

The Synapse: Postsynaptic mechanisms

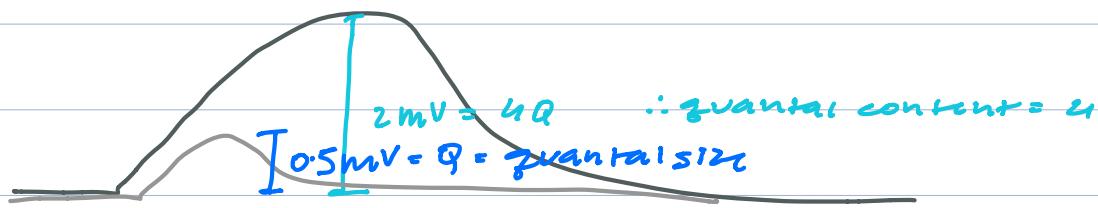
ligand-gated
ion channelsreversal
potentials

EPSPs

Quantal size versus content

↓
Response to one
vesicle
= minisize = Q

↓
of vesicles released



Membrane spanning domain of excitatory receptor

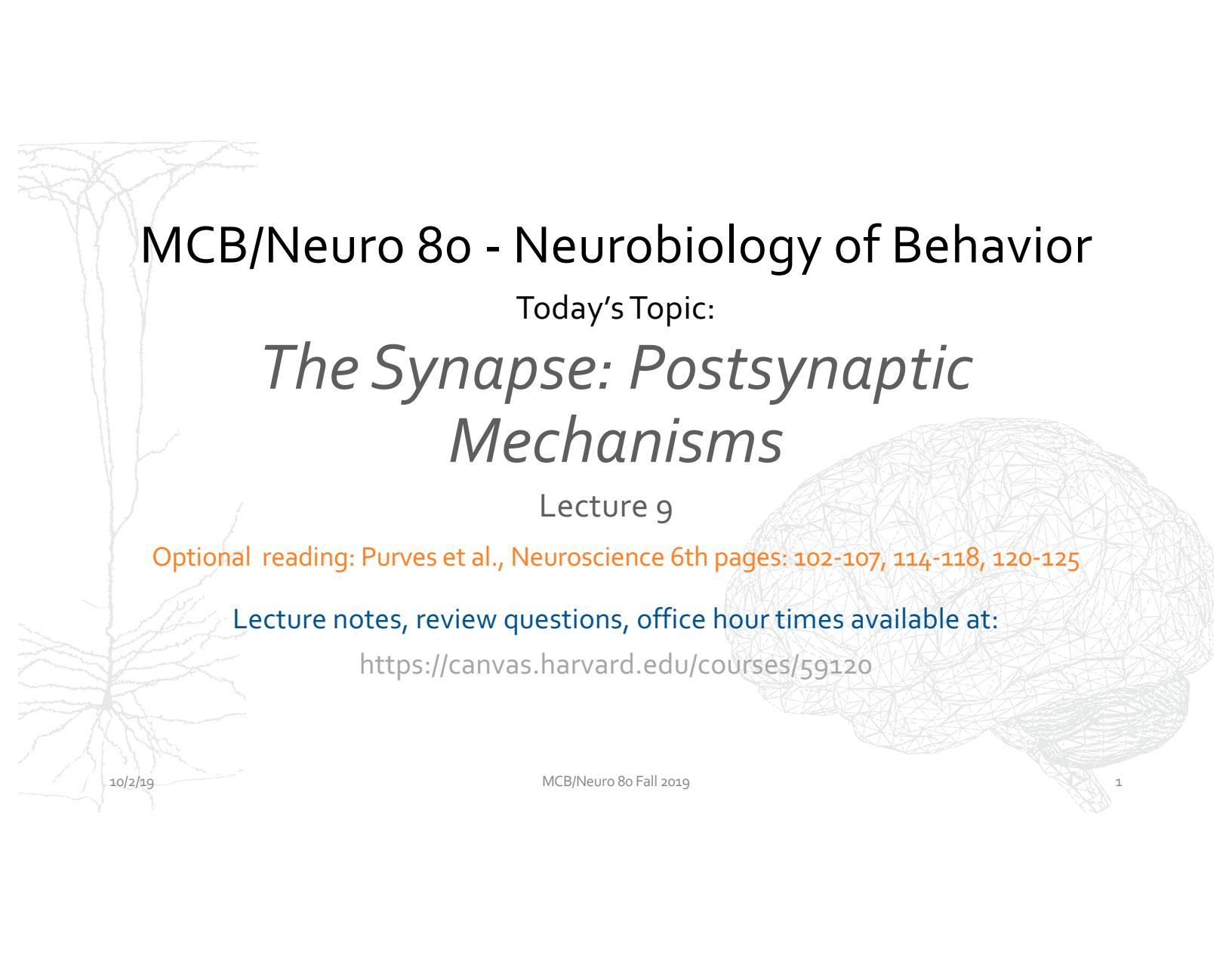
- lined w/ - charged amino acids

Record minis from 2 runs: one w/ quantal size of
 0.4mV and one w/ 0.7mV , why different?

of postsynaptic neurotransmitter receptors

What ion has reversal potential of -10mV ?

- there is no one ion



MCB/Neuro 80 - Neurobiology of Behavior

Today's Topic:

The Synapse: Postsynaptic Mechanisms

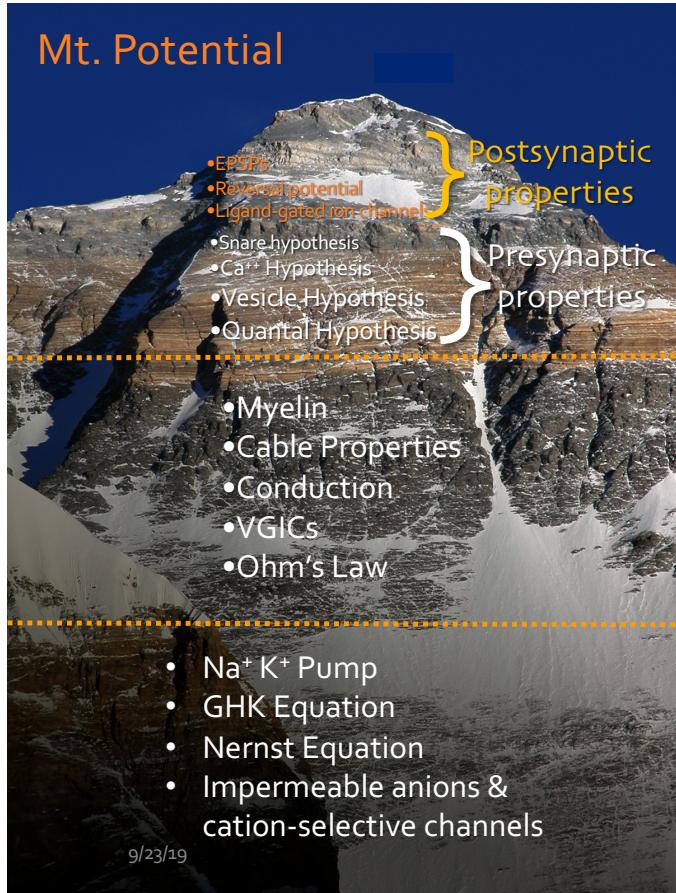
Lecture 9

Optional reading: Purves et al., Neuroscience 6th pages: 102-107, 114-118, 120-125

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

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

Mt. Potential

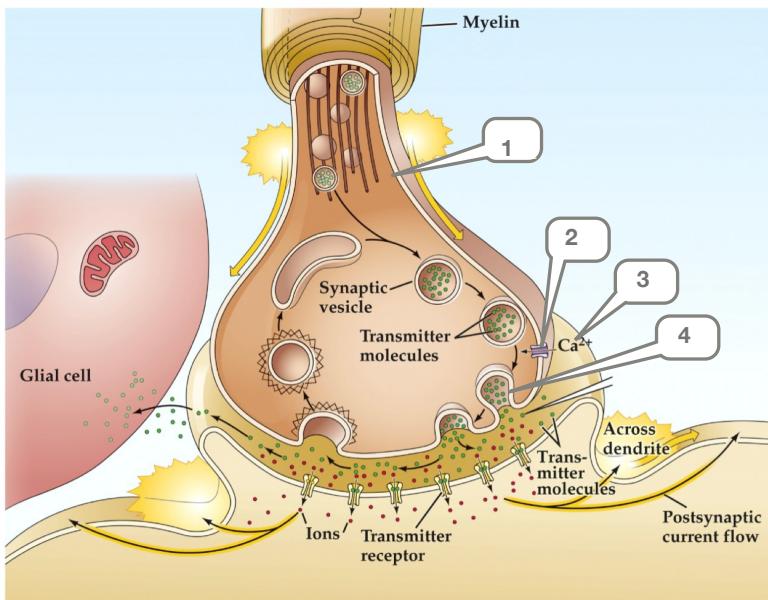


Synaptic Potential

Action Potential

Resting Potential

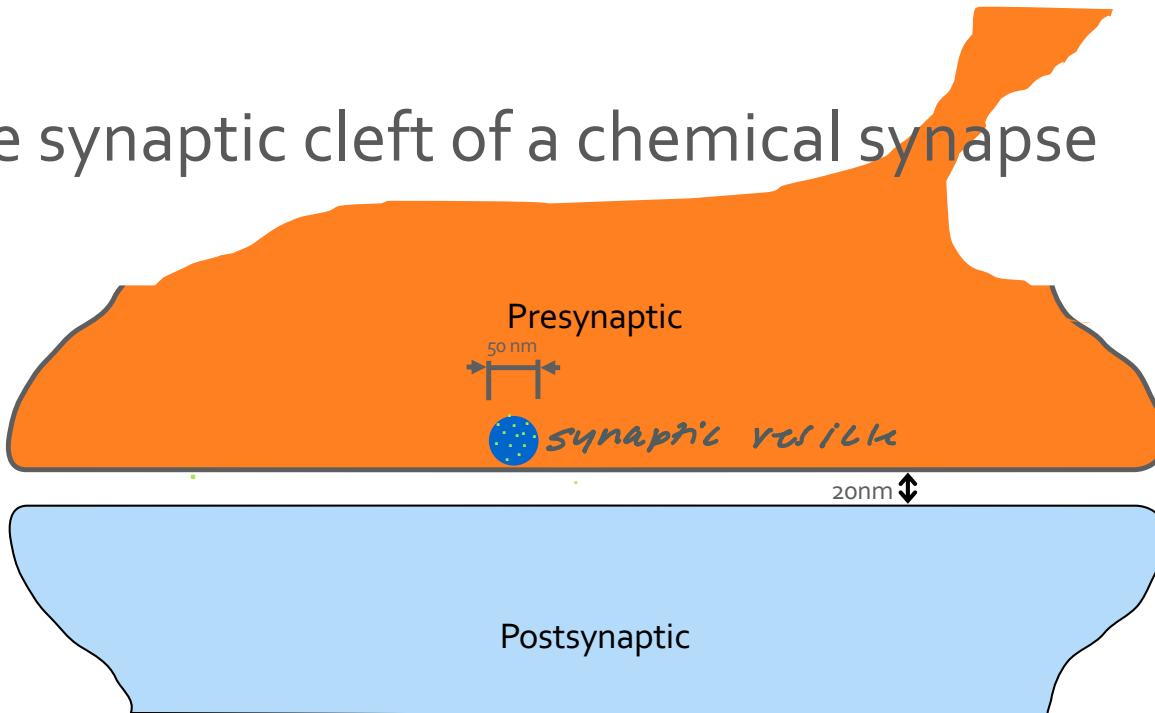
Voltage-dependent calcium entry couples presynaptic action potentials to quantal release of NT



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

postsynaptic spines ~1 micron

The synaptic cleft of a chemical synapse



Time for transmitter to diffuse *across* cleft (<1/25 mile/hr): $< 1\mu\text{s}$

Time for transmitter to diffuse *out* of the cleft: $\sim 1\text{ms}$

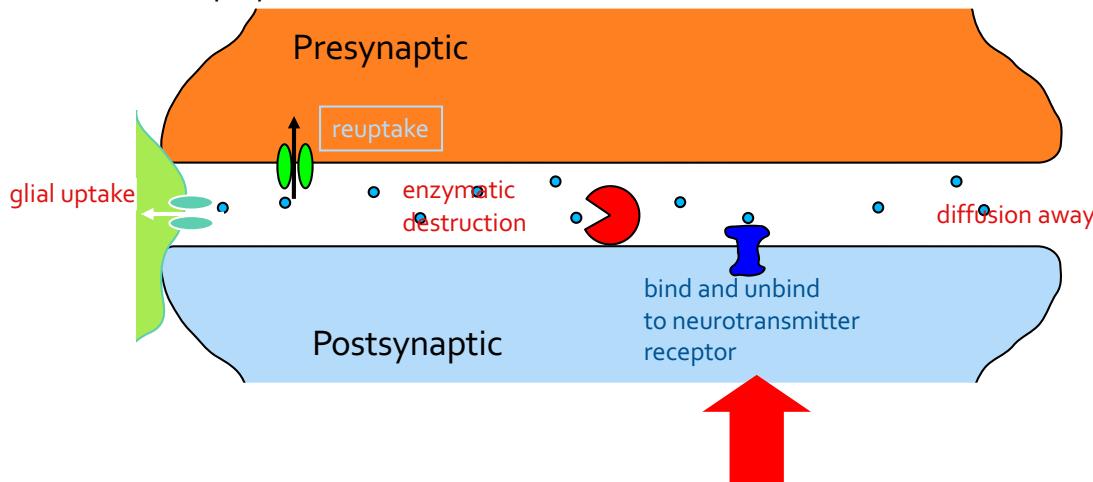
So mechanisms needed to shut off neurotransmitter action

The lifecycle of transmitter

Released neurotransmitter can be:

- 1) Taken up by the presynaptic nerve terminal (e.g., serotonin)
- 2) Broken down by synaptic cleft enzymes (e.g., acetylcholine)
- 3) Taken up by glial cells (e.g. glutamate)
- 4) Diluted to low concentrations by diffusing away (all of them)
- 5) Removed by multiple factors (e.g., acetylcholine (ACh) is cleaved by ACh esterase in cleft and the choline is taken up by the nerve terminal to be used to make more ACh)

SSR1
serotonin reuptake blockers : ↑ serotonin



Ways of classifying synapses:

Largely explained by properties of the postsynaptic cell, especially its neurotransmitter (NT) receptors

Today!

- Fast (5-10's ms): **Ionotropic**
 - NT gated ion channels
- **Excitatory**: brings the membrane potential closer to threshold
- Strength: **strong** (suprathreshold)
 - Single EPSP can cause a postsynaptic action potential

- Slow (10's-minutes): **Metabotropic**
 - NT binding leads to signaling cascade
- **Inhibitory**: bring the membrane potential away from threshold
- Strength: **weak** (subthreshold)
 - Need to integrate multiple smaller EPSPs to cause postsynaptic action potential

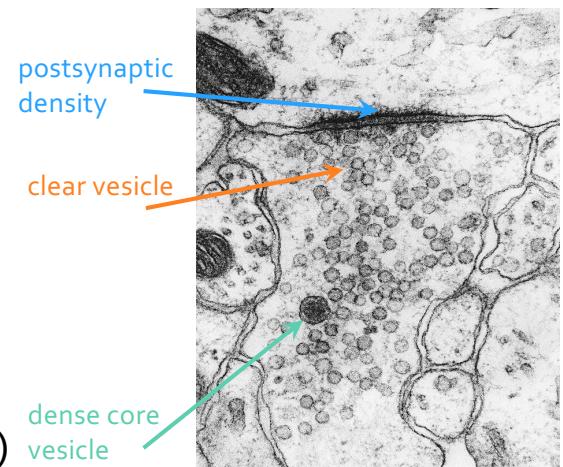
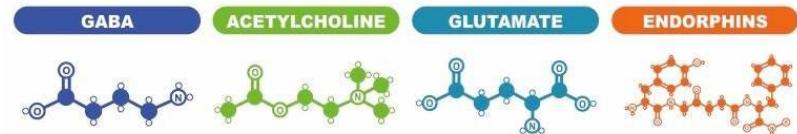
- Neurotransmitter effect depends on the receptor type
- Some neurotransmitters can bind to several different types of receptors.

(Next week)

*ligand-gated ion channel
neuromuscular junction*

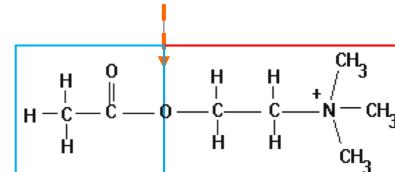
Types of neurotransmitters

- 100s of NTs have been identified
- 2 major classes:
 1. Small molecules – kept in 50 µm clear vesicles, at the active zone
 - Amino acids – glutamate, GABA, glycine
 - Amines – dopamine, serotonin, norepinephrine
 - Other – acetylcholine, APT, endocannabinoids
 2. Peptides – kept in large (150 µm) “dense” vesicles, away from active zone. Typically released after prolonged activity
 - 3-36 AA long
 - Endorphins, substance P, somatostatin, etc...
- Dale's Rule: all terminals of a neuron release the same NTs, (may release more than 1 type)



NMJ (neuromuscular junction) : Exemplar Fast Strong EPSP

- Suprathreshold depolarization in response to **acetylcholine** (ACh)
- ~50-100s of quanta released by motor axon at each NMJ per stimulus (very large quantal content)
- Huge “safety factor” : 15 quanta sufficient to get muscle action potential. Why so much excess?
- Chemical Specificity: muscle membrane has high density of ACh Receptors (AChRs) at NMJ
- Spatial Specificity: AChRs closely aligned with the synaptic terminal
- Mechanism: AChRs are **ligand-gated ion channels** (ACh is the “ligand”)



Alpha bungarotoxin, a venom component that blocks the AChR



Chinese Krait: *Bungarus multicinctus*

6/17/10



- Elapid snakes (cobras, coral, mambas and kraits)
- Have short non-retractable fangs
- Use toxin to incapacitate prey

A MUSCLE:

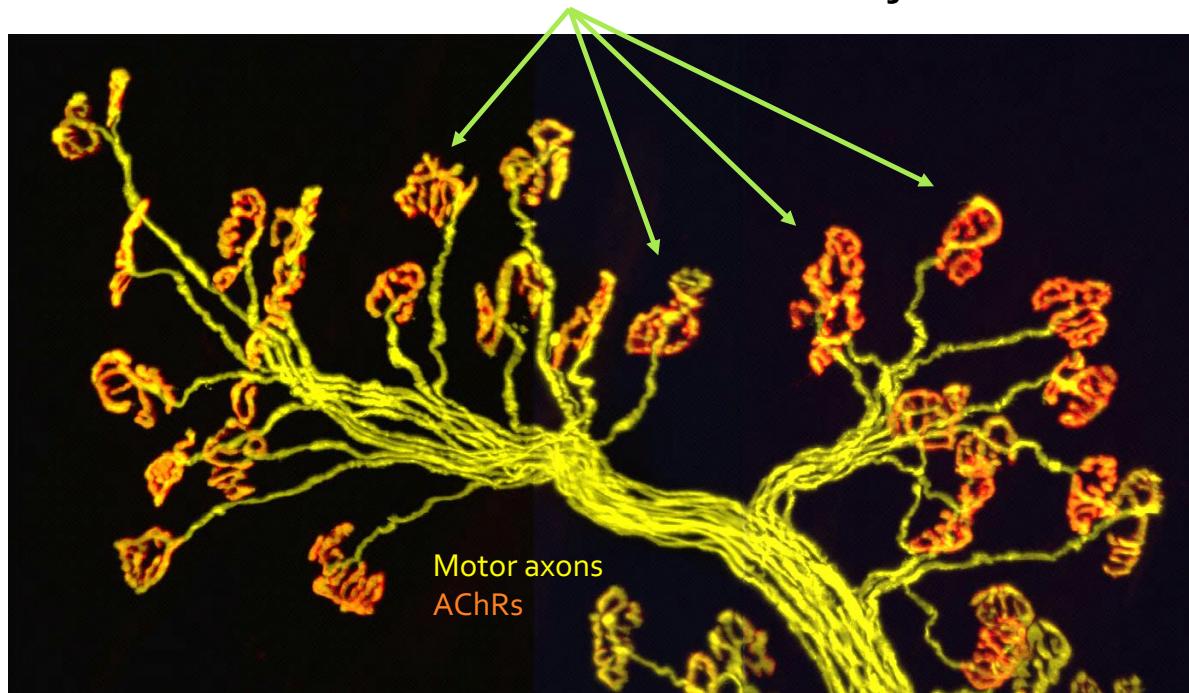
Motor axons (transgenically labeled with a yellow fluorescent protein)

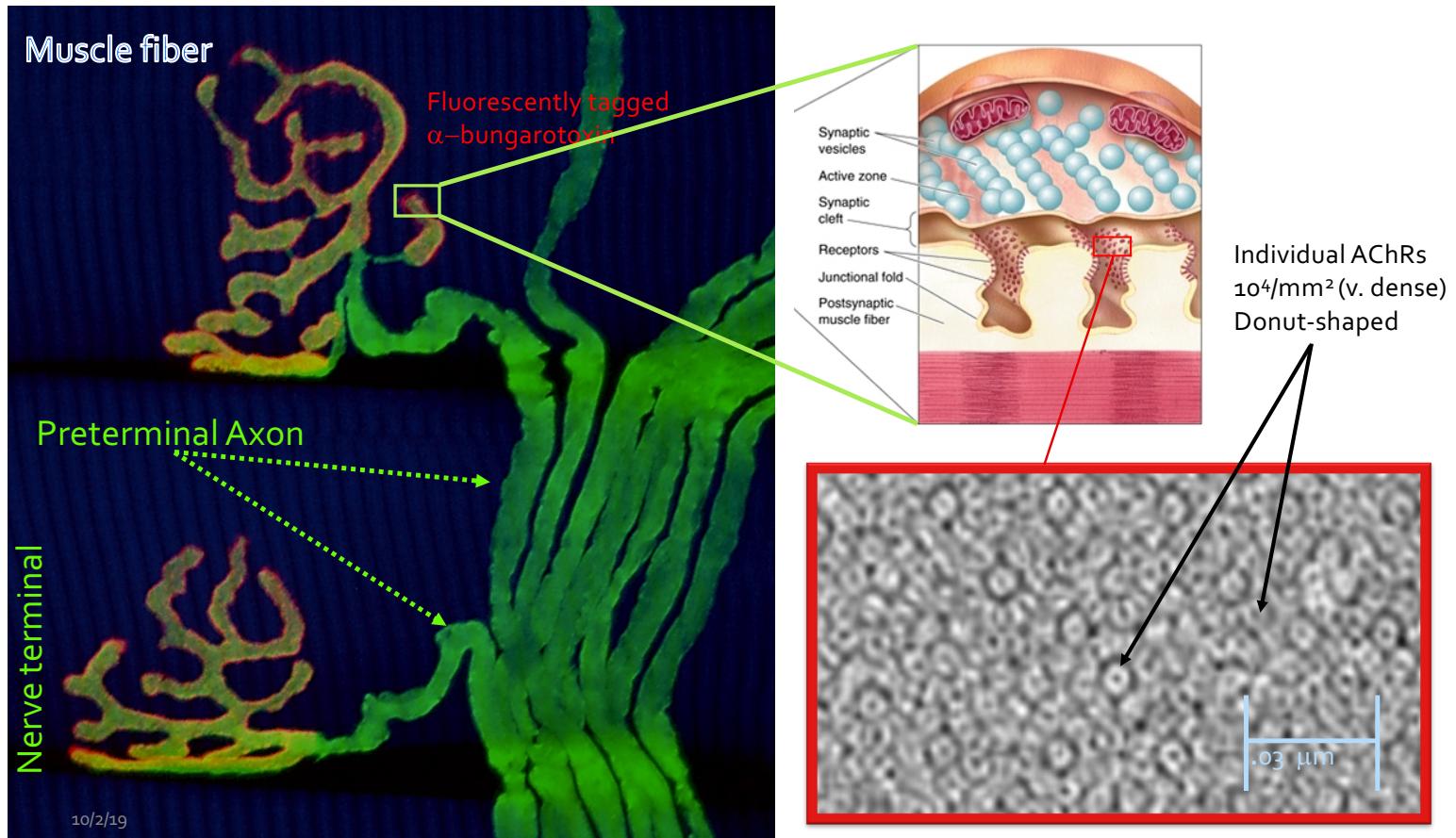
AChRs (labeled with fluorescently tagged alpha bungarotoxin)



Nerve: bundle containing lots of motor and some sensory axons

Neuromuscular junctions

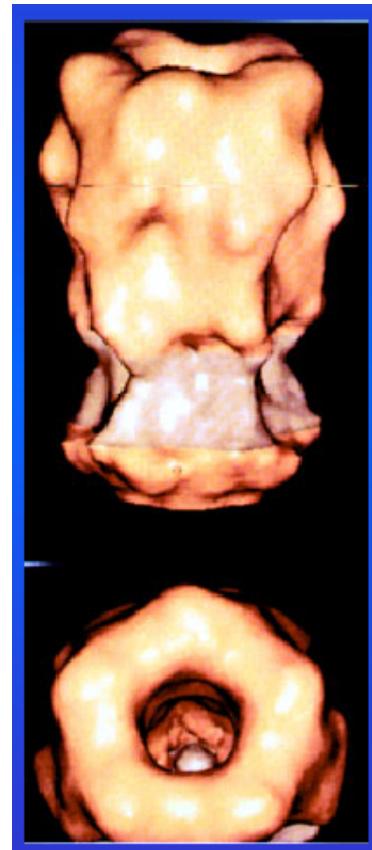
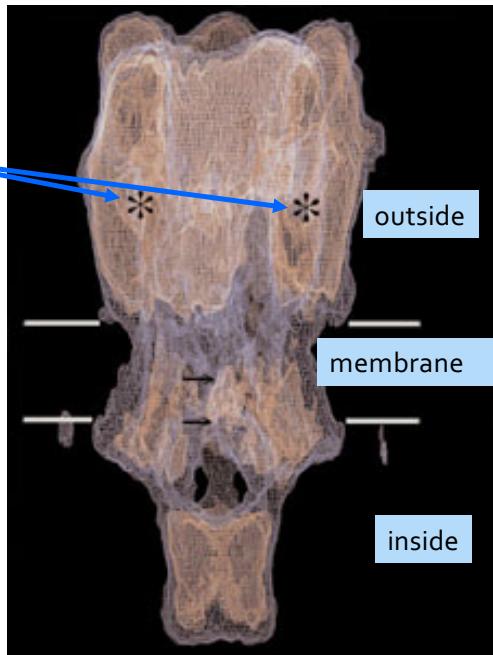




AChR channel structure

ACh binding sites
(**2** Per AChR,
and both need to be
occupied for channel
to open)

- Pore is 0.8 nm
- Bigger than V-gated channels
- Ions plus water entourage goes through
- Less ion specific

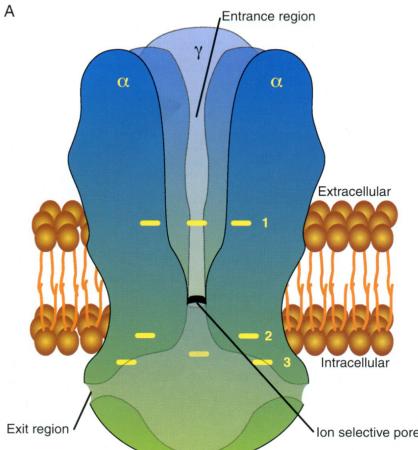


X-ray crystallography

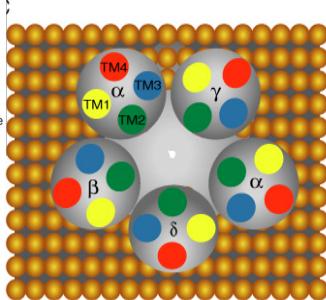
"nicotinic"

ACh receptor structure – a ligand gated ion channel

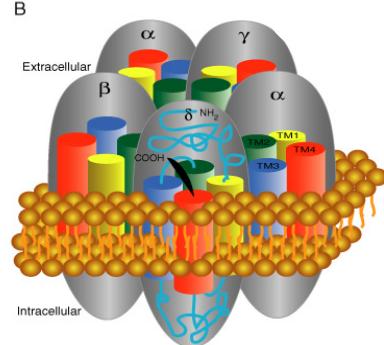
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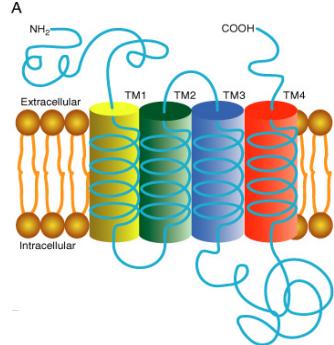
- 5 **separate** proteins ("subunits") $\alpha_2\beta\gamma\delta$
 - (vs. 1 protein for the voltage gated Na channel)
- Each subunit has 4 membrane spanning domains (M1-M4)
- M2 domains line the pore



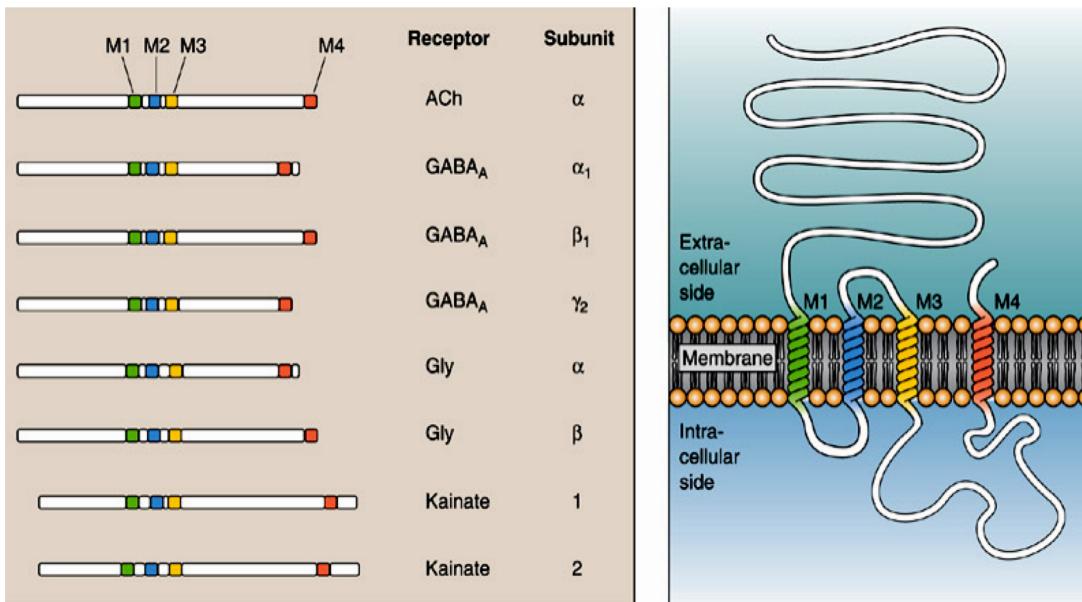
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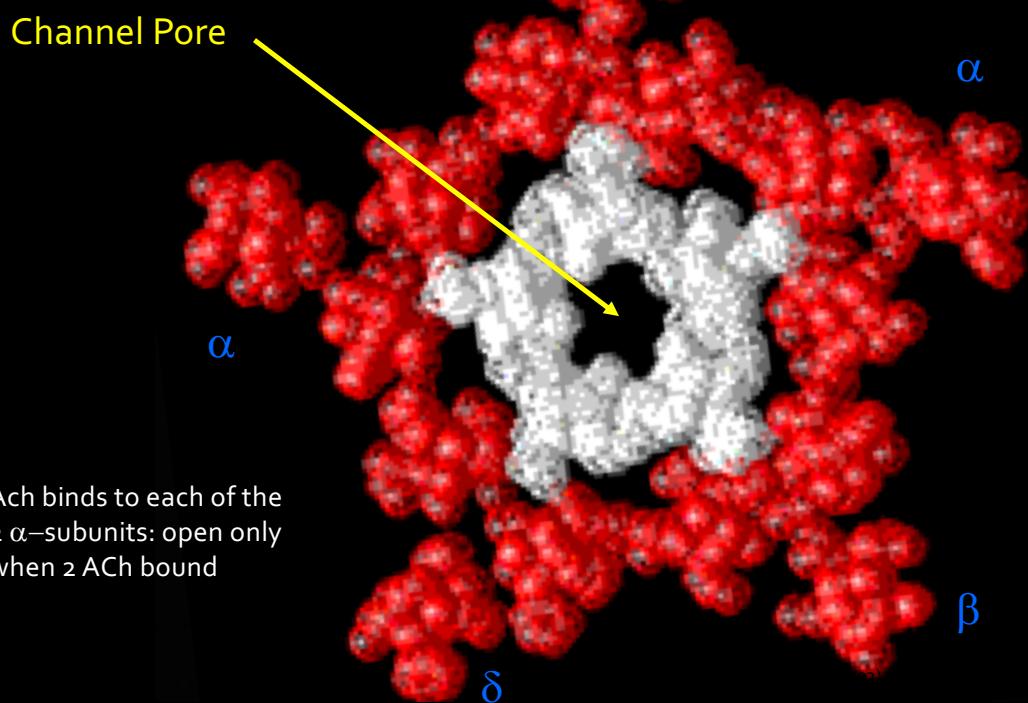
C



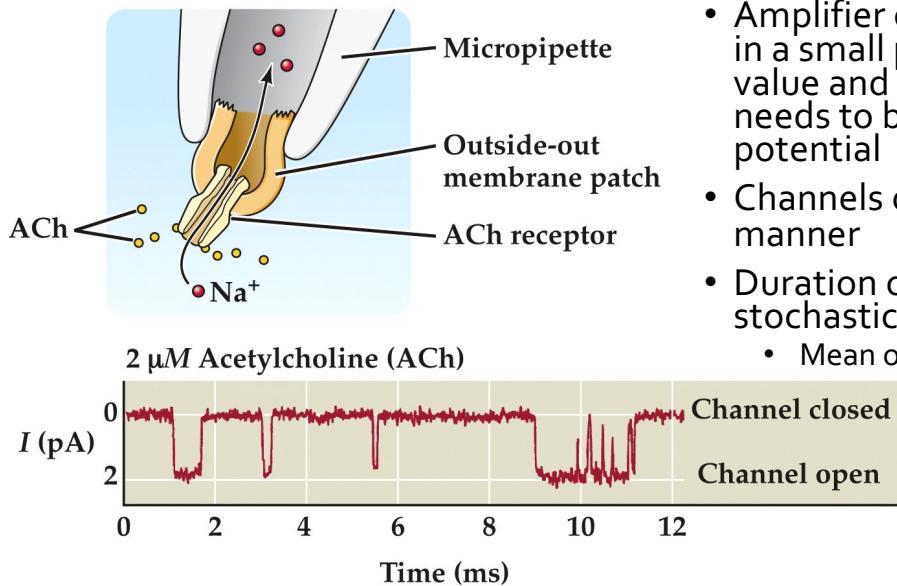
Many neurotransmitter receptor subunits are part of the same gene family



ACh (the “ligand”) causes conformational change in the AChR, (its “receptor”). Therefore the AChR is a “ligand-gated ion channel”

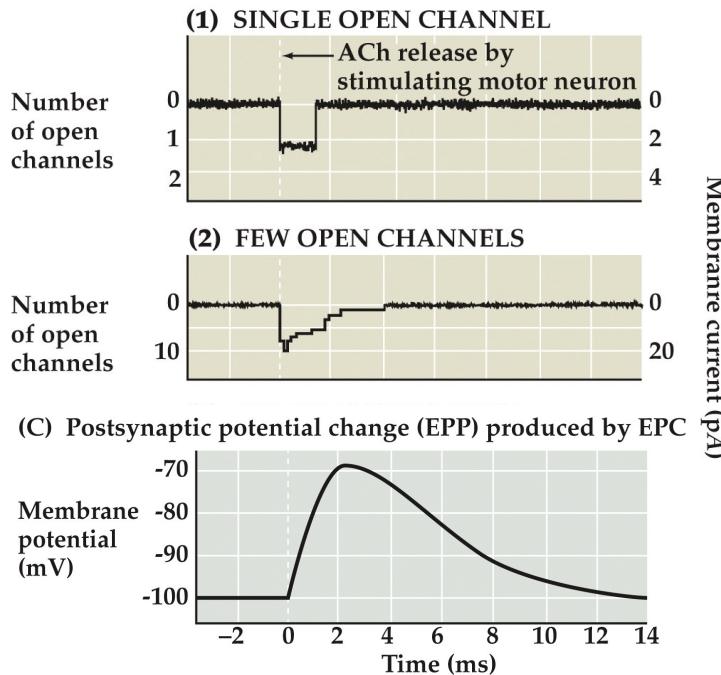


Single channel “Patch clamp” technique to isolate single channel currents



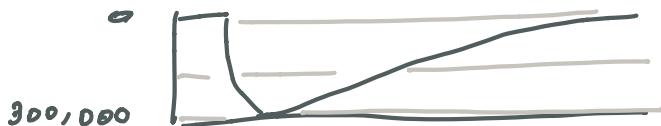
- Amplifier clamps the membrane voltage in a small patch of membrane to a set value and records how much current needs to be applied to maintain that potential
- Channels open and close in a stepwise manner
- Duration of opening of AchR is stochastic
 - Mean open time ~1 msec

The postsynaptic potential is due to the sum of the currents through many channels

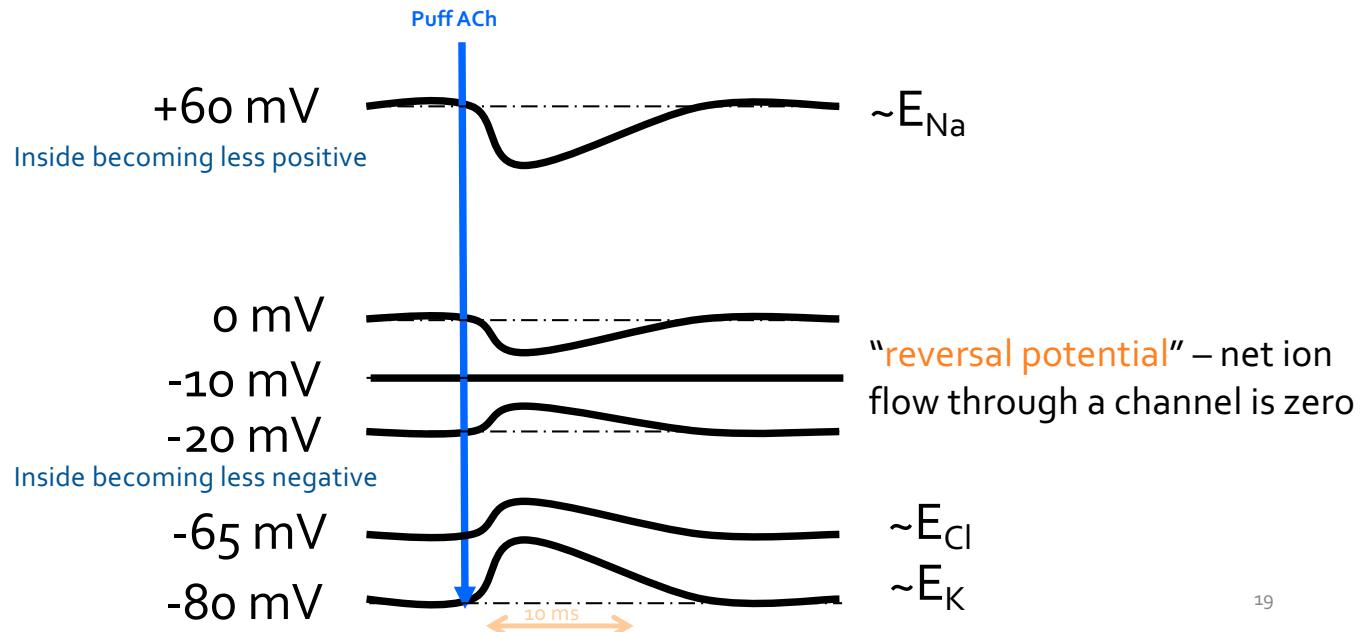


- All channels open at nearly the same time but close randomly
- Channel current sum together to produce the total membrane current
- Current flow through AChR channels
 - Remember downward deflection (negative; inward) is depolarizing
- Change in membrane potential in the muscle (EPP)
 - Upward deflection (positive voltage) is depolarizing
- Rising phase ligand gated ion channels **open**
- Falling phase ligand gated ion channels **closed** - K⁺ through leak channels (always open) repolarize the membrane

All channels open



What ions go through the channel? By changing the membrane potential by current injection we can get an idea



Reversal Potential of the AChR is due to synchronous conductance of several cations

AChR is a **promiscuous cation channel**

With conductance for Na^+ , K^+ , and some small amount of Ca^{++}

At **reversal potential** there is **no net charge transfer**, i.e., inward ion flow equal and opposite to outward ion flow

$$I_{\text{Na}} + I_K = 0$$

$$I_{\text{Na}} = -I_K$$

$$g_{\text{Na}}(V_m - E_{\text{Na}}) = -g_K(V_m - E_K)$$

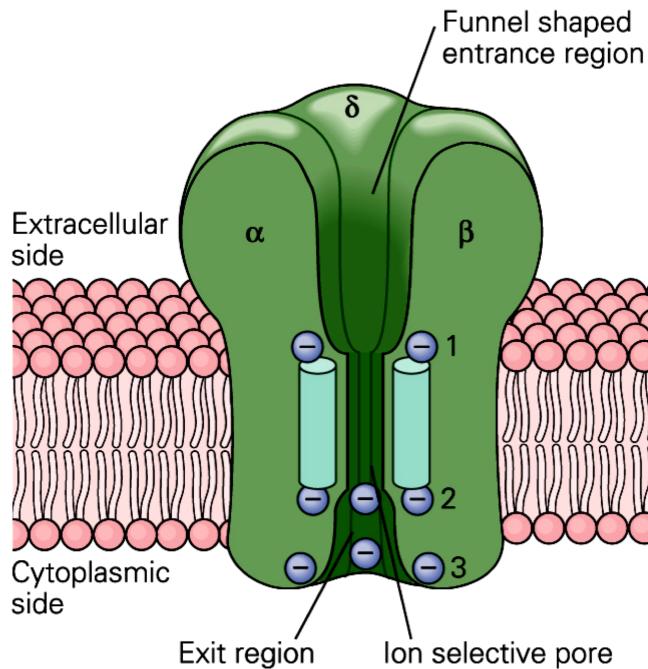
solve for V_m

$$V_m = \frac{g_K \cdot E_K + g_{\text{Na}} \cdot E_{\text{Na}}}{g_K + g_{\text{Na}}}$$

If $g_K = g_{\text{Na}}$ then

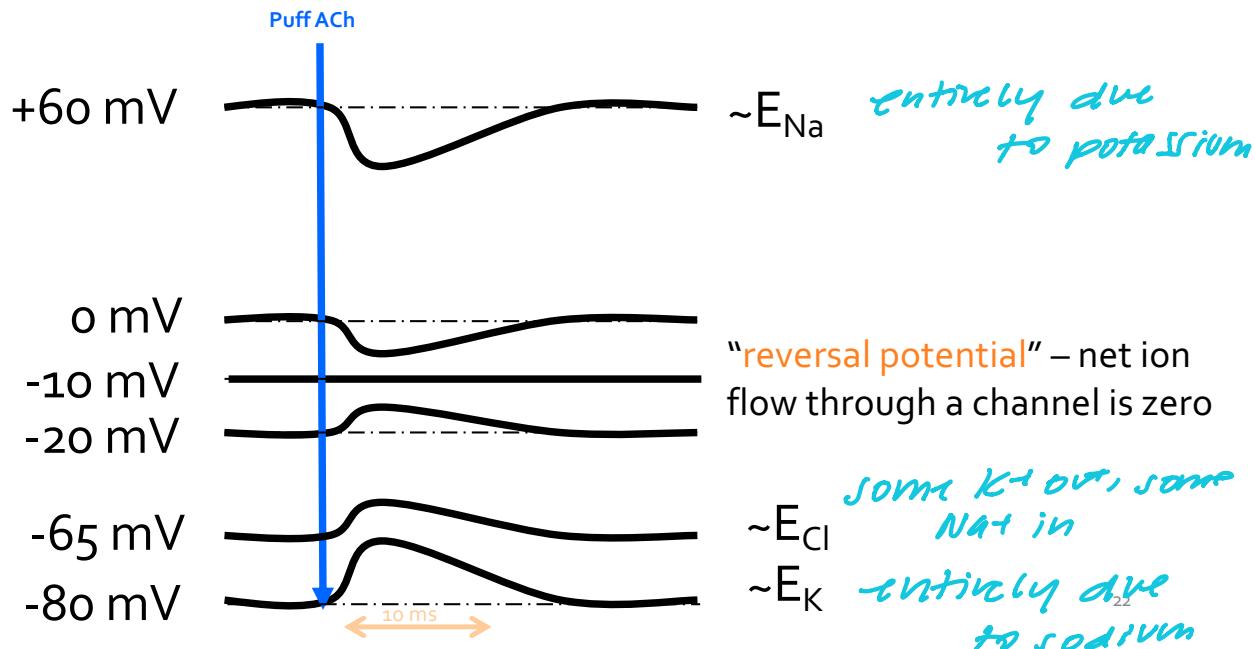
$$V_m = \frac{E_K + E_{\text{Na}}}{2} = \frac{-80 + 60}{2} = -10 \text{ mV}$$

Functional model of ACh receptor-channel



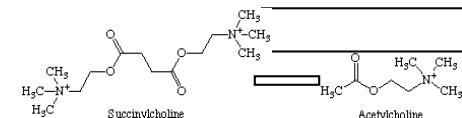
Cation (Na^+ & K^+) selectivity of acetylcholine receptor due to negatively charged amino acids lining the pore of the channel, but size allows both ions to flow with approximately equal conductance

What ions go through the channel? By changing the membrane potential by current injection we can get an idea



Some ligand gated channels (e.g. AChR) undergo desensitization when in the presence of too much neurotransmitter

- If AChRs or other ligand gated ion channels are open for a long time (e.g., if too much neurotransmitter is released/bound too long) then the channel closes
 - analogous to Na^+ inactivation in voltage gated channel
- **Succinyl choline** not cleaved easily by ACh esterase and is thus a muscle relaxant used commonly in surgery because it causes desensitization of the AChR and paralysis
- Pesticides and Nerve Gas (e.g., Sarin) use this mechanism of paralysis too



some muscle relaxants bind to AChR receptors
and desensitize them
Same w/ sarin

Comparing AP channels with AChR channels

# of channel types?	2 channels (Na^+ , K^+)	1 channel
Gating mechanism?	voltage gated	ligand-gated
Sequence of conductances?	gNa^+ then gK^+	simultaneous g's
Blockers?	TTX	$\alpha\text{-BTX}$
Diseases?	Some channelopathies	myasthenia gravis

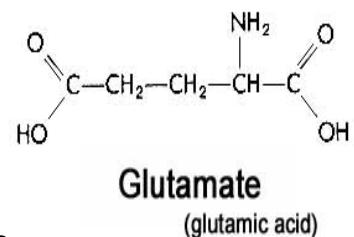
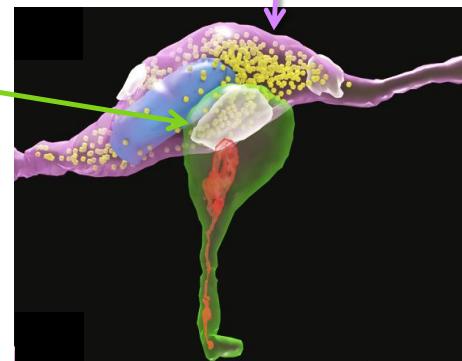
In CNS: the fast (but usually weak) EPSPs are due to Glutamate release from **axon varicosities** onto **dendritic spines**

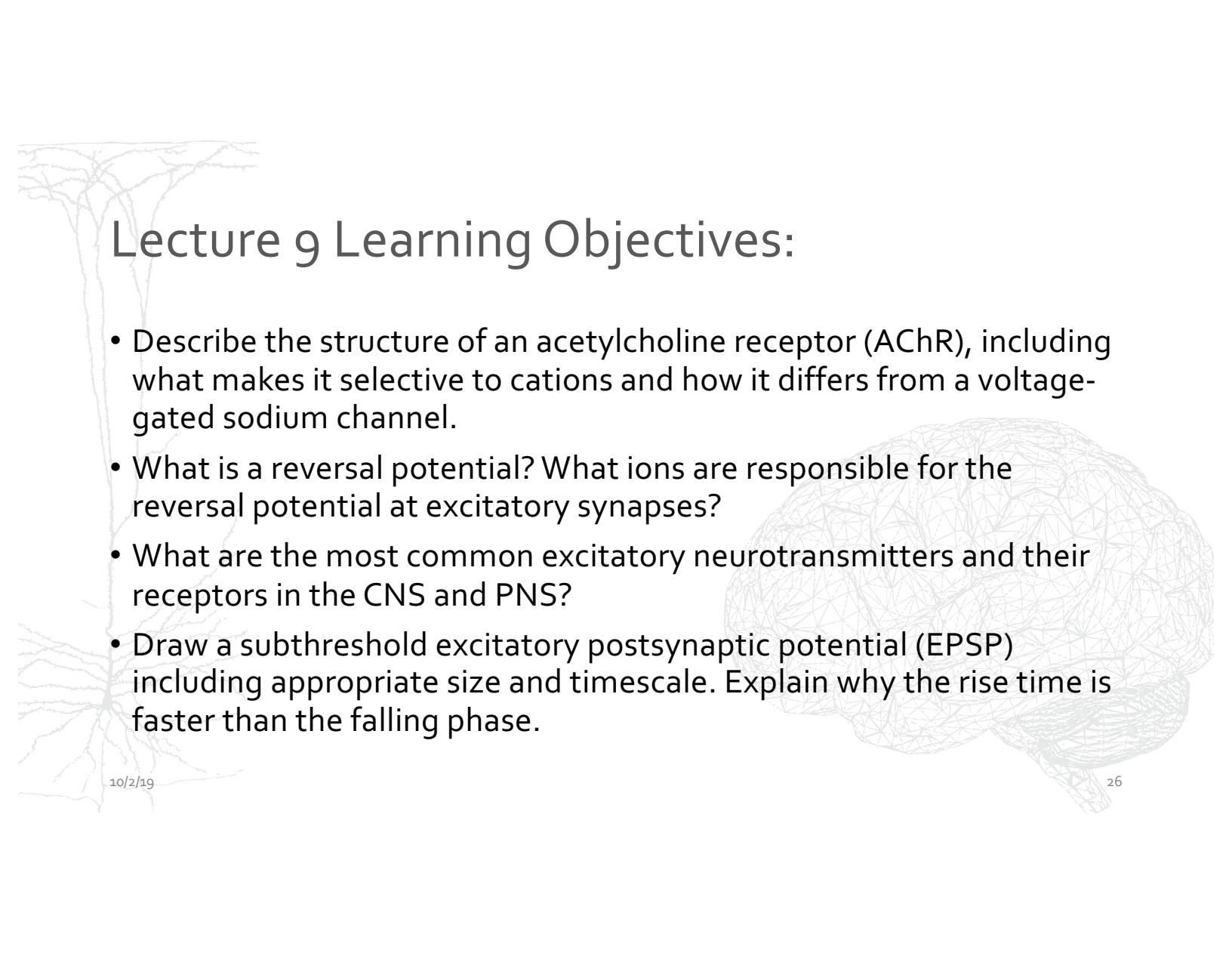
Many types of glutamate receptors
Ionotropic (let ions flow):

- **AMPA** Receptor
 - Main glutamate channel
- **NMDA** Receptor
 - Voltage and Ligand Gated
 - Lets Ca^{++} enter during large depolarization important for **plasticity** (more later)

Metabotropic (change metabolism)

G protein coupled – you'll hear about these in another lecture





Lecture 9 Learning Objectives:

- Describe the structure of an acetylcholine receptor (AChR), including what makes it selective to cations and how it differs from a voltage-gated sodium channel.
- What is a reversal potential? What ions are responsible for the reversal potential at excitatory synapses?
- What are the most common excitatory neurotransmitters and their receptors in the CNS and PNS?
- Draw a subthreshold excitatory postsynaptic potential (EPSP) including appropriate size and timescale. Explain why the rise time is faster than the falling phase.

Lecture 9 - Postsynaptic Potentials at Excitatory Synapses

Pre-class notes for October 2, 2019

Reading: *Neuroscience* by Purves et al., pages 102-107, 114-118, 120-125

After the vesicle fuses to the presynaptic membrane, the neurotransmitter molecules must diffuse across the synapse and bind to the receptors on the postsynaptic cell. In addition to binding to a postsynaptic receptor, there are several other possible outcomes of the neurotransmitter including, **diffusion** and dilution away from the synapse, and:

Reuptake - method of removing neurotransmitter from the synaptic cleft through transporters located the presynaptic terminal. There may instead (or also) be **uptake**, where neurotransmitter is taken up by glia.

Enzymatic breakdown - there are enzymes in the synaptic cleft that break down neurotransmitter molecules so they are no longer able to bind to postsynaptic receptors.

Much of what type of message the synapse conveys depends on the type of receptor. Today we will be focusing on fast, excitatory synaptic transmission.

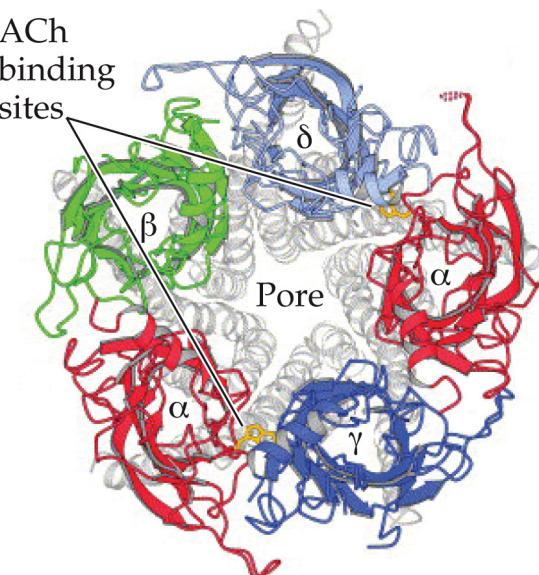
Excitatory synapse - a synapse where the release of neurotransmitter increases the likelihood that the *postsynaptic* neuron fires an action potential. Usually by bringing the membrane potential closer to or beyond the threshold potential.

Ligand-gated ion channel - type of neurotransmitter receptor in the postsynaptic membrane, also known as *inotropic receptor*, where the binding of a neurotransmitter molecule, causes the opening of a channel and allows ions to flow through the channel.

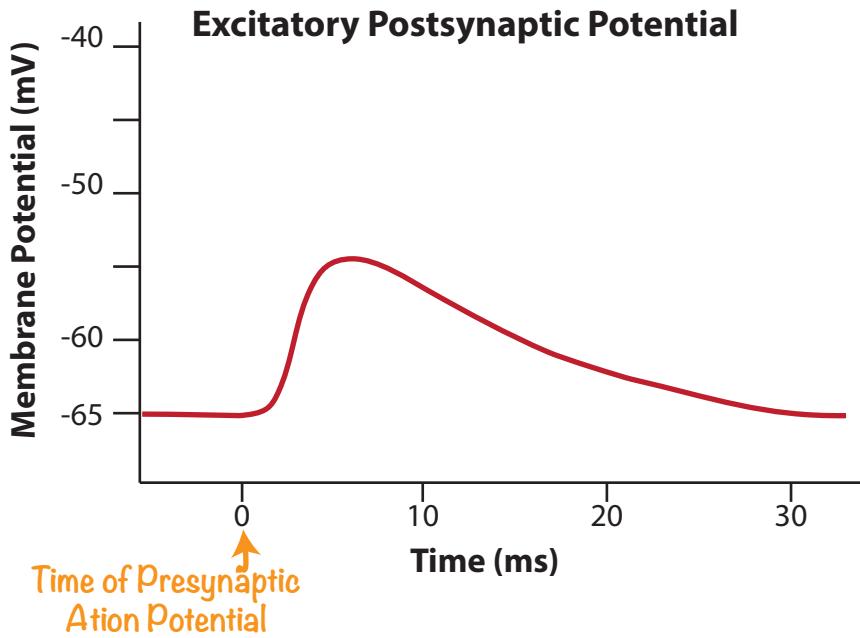
Acetylcholine (ACh) - the excitatory neurotransmitter at the neuromuscular junction (NMJ).

Acetylcholine receptor (AChR) - type of synaptic receptor at the NMJ, located on the muscle. NMJ AChR are ligand-gated ion channels that desensitize and are activated by nicotine so sometimes referred to as a *nicotinic* AChR or nAChR. This channel, when opened, permits the flow of Na^+ and K^+ currents.

Alpha-bungarotoxin - competitive agonist for nAChRs that are purified from snake venom. α -bungarotoxin specifically binds to and blocks the receptor.



From Unwin (2005) J. Mol. Biol. 346: 967-989



Excitatory postsynaptic potential (EPSP) - a transient depolarization of the membrane potential as a result of the activation of an excitatory chemical synaptic. An EPSP is an EPSP at the NMJ. An EPSP typically has a fast rise and a slower decay.

An EPSP mediated by ligand-gated ion channels like the nAChR, will rise as long as the ion channel is open and there is a net flow of cations into the cell.

Reversal potential (V_{Rev}) - membrane potential where the *net current* through an open ligand-gated ion channel (or receptor) would be zero. Depends on the permeability of the channel to different ions and the equilibrium potential for those ions. For example the reversal potential at the NMJ is ~ 10 mV because the conductance of sodium equals the conductance of potassium through the nAChR.

$$I_{\text{Na}} + I_K = 0 \Rightarrow I_{\text{Na}} = -I_K$$

$$[I = g * \text{driving force}]$$

$$g_{\text{Na}} (V_M - E_{\text{Na}}) = g_K (V_M - E_K) \Rightarrow V_M = \frac{g_K * E_K + g_{\text{Na}} * E_{\text{Na}}}{g_K + g_{\text{Na}}}$$

$$\text{If } g_K = g_{\text{Na}}, \text{ then } V_M = \frac{E_K + E_{\text{Na}}}{2}$$

Desensitization - property of some ligand-gated ion channels such that if they are open for a long time, they will desensitize or close even though neurotransmitter molecules may still be bound to the receptor. Typically, AChRs and AMPA receptors desensitize.

Glutamate - major neurotransmitter at excitatory synapses in the central nervous system.

AMPA receptor - glutamate-gated ion channel. Typical AMPA receptors desensitize, are permeable to Na^+ and K^+ but impermeable to Ca^{2+} . Has fast rise and decay times. AMPA channels are the most common receptor type in the brain.

NMDA receptor - glutamate-gated ion channel, that is also voltage dependent. Mg^{2+} blocks the receptor unless depolarization pushed the Mg^{2+} out of the pore. Typical AMPA receptors do not desensitize, are permeable to Na^+ and K^+ and Ca^{2+} . Has slower rise and decay times than AMPA receptors. NMDA receptors are important in learning and synaptic plasticity.

Learning Objectives: (By the end of Lecture 9 you should be able answer the following)

1. Describe the structure of an acetylcholine receptor (AChR), including what makes it selective to cations and how it differs from a voltage-gated sodium channel.
2. What is a reversal potential? What ions are responsible for the reversal potential at fast excitatory synapses?
3. List the most common excitatory neurotransmitters in the CNS and PNS.
4. Draw a subthreshold excitatory postsynaptic potential (EPSP) including appropriate size and timescale. Explain why the rise time is faster than the falling phase.