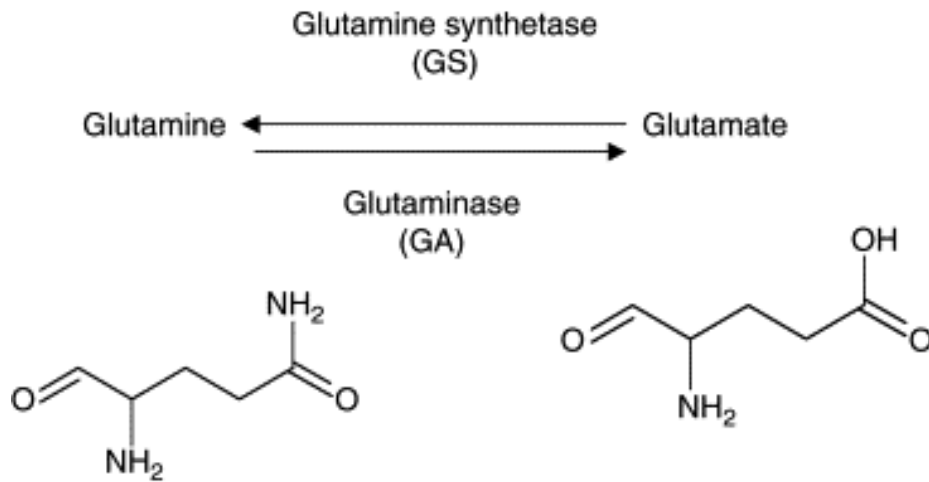


Structure of a chemical synapse



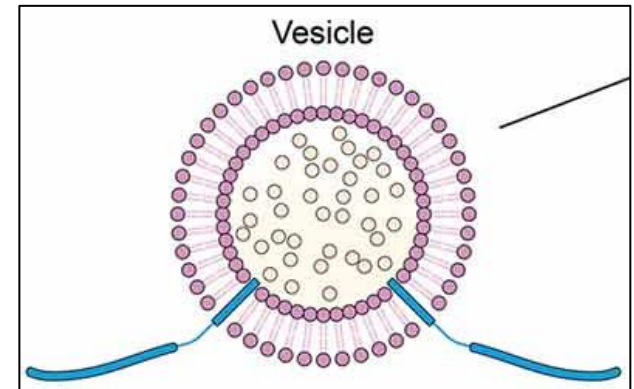
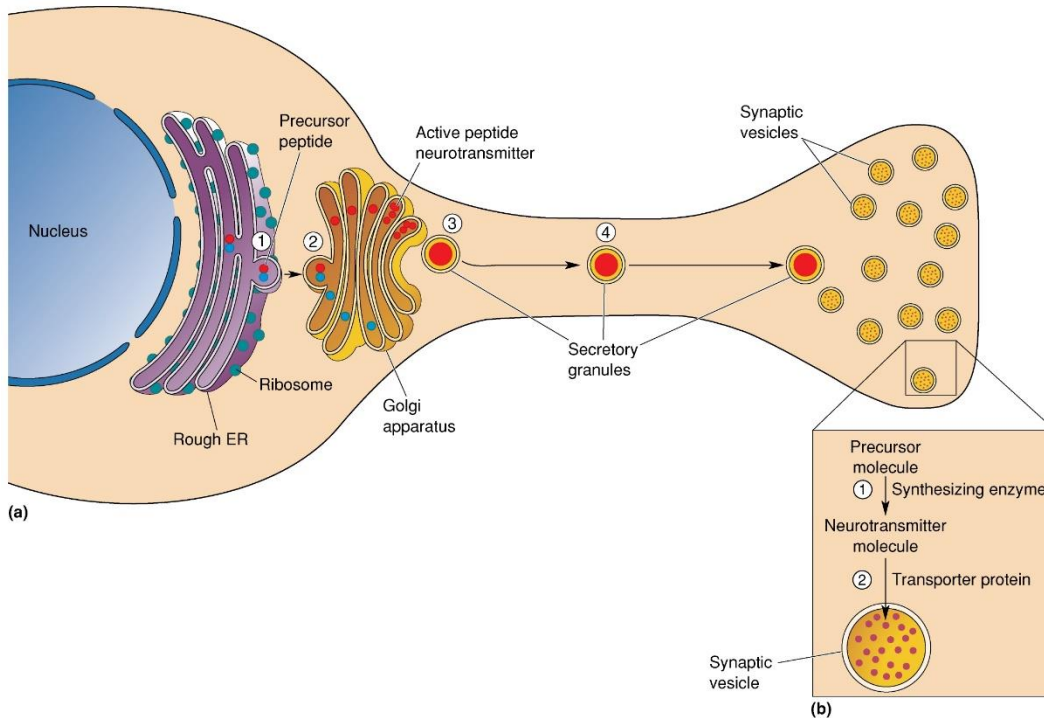
- Presynaptic terminal contains spherical vesicles filled with neurotransmitters
- Neurotransmitters diffuse across the synaptic cleft to bind to receptors on the postsynaptic side.

An excitatory neurotransmitter; Glutamate



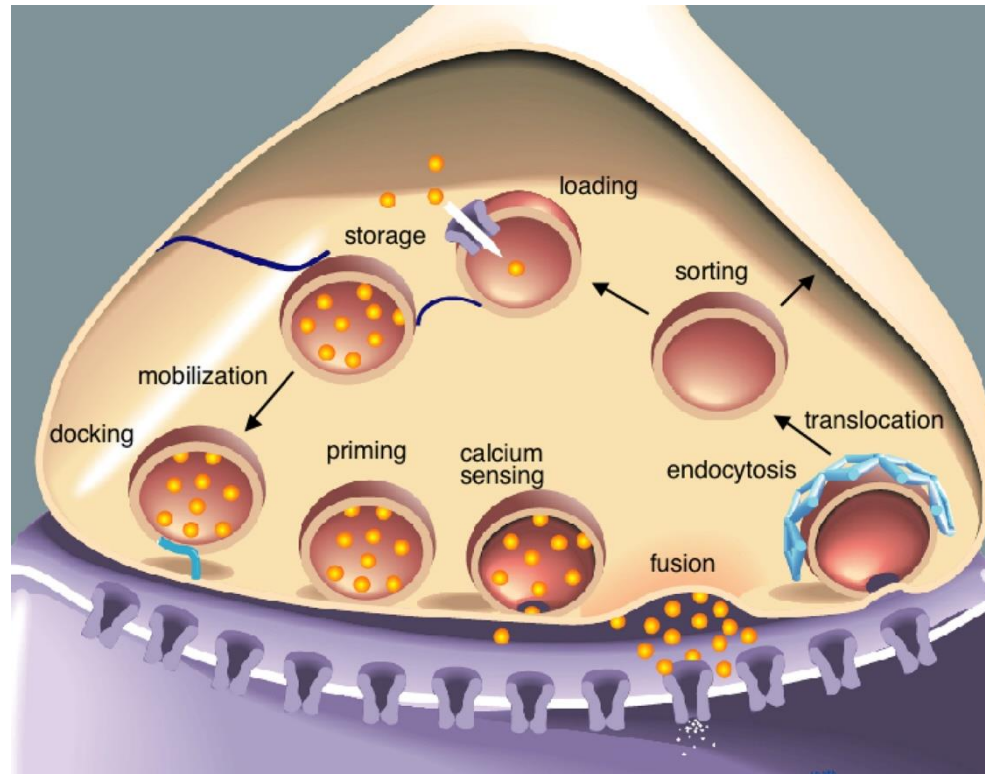
- Neurons that release glutamate (glutamatergic neurons) convert the amino acid glutamine into glutamate.
- Glutamate is then pumped into vesicles
- Vesicles go to the presynaptic terminal

Vesicle formation



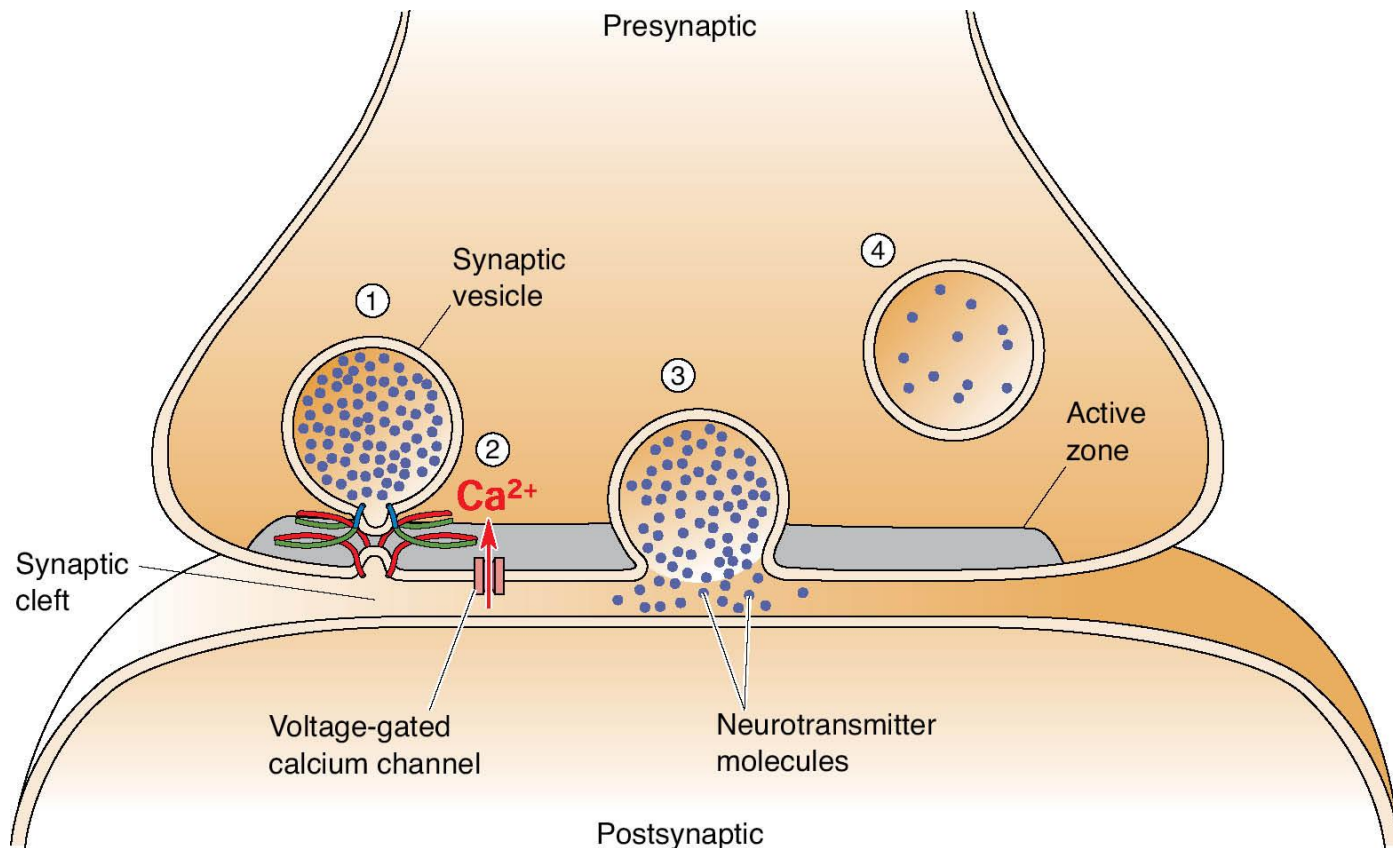
- Vesicles bud off of the Golgi apparatus
- They are made up of the same phospholipid bilayer as the cell membrane

Synaptic vesicle life cycle



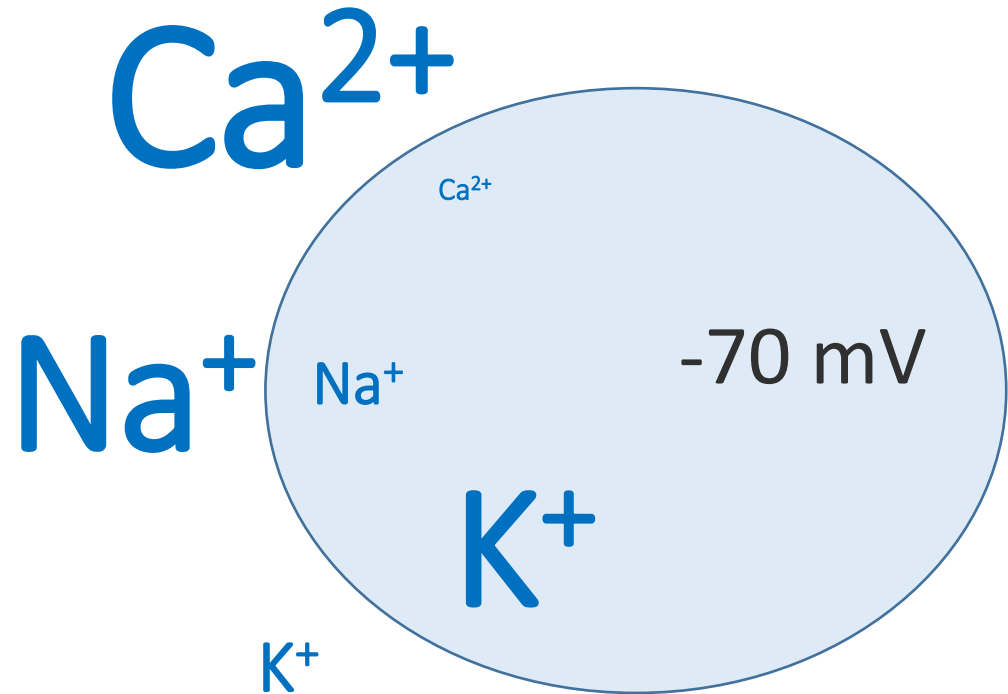
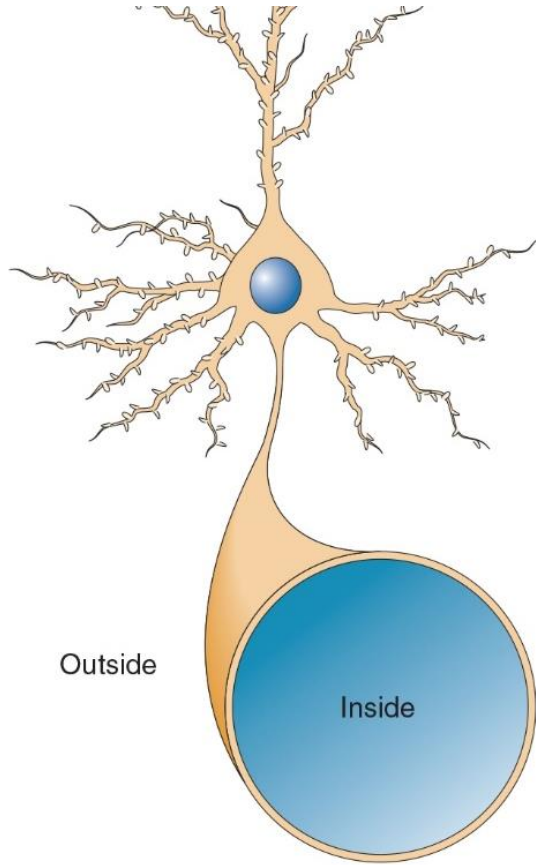
- After loading, vesicles go to the presynaptic membrane and dock
- Then, when an action potential comes down the axon, fusion is triggered
- Vesicles can re-fuse with cell membrane and get recycled

Neurotransmitter release



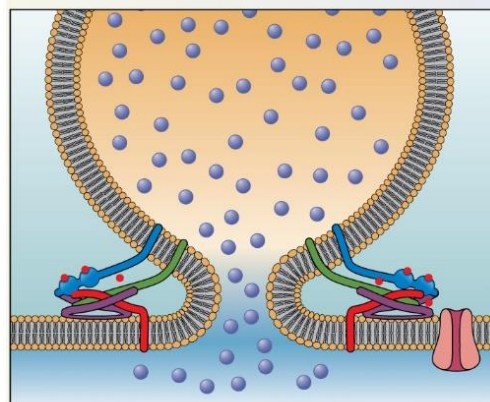
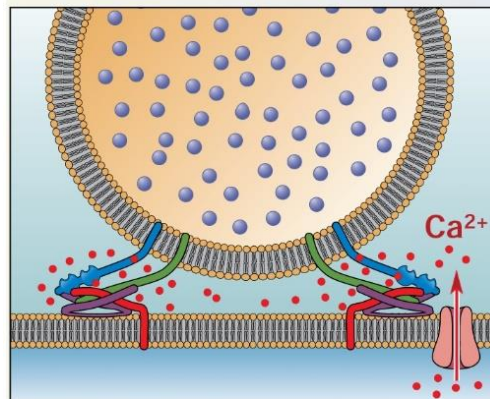
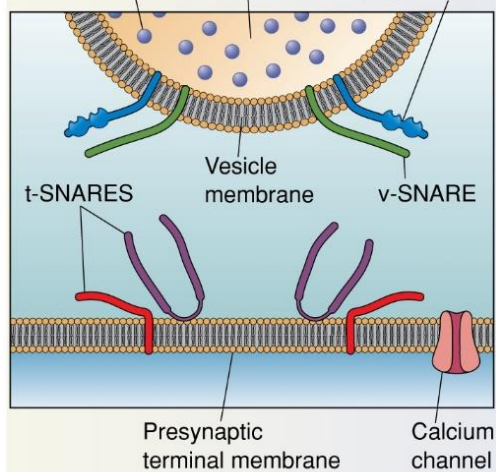
- When an action potential enters the presynaptic terminal, the depolarization opens voltage gated calcium channels

Calcium concentration



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Cl ⁻	150	13	11.5 : 1	-65 mV

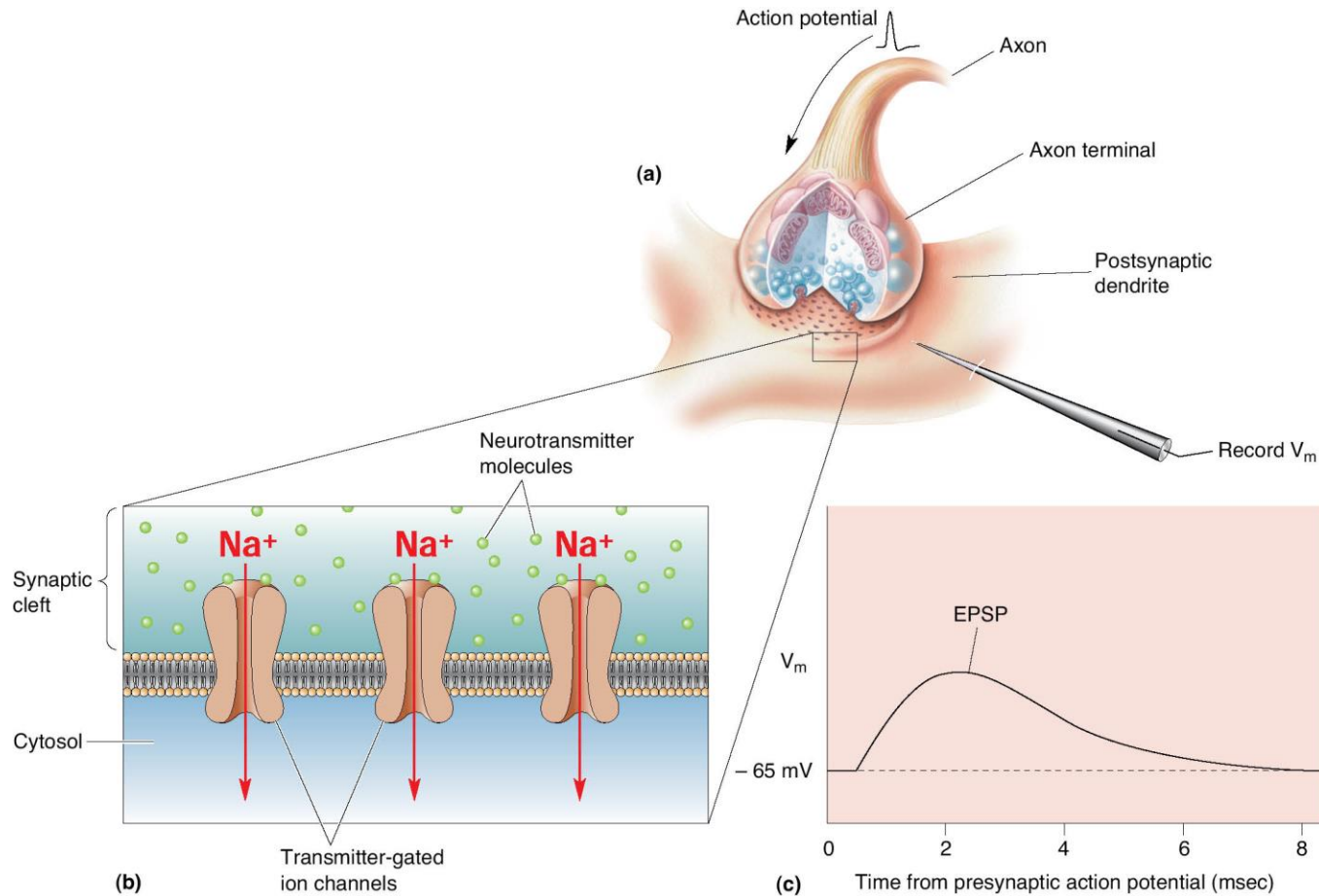
Neurotransmitter Vesicle Synaptotagmin



Neurotransmitter release

- Synaptic vesicles have membrane proteins called v-SNAREs
- The presynaptic membrane has t (target) SNAREs.
- The SNAREs bind to each other to dock the vesicle
- AP enters terminal
- Calcium influx causes a conformational change that makes the vesicle membrane fuse with the presynaptic membrane
- Neurotransmitter is released into the synaptic cleft

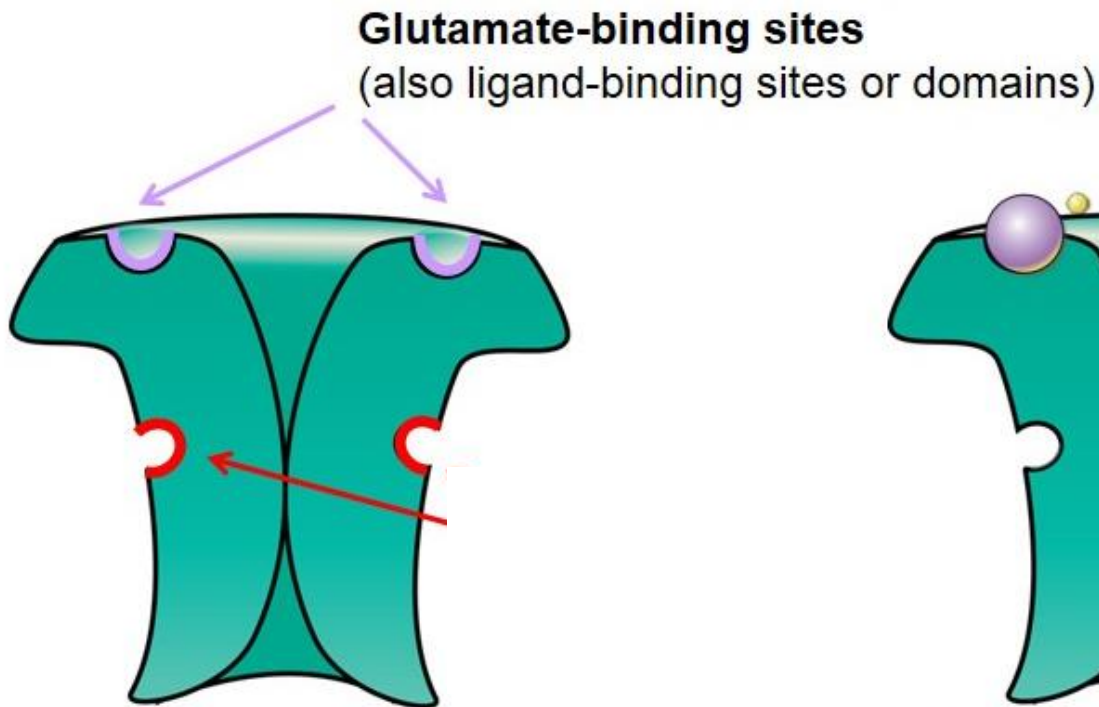
Effect of neurotransmitter on postsynaptic membrane



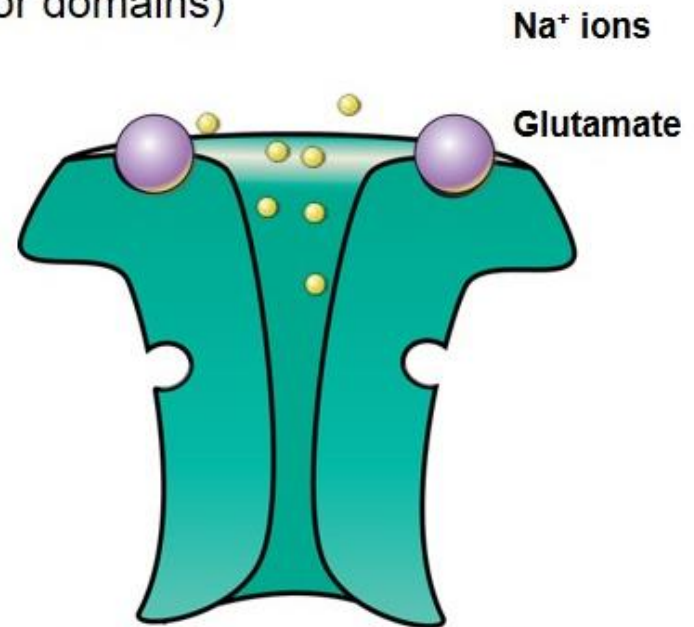
- Neurotransmitter diffuses to postsynaptic membrane, interacts with receptors there

Glutamate activates a ligand-gated sodium channel

AMPA receptor
(closed, inactive state)

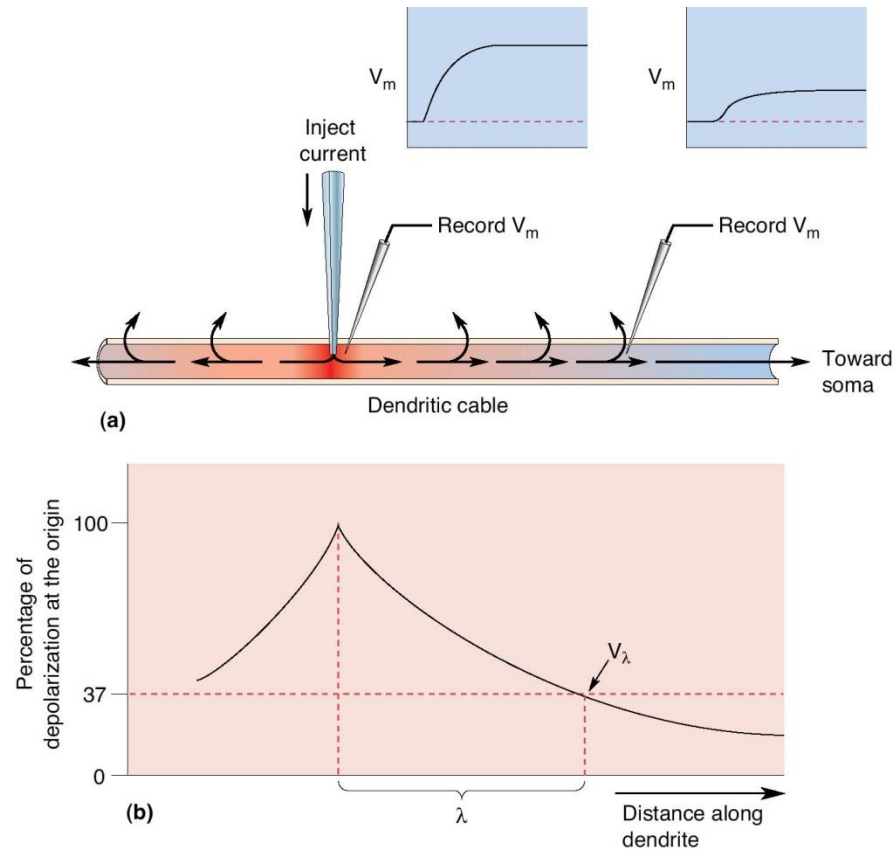


AMPA receptor
(open, active state)



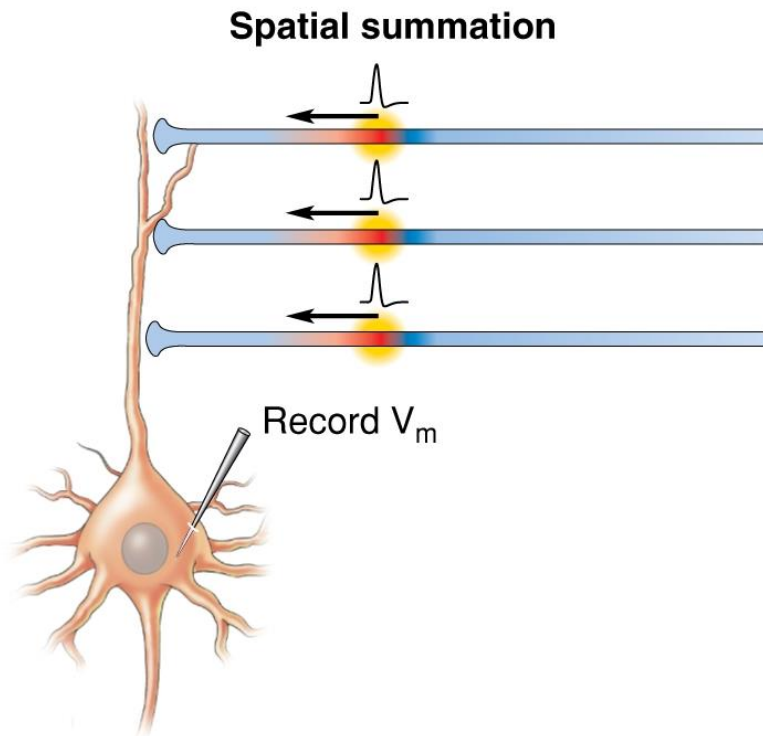
The receptor's ion channel allows influx of Na⁺ ions (and sometimes Ca²⁺ ions) into the neuron

Passive conduction

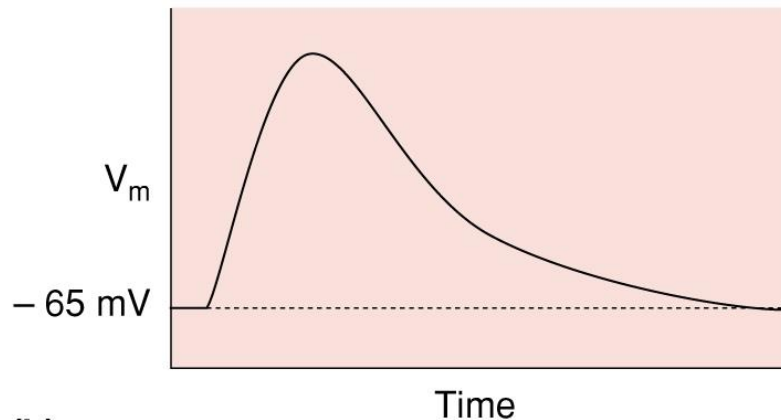


- Sodium enters postsynaptic neuron via AMPA receptors
- Postsynaptic membrane is depolarized
- Subthreshold depolarization decays as ions flow down dendrite

EPSP summation

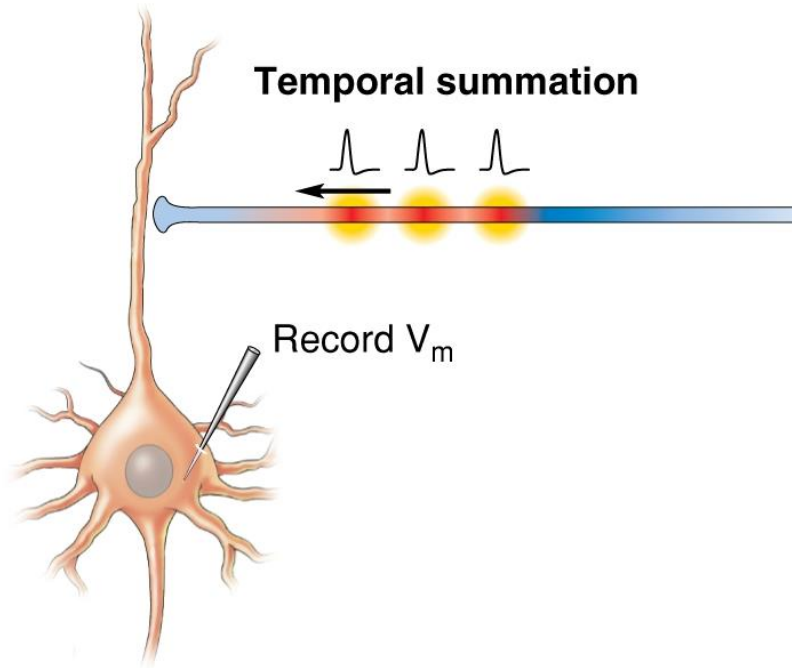


- If multiple synapses are active in the postsynaptic neuron over a short time scale, the input sums to a larger depolarization
- May trigger an action potential

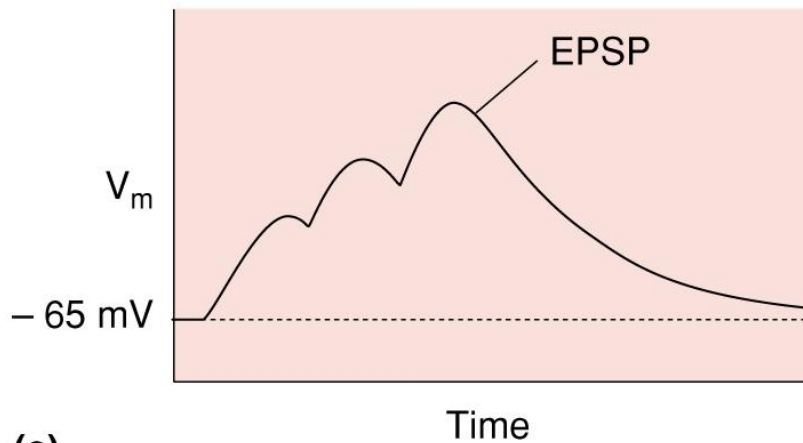


(b)

EPSP summation

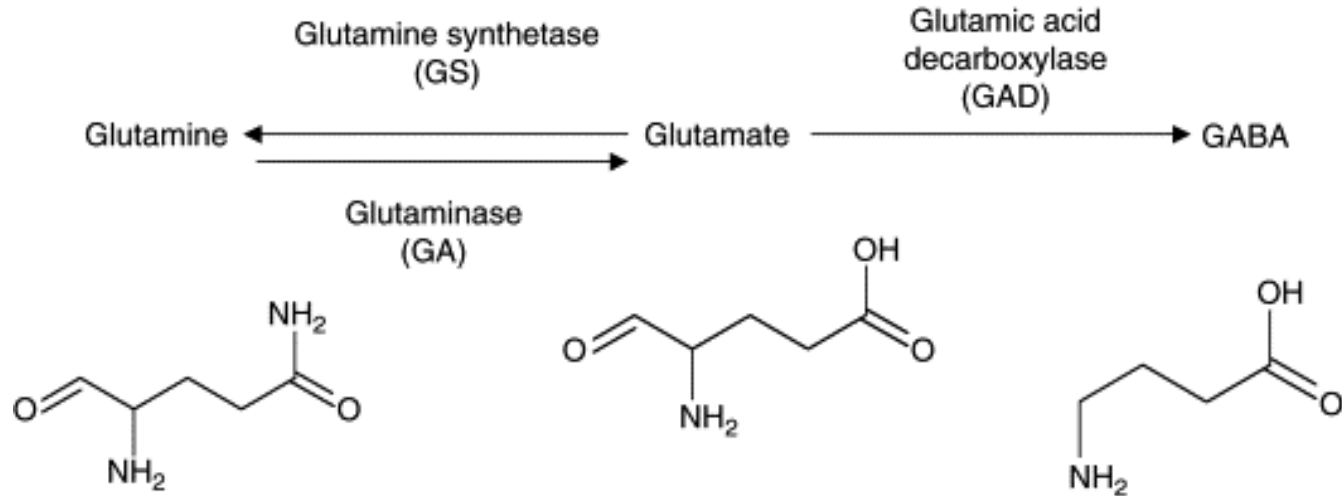


- If a single synapse is releasing a lot of vesicles, the EPSPs will add up to a larger depolarization



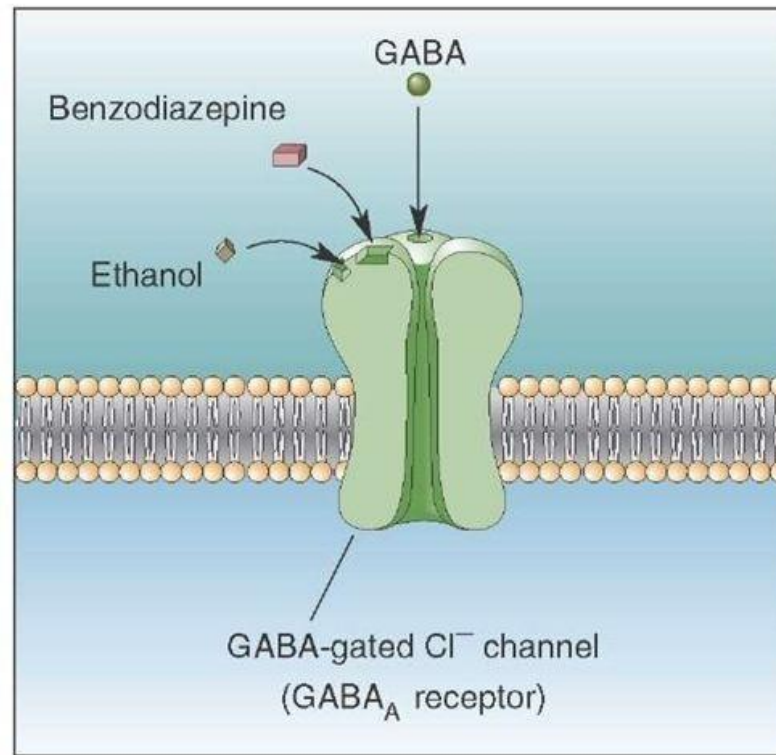
(c)

An inhibitory neurotransmitter; GABA



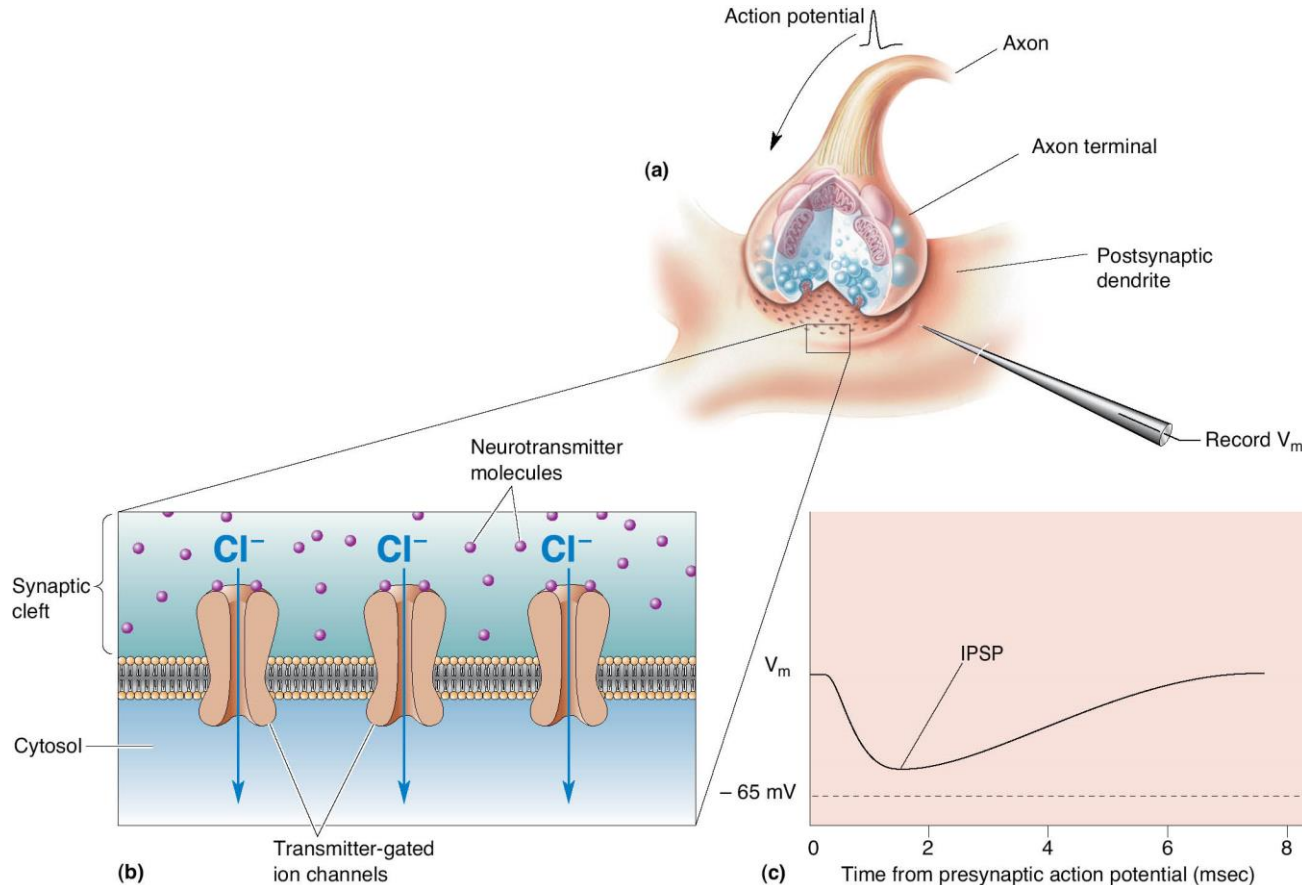
- Neurons that express GAD make GABA from glutamate
- They are called Gabaergic neurons
- GABA is transported into vesicles

An inhibitory neurotransmitter; GABA



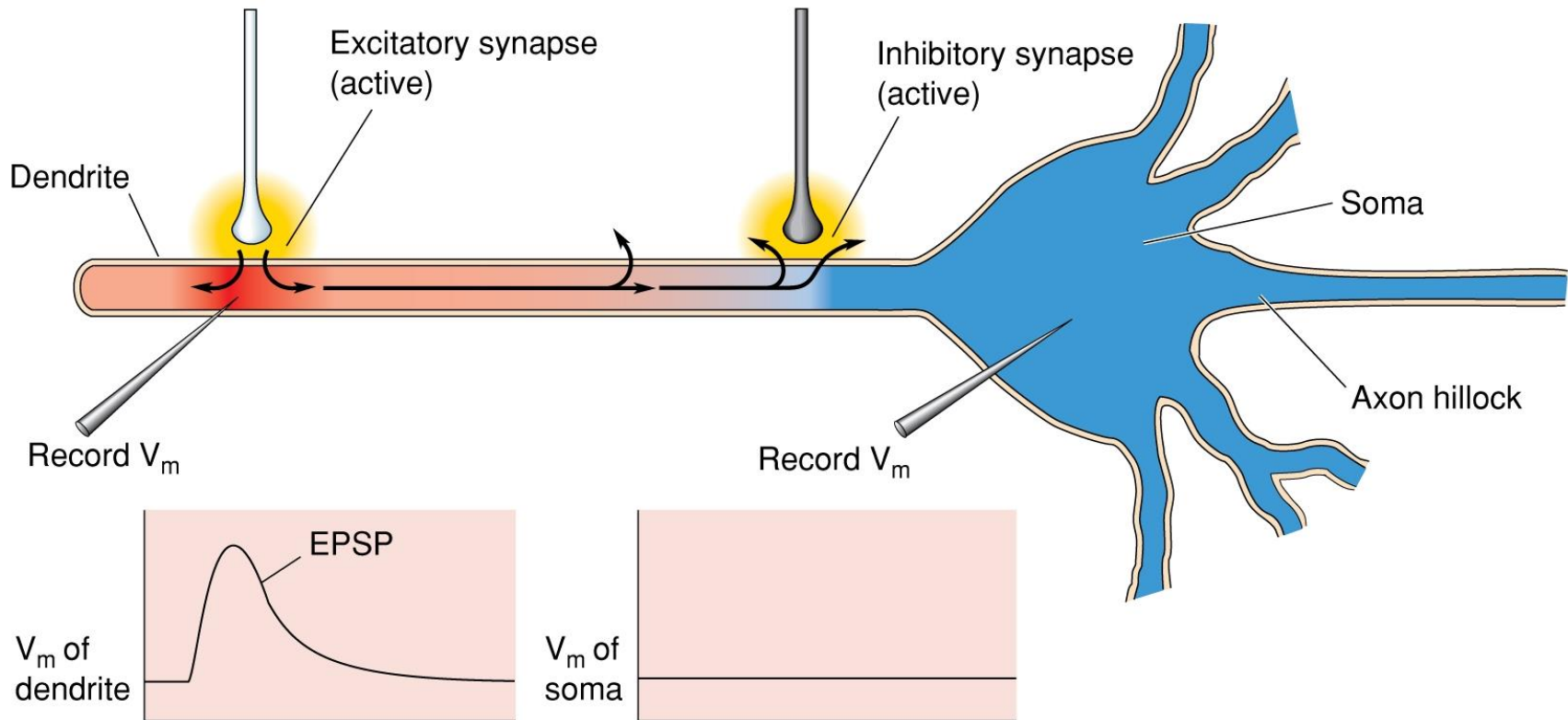
- GABA_A Receptor is a Chloride channel
- Other compounds such as Benzodiazepines (e.g. Valium) and alcohol increase the effect of GABA; they are agonists of the receptor

Generation of an IPSP (Inhibitory post synaptic potential)



- Action potential enters presynaptic terminal in GABAergic neuron
- GABA is released, binds to GABA_A receptors on postsyn membrane
- Chloride enters, hyperpolarizing the postsynaptic membrane

IPSP and EPSP interactions

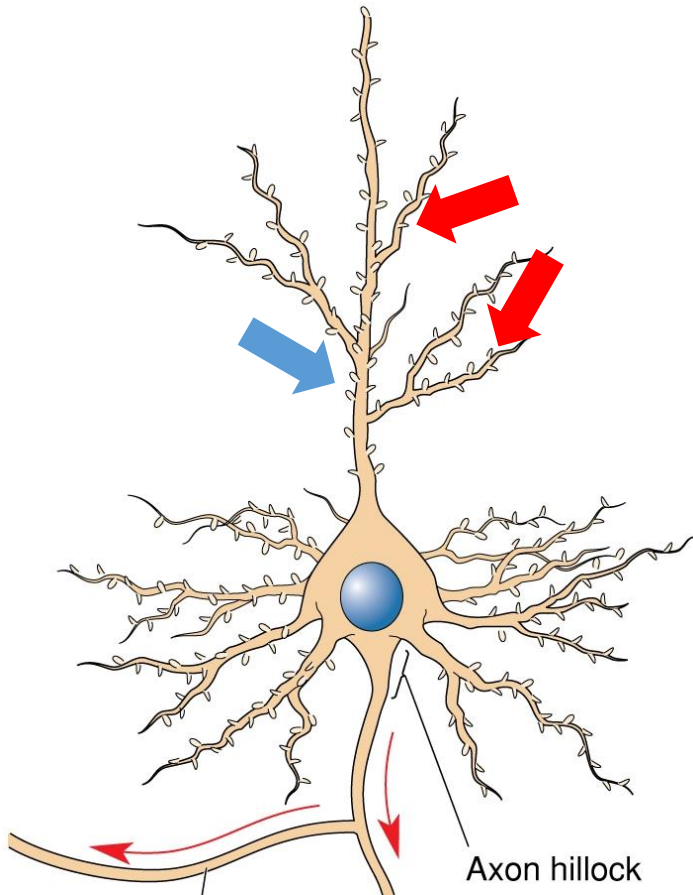


(b)

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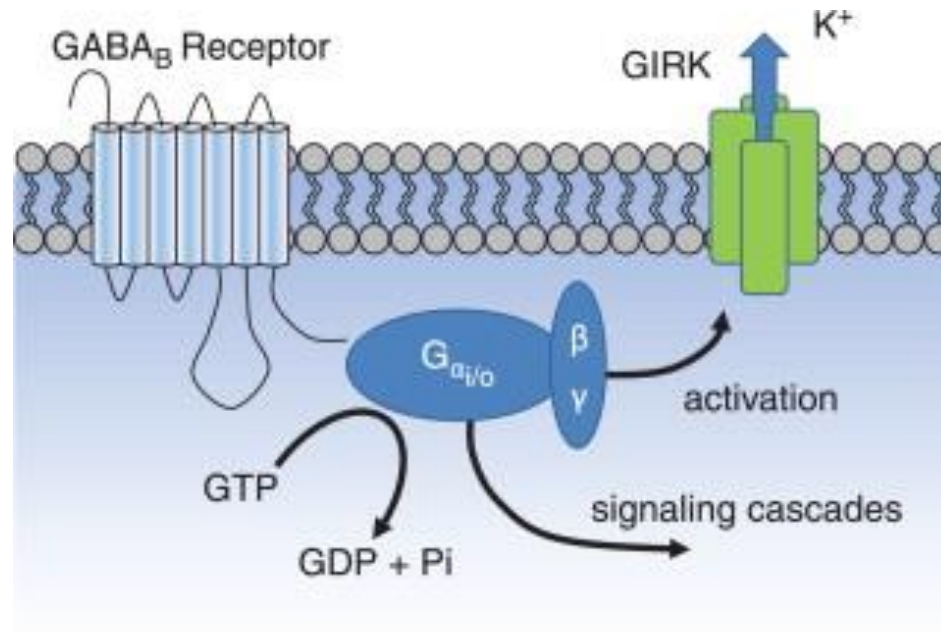
- Excitatory synapse causes EPSP in dendrite
- If inhibitory synapse is active at the same time, it can cancel out

Integration across a dendritic tree



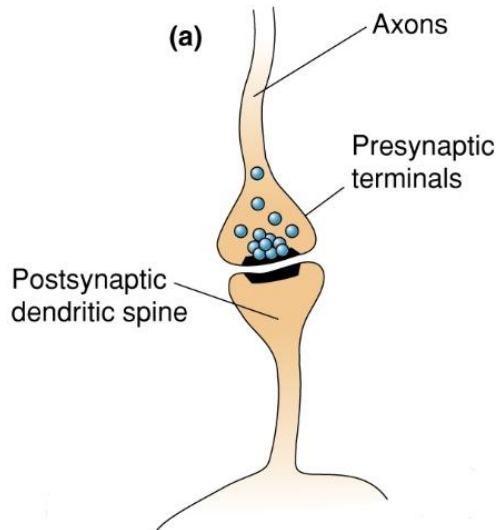
- Decision to spike or not occurs at axon hillock
- Depends on whether AP threshold is exceeded
- That depends on interactions between excitatory and inhibitory synapses onto the neuron

There is more than one kind of receptor for each neurotransmitter



- GABAB receptor; a GPCR (G protein coupled receptor)
- GABA binding allows beta/gamma subunit to bind to a potassium channel, activating it and allowing potassium to leave the cell
- hyperpolarization

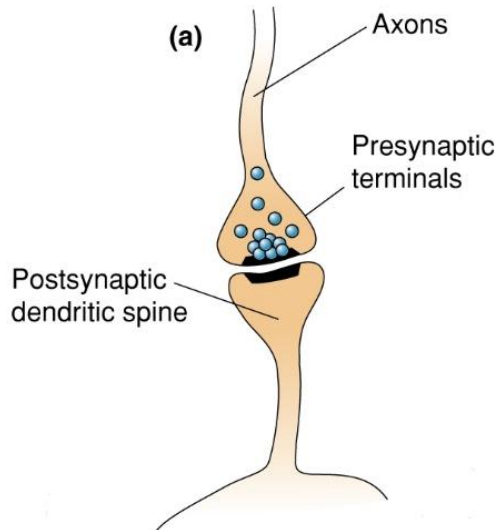
Decision tree for synaptic output



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What impact does an action potential in the presynaptic neuron have on the postsynaptic neuron? That depends...

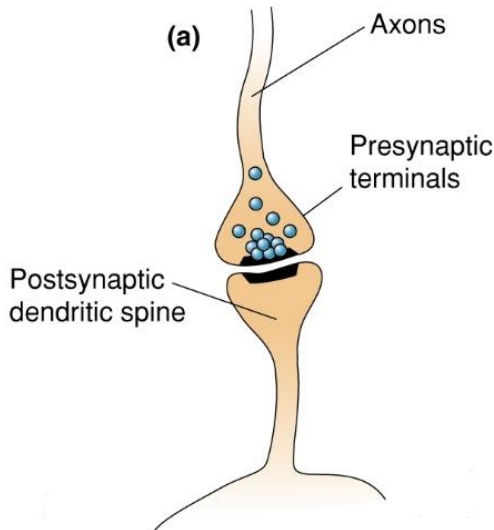
Decision tree for synaptic output



1. What neurotransmitter does the presynaptic neuron release?

Gaba Dopamine
 ↓
 Glutamate
 Acetylcholine

Decision tree for synaptic output



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1. What neurotransmitter does the presynaptic neuron release?

Glutamate

2. What Glutamate receptor does the postsynaptic neuron express?

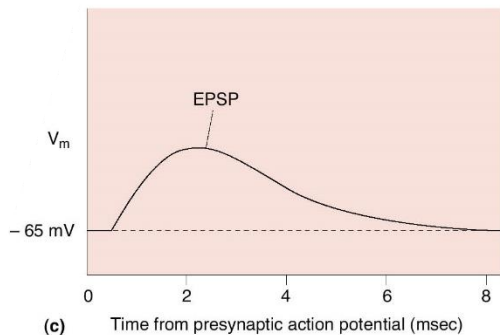
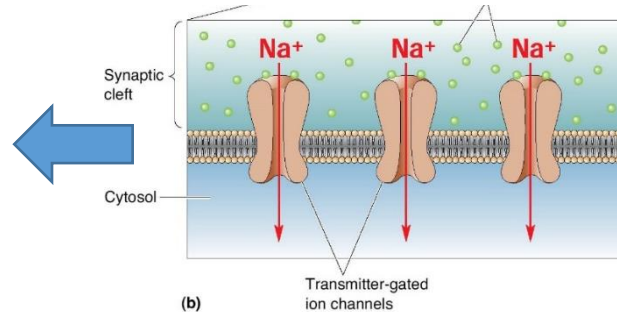
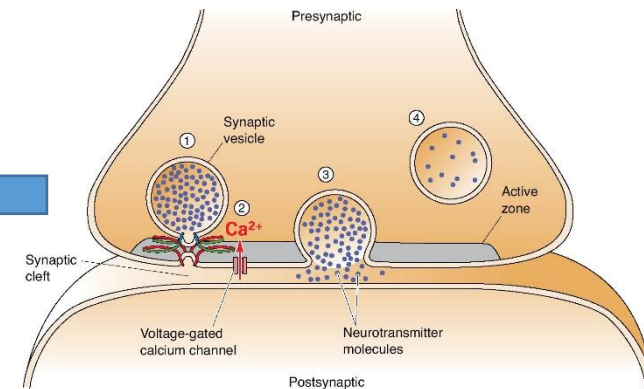
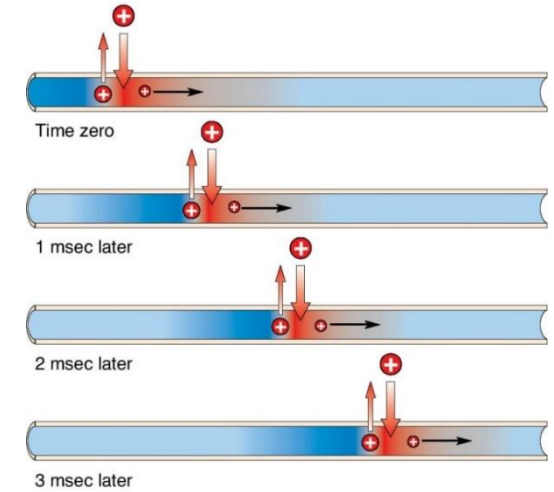
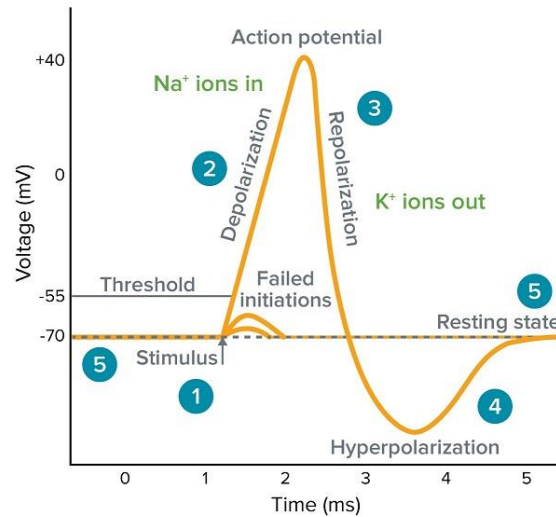
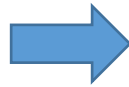
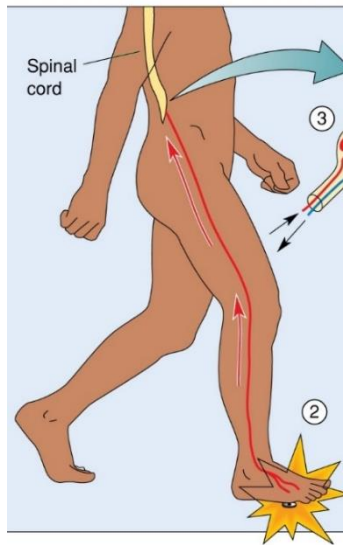
NMDA

mGluR1-8

AMPA

Sodium ion influx, depolarization of postsynaptic neuron

The full pathway; what happens when you step on a thumbtack?



Questions?