Lecture 3
Neurophysiology I/II

PSYC 304

Announcements

Reading club articles

- Sign up will be open at 9pm tonight.
- Readings will be released at 12pm tomorrow.
- Anyone not signed up by Thursday, will be auto-assigned into a group by Friday end of day.
- Reading club 1 is on May 29th.

Midterm 1

- June 3rd
- Covers all material up to that point
- 6pm, zoom link and test in modules in Canvas, 60-90 minutes

Reading club details

PSYC 370 Reading club presentation submissions (12% of final grade)

- Once each semester, students will sign up for a reading, for which they will provide a brief recorded PPT presentation summary.
- On the due date of your article summary, we will have an in-class discussion regarding the reading. We will get in small group (4-5 people and you will give an informal presentation of your summary. After, we will discuss the important take-aways as a class.
- The readings are examinable (about 1 questions per reading), and the discussion is not recorded.
- The summary is meant to describe the article in simple terms that can be understood by a broad audience (i.e., your non-academically inclined family and friends could watch your summary and understand the main takeaways from the reading)
- Length: 9-10 slides (including title page), 7-8 minutes long, pre-recorded PPT (.mp4).

Reading club details

Basic Rubric (based on a score /10)

- ***Overall impression*** – Quality, style, and clarity. The presentation was clear, concise, enjoyable to watch. /2
- ***Narrative/Introduction*** – The stage is set for an explanation of the research: Why is it important? what questions are being asked/investigated? what lead to this research? Who cares? /2
- ***Methods*** – A concise explanation of the general methodology used is provided. It is clear how the study was conducted, what the variables of interest were, and how changes in those variables were assessed. Definitions are provided for all key term, key variables, and jargony words. /1

Reading club details

Basic Rubric (based on a score /10)

- ***Results*** – Important/relevant results are summarized including the key take-aways. Although the audience may not have the technical knowledge to understand the results themselves, they are able to understand what the results mean. /2
- ***Discussion*** – The results are discussed in the context of the narrative provided. The reader has a clear understanding of the impact of those results in the research field, and where the results may lead (or may have led). /2
- ***References*** – Claims requiring references had references. Please include at least 3 references (in addition to the paper itself). You may use references from the paperitself. References can use in-text APA styling. /1

Reading club details

Basic Rubric (based on a score /10)

Removal of points

- Inconsistent referencing style
- Stretched on out of focus images
- Clunky slides (too much text, lack of formatting, hidden images/text etc.)
- Time or slide limit not followed.

Example of reading club day timing:

- 7:50pm – Reading Club 1A, those in group 1A will present to a small group of peers that aren't in group 1A
- 8:00pm – presentation ends – question period begins
- 8:10pm – Large class discussion of the paper
- 8:20pm – Reading club 2A (repeat same as above for 2A)

Review

Visualizing neurons

Resting Membrane potential

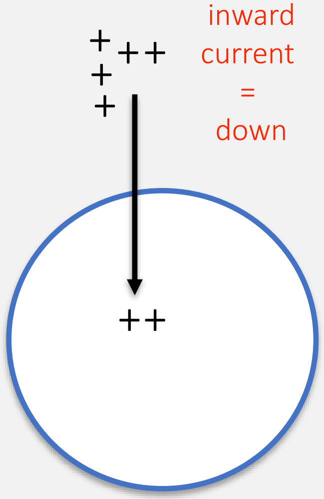
Exercise (Answers):

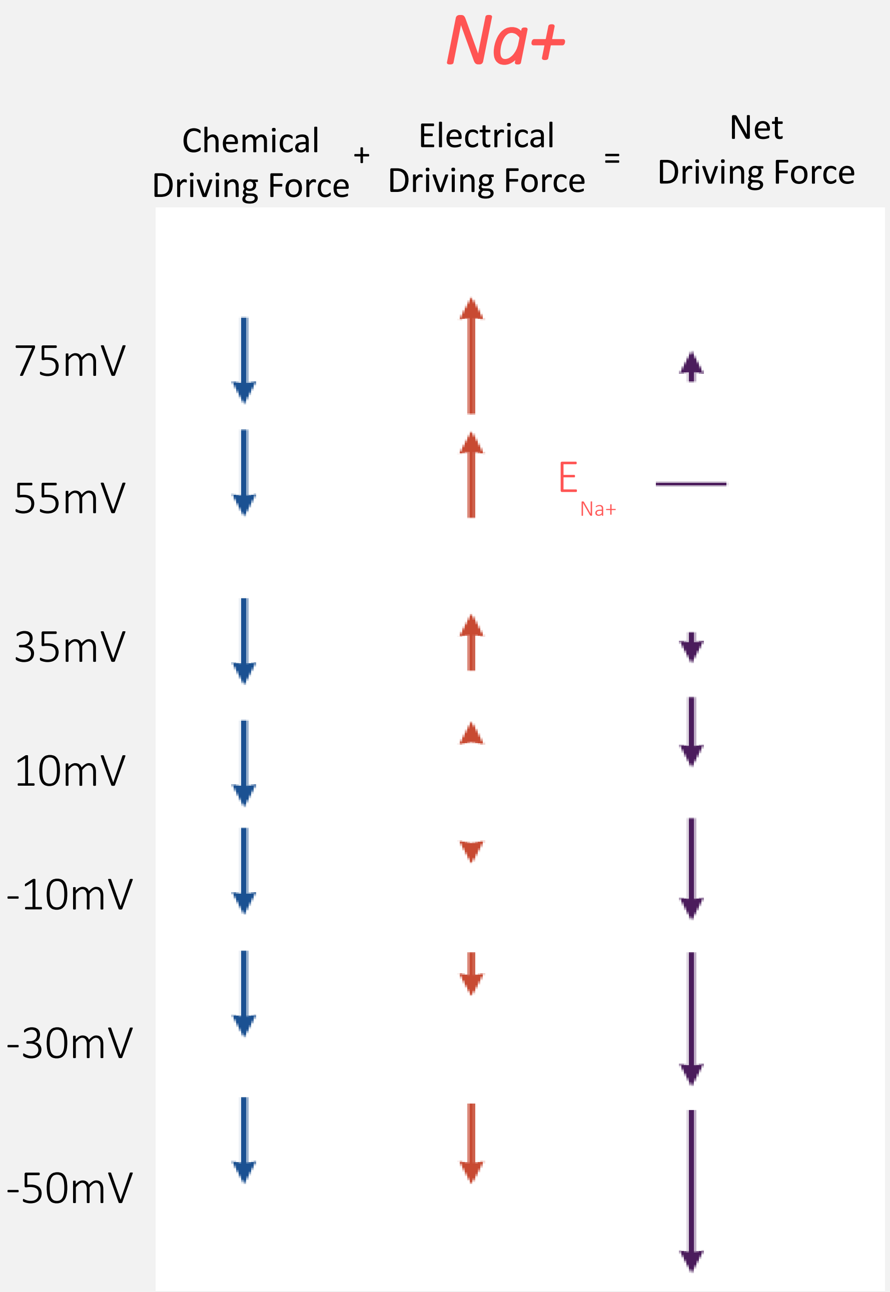
Graphically represent chemical and electric driving forces for Na+ and K+, at different membrane potentials

--use downward arrows of various sizes to indicate magnitude of inward current (ie positive charge flowing into the cell), upward arrows for outward current

—hint: is the chemical driving force different depending on the membrane potential?

To help you remember that inward current is down, and outward current is up:





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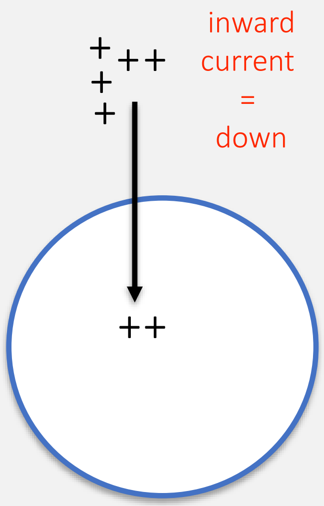
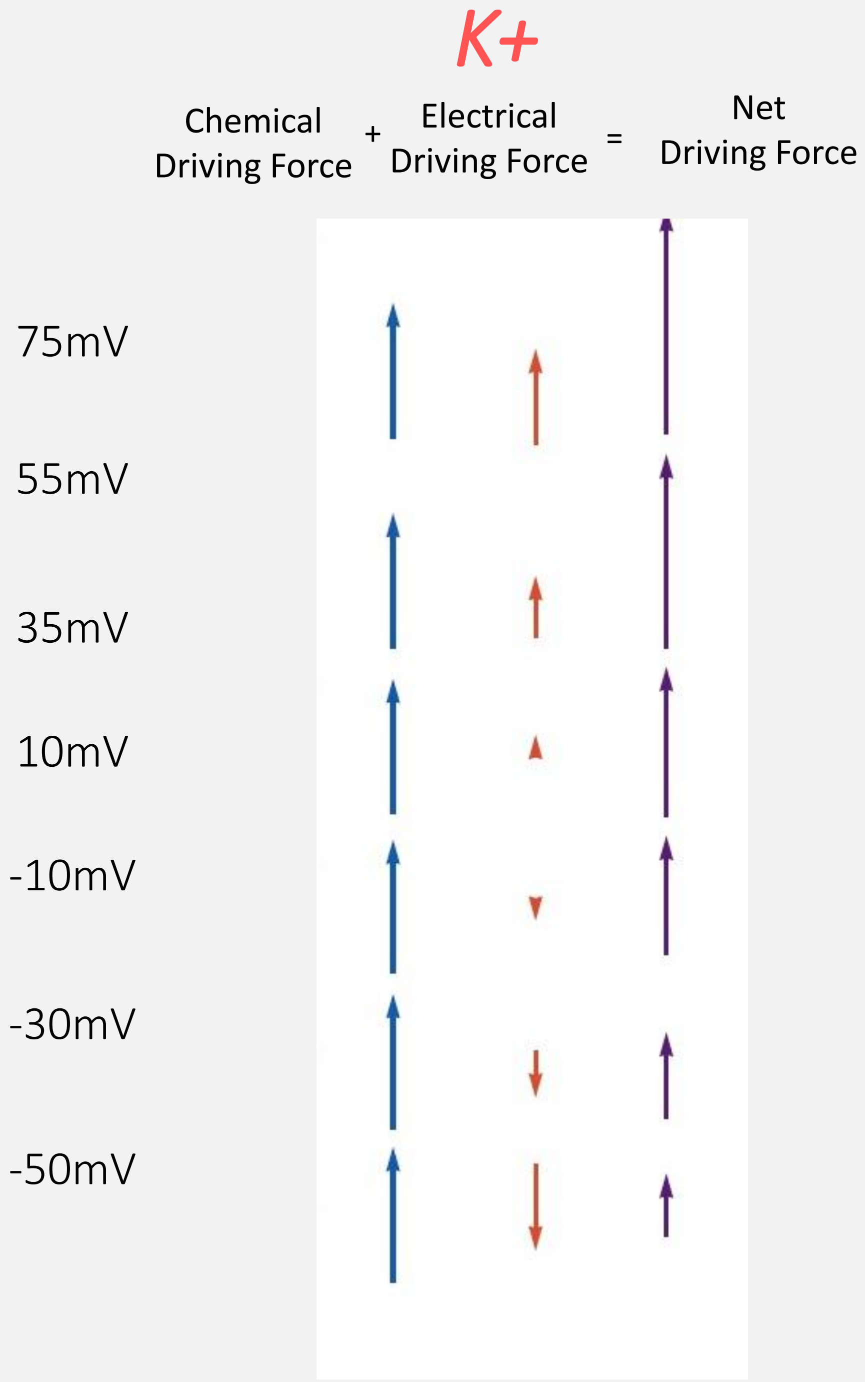




The Action Potential (AP)

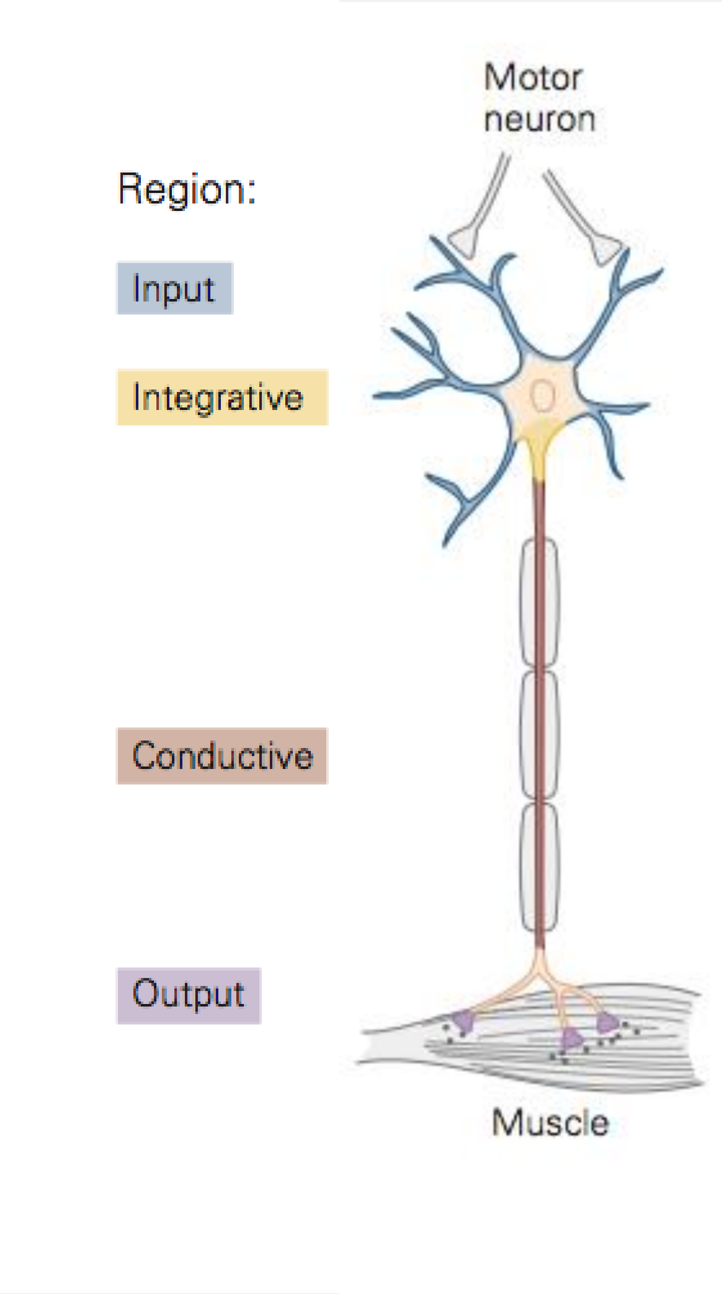
The Action Potential

The starting point of an electrical signal

- Hyperpolarized Vm (membrane potential)
- Hyperpolarized meaning further from 0 mV membrane potential
- i.e. -65mV
- Can think of voltage as force that is going to push ions in or out of the cell
  - Like water pressure pushing water down a hose

What do we need?

- Regeneration of signal



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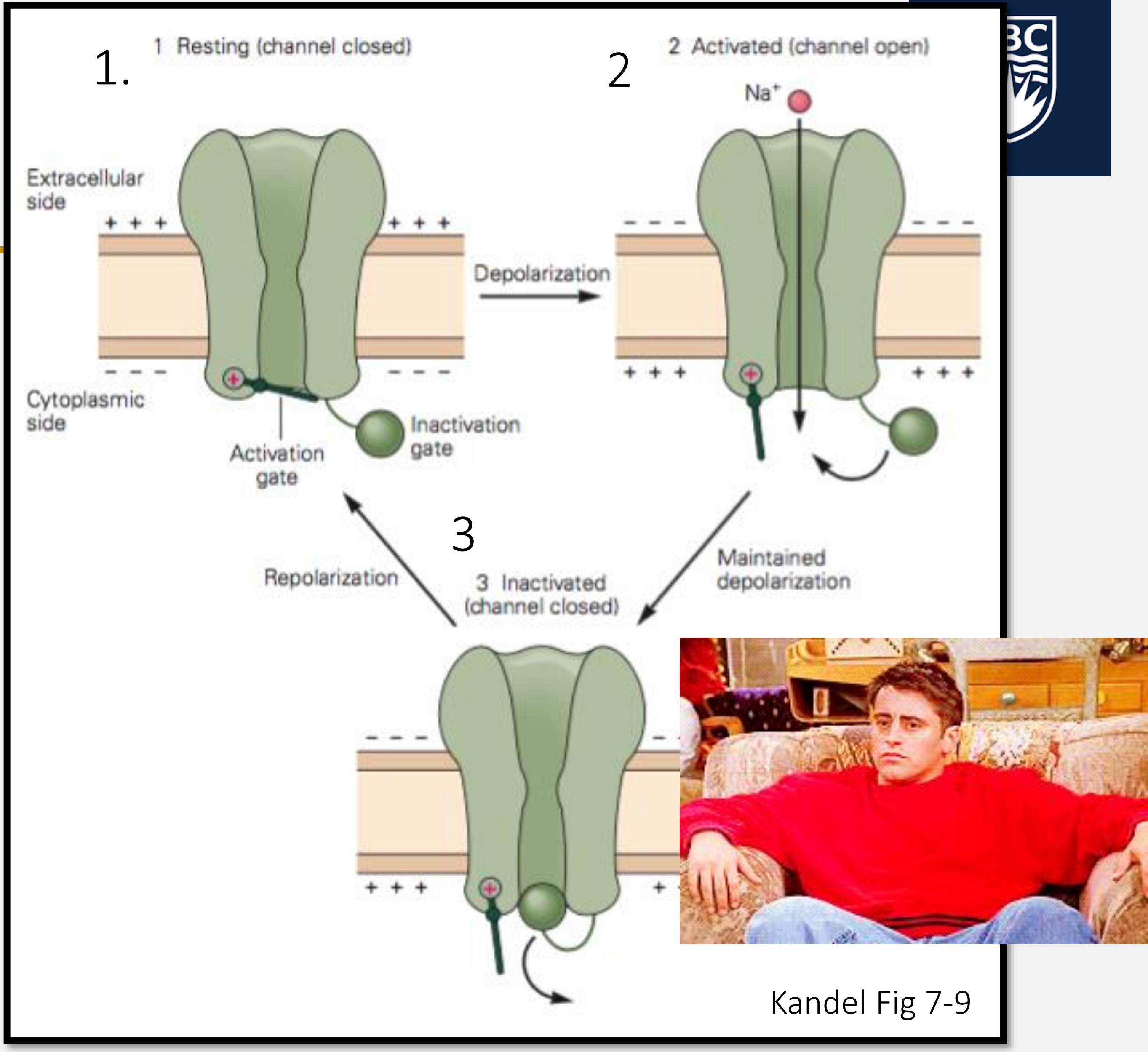
Voltage-gated ion channels

1. Resting

- VG channel closed
- Positively charged extracellular side
- Negatively charged cytoplasmic side

2. Activated

- Caused by potential change
- VG channel open
- Activation gate open
- Inactivation gate closing (but is a little slow)

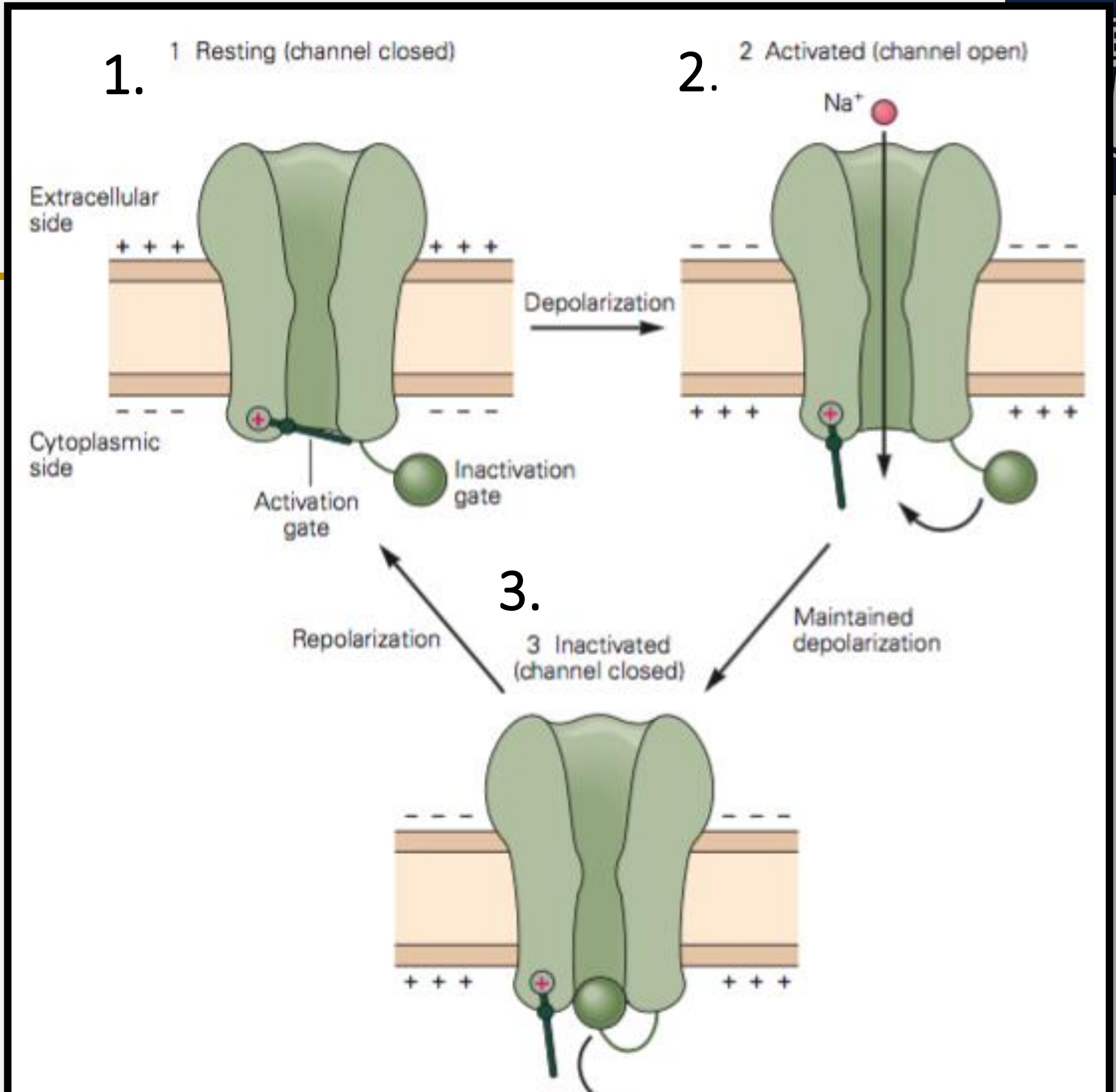


Kandel Fig 7-9

Voltage-gated ion channels

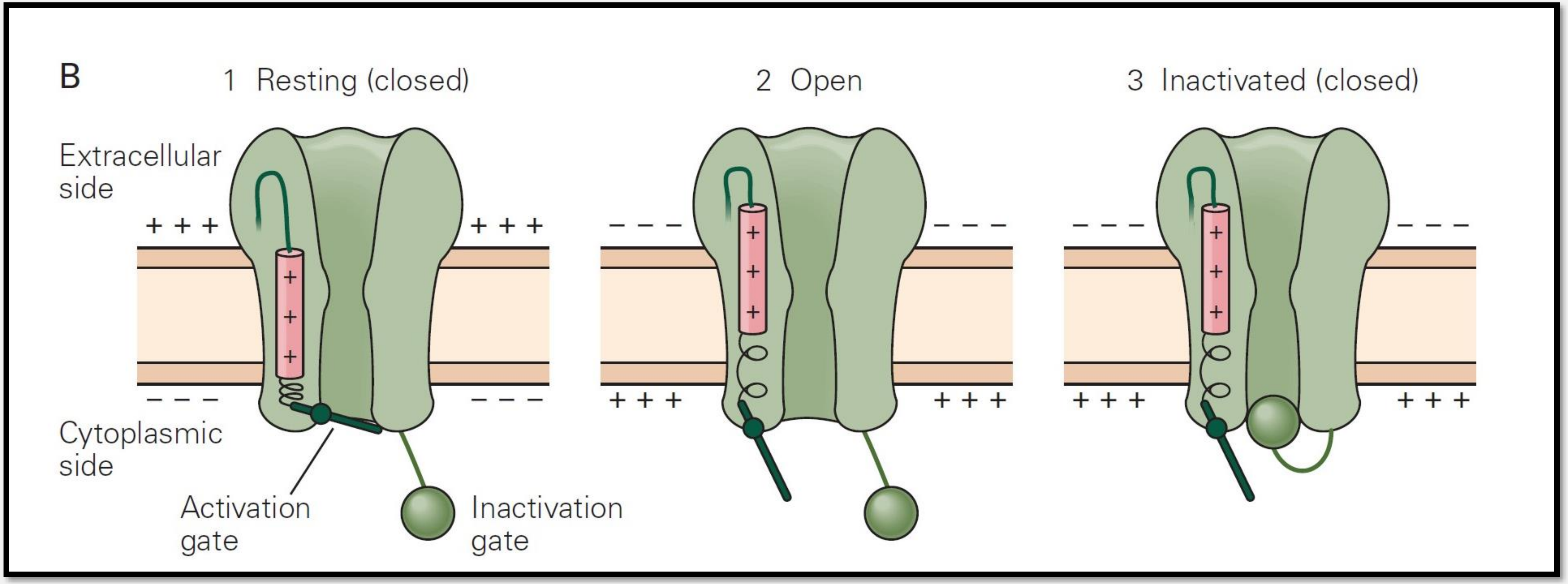
3. Inactivated

- Channel closed by the inactivation gate
- While inactivated the cell cannot fire another action potential – *Refractory period*



Kandel Fig 7-9

Voltage gated ion channels

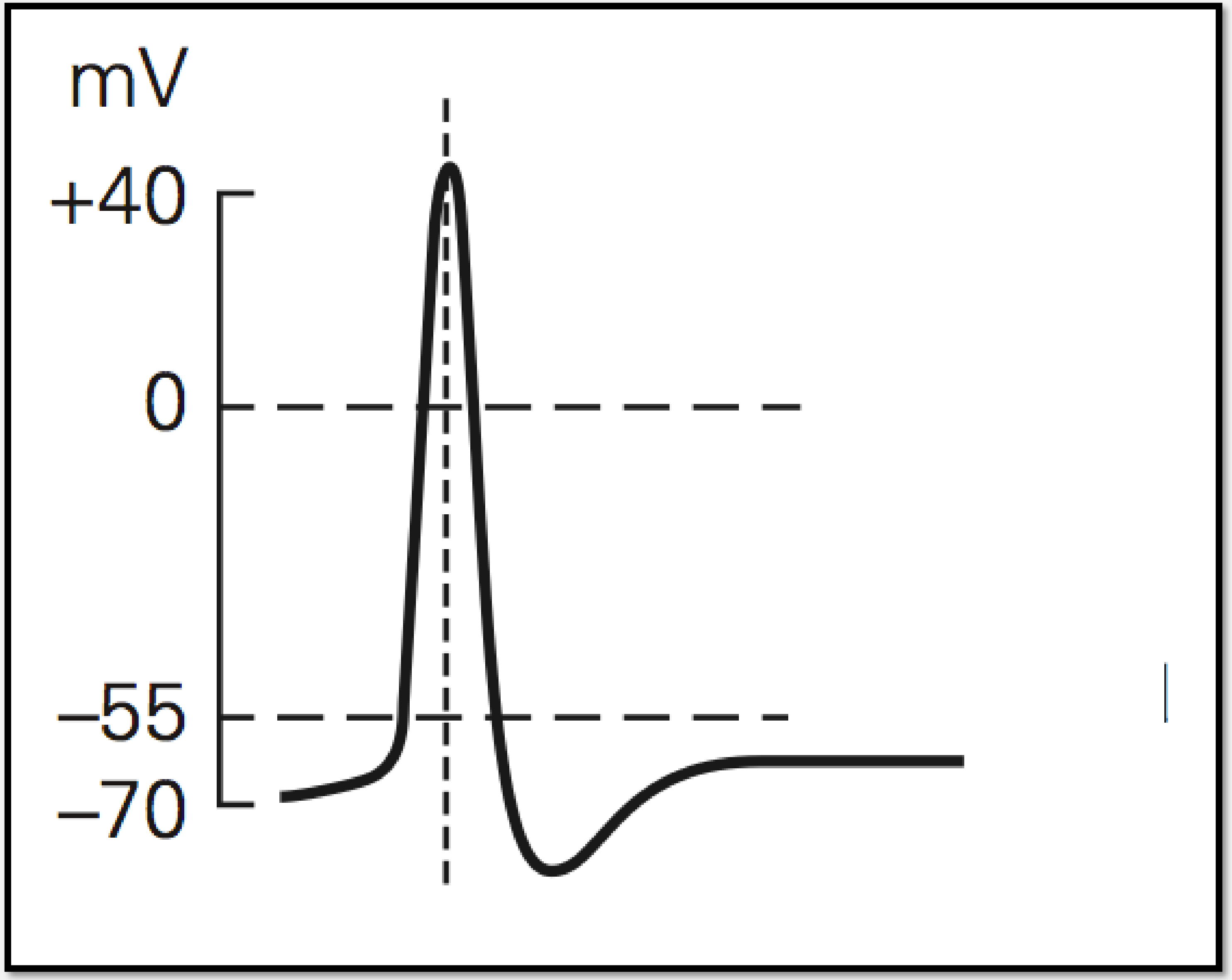


Exercise:

1) Please label when the typical neuron is: at resting membrane potential, and at threshold, is depolarized, and is hyperpolarized

2) VG Na+ channels.
Please indicate where on the plot the VG sodium channels are at rest, activated and inactivated

3) After Peaking at around +40 mV, the membrane potential plumets back down to past -70 mV. What is responsible for this shift? Label any important spots on the graph



$$V_m = \frac{RT}{F}\ln\frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}$$

Remember: Vm at rest determined by:

$$P_K : P_{Na} : P_{Cl} = 1.0 : 0.04 : 0.45$$

But when Na+ channels open:

$$P_K : P_{Na} : P_{Cl} = 1.0 : 20 : 0.45$$

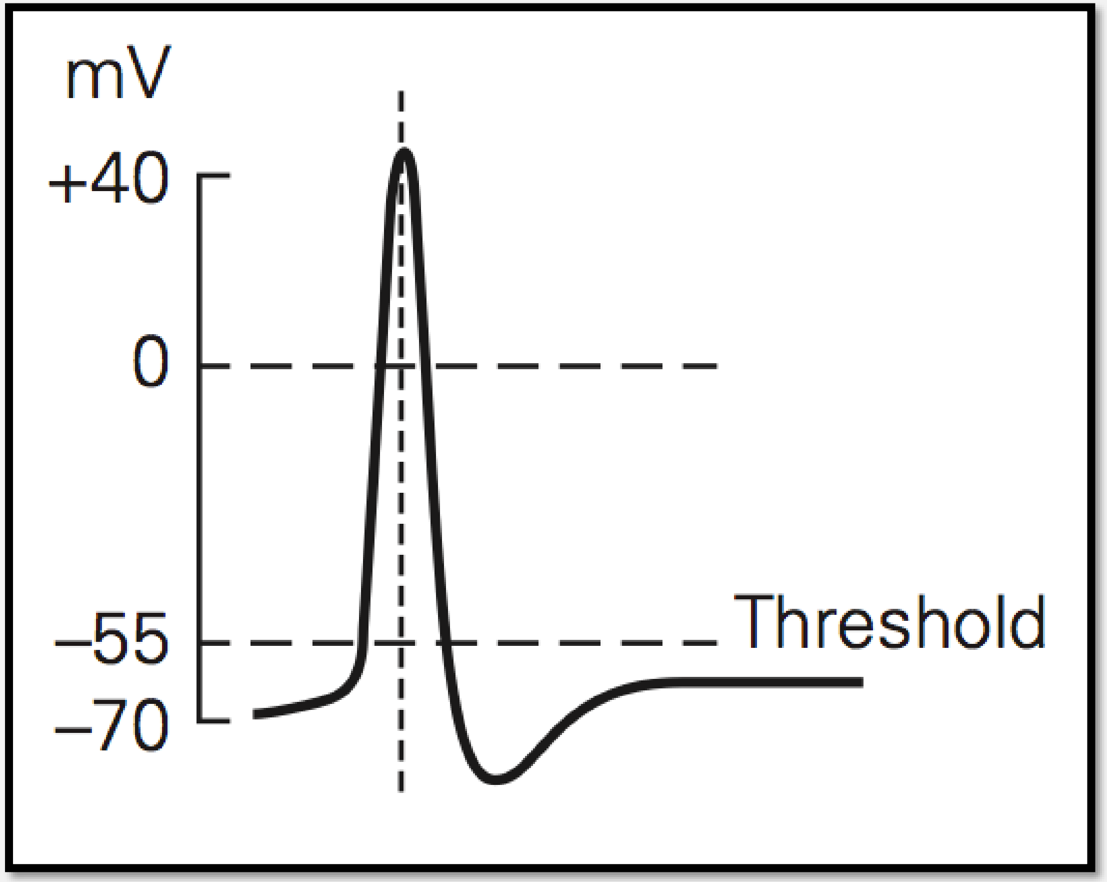
Action potentials

VG Na+ channels

- At rest: -70mV
- Activated: -55mV - +40mV
- Inactivated: =+40 mV - -70mV

VG K+ channels

- The opening and closing of VG K+ channels rapidly brings the vicinity back to a hyperpolarized state
- Positive charge inside the cell and high [K+] forces K+ out
- Slow closing of VG K+ channels causes hyperpolarization



Conducting an electrophysiological experiment

Hodgkin & Huxley model

Needs

- Healthy cells: (in vitro (culture/expression), ex vivo (an acute slice), in vivo (an alive animal)
- Microscope, fine electrodes, noise reduction (grounding wires, faraday cages, vibration dampening)
- Signal acquisition device: amplifier, digitizer, computer software

Consideration of Ohms Law

- V = Voltage (volts)
- I = Current (amperes)
- R = resistance (ohmns)
- G = Conductance (Siemens)

$$G = 1/R$$

- Insulators – separate electrical conductors, are really good resistors

$$\boxed{\Delta V = IR}$$

Experimental set-up

Needs

Health cell(s)

<u>Electrodes</u>

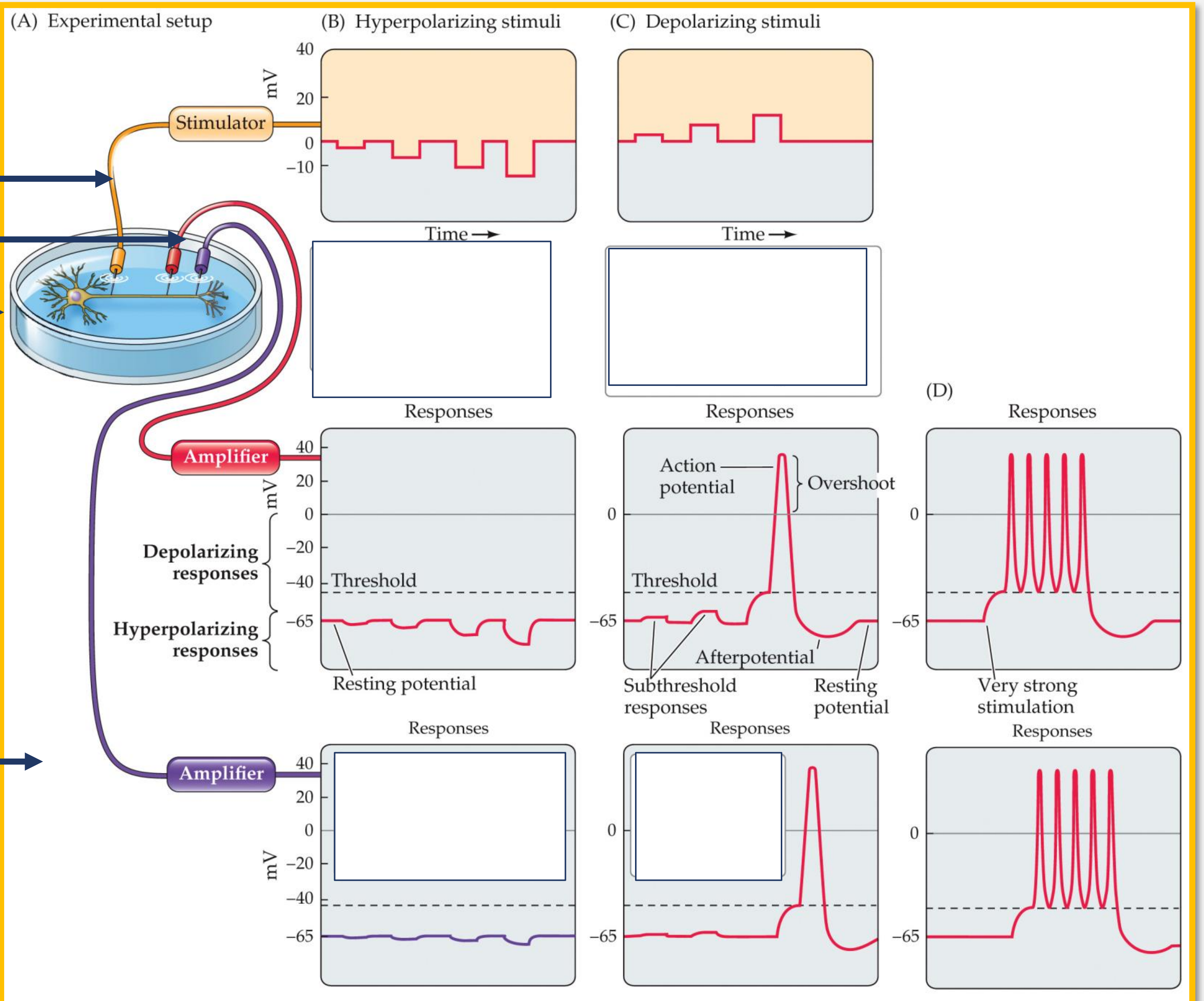
Stimulating

Recording

<u>Signal acquisition device</u>

Amplifiers

Computer software



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.6

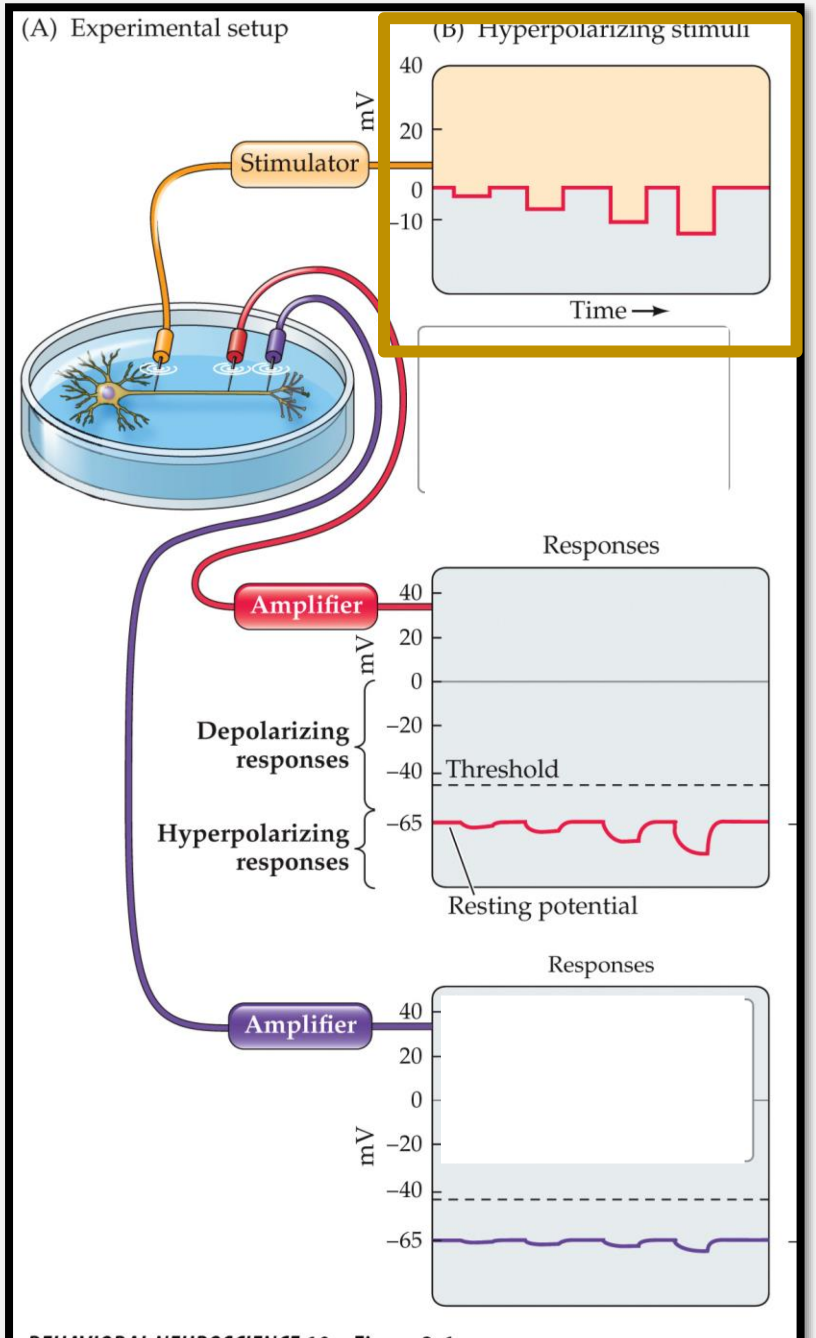
Hyperpolarizing Stimulus

Question:

What happens if we hyperpolarize the cell, i.e., make the membrane potential more negative?

Step 1:

Provide hyperpolarizing stimuli of increasing strength over time



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.6

Step 1:

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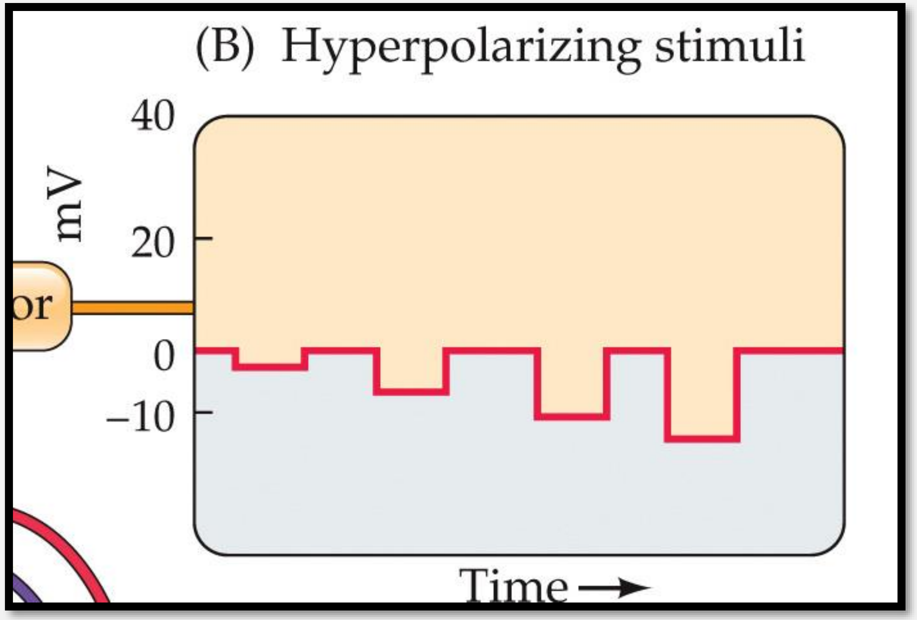


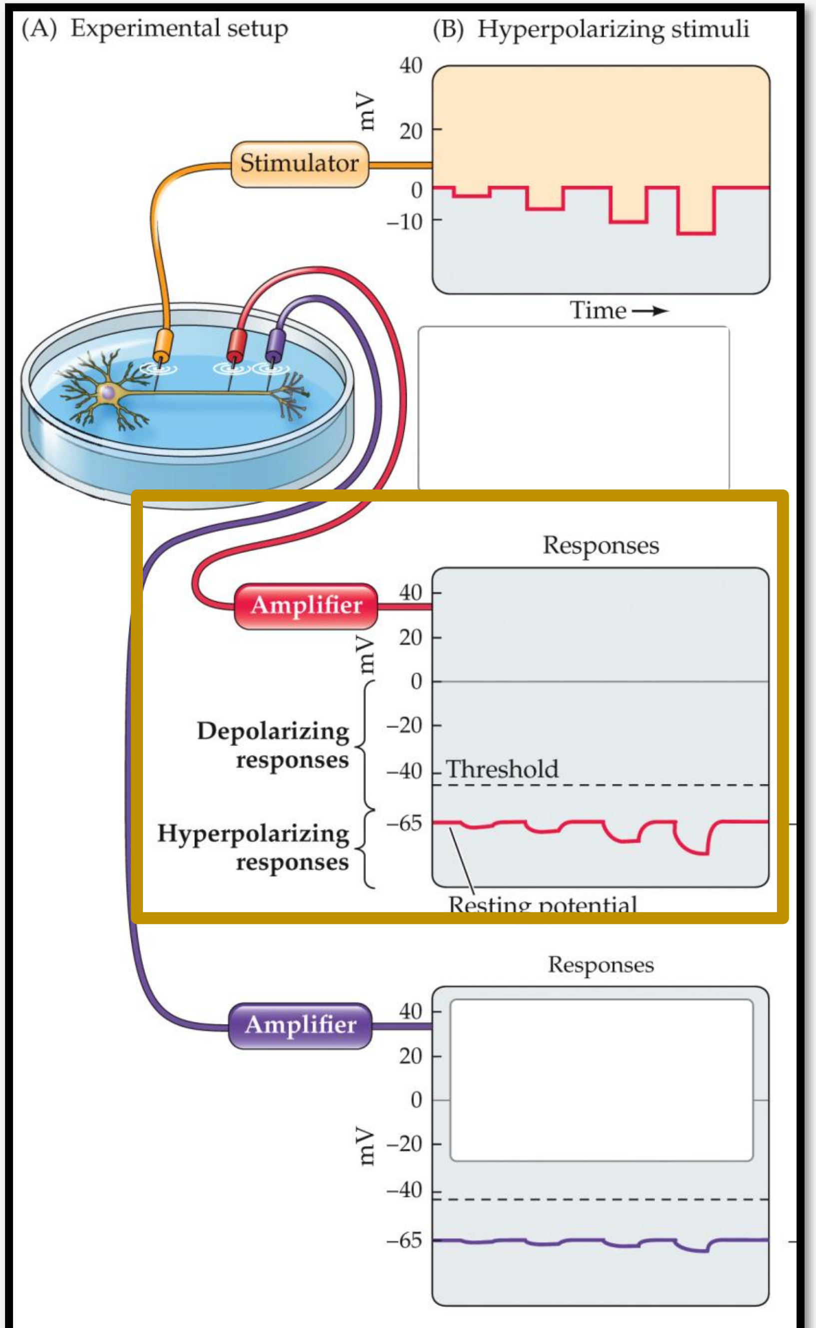
Hyperpolarizing Stimulus

Question:

What happens if we hyperpolarize the cell, i.e., make the membrane potential more negative?

Step 2:

Observe response down the axon (what happens to the membrane potential?)



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.6

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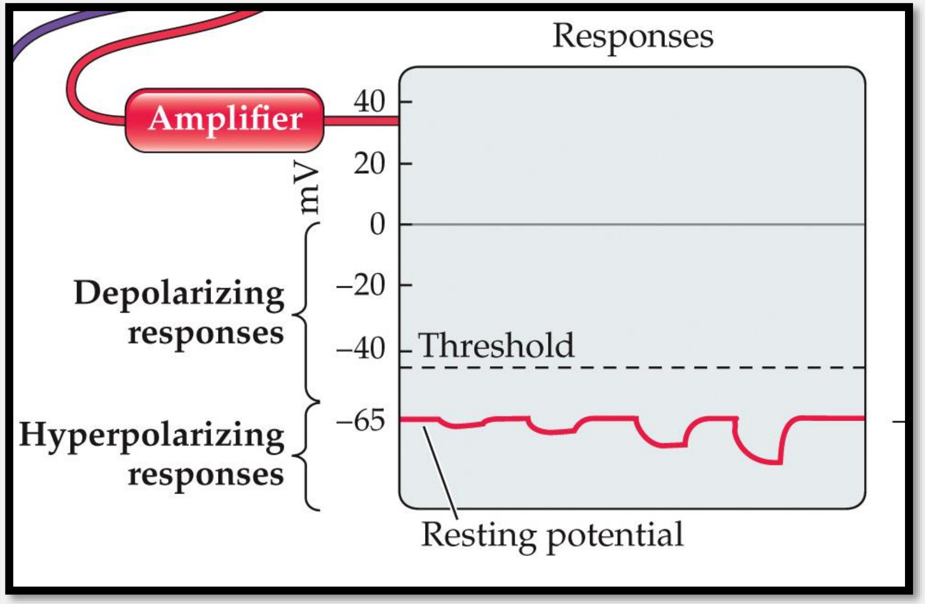


Observation:

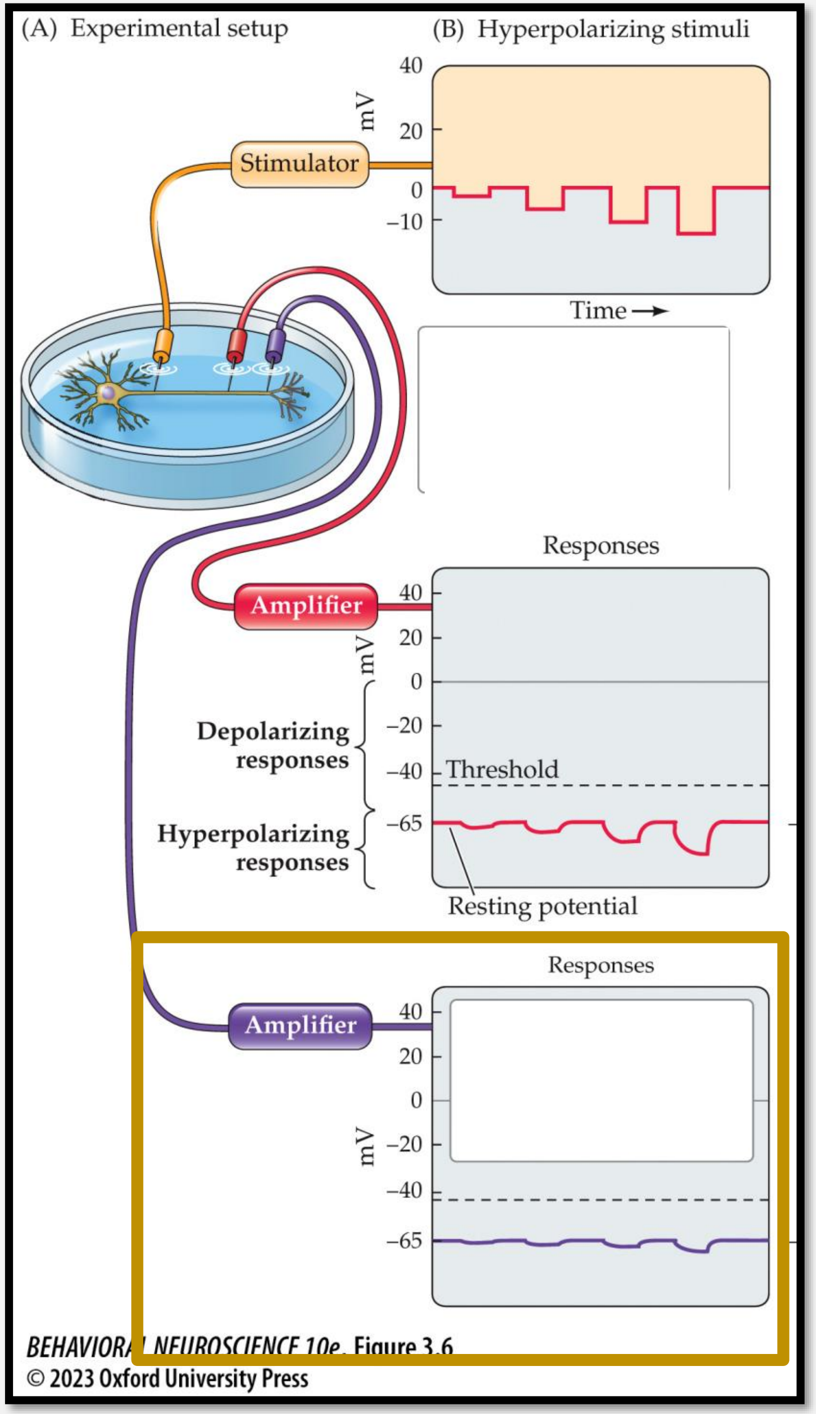
Hyperpolarizing Stimulus

Question:

What happens if we hyperpolarize the cell, i.e., make the membrane potential more negative?

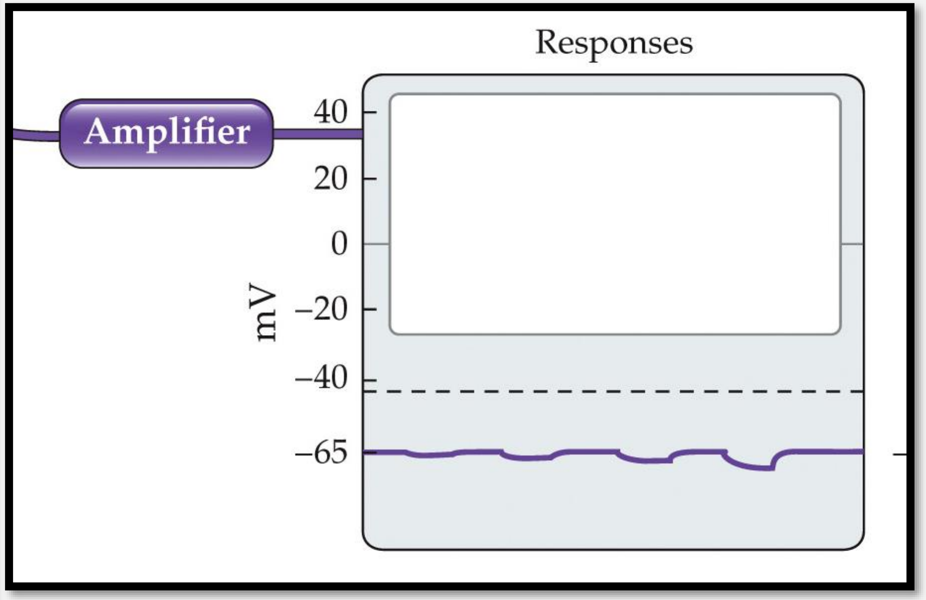
Step 3:

Observe response a bit further down the axon (what happens to the membrane potential?)



Step 3:

Observe response a bit further down the axon (what happens to the membrane potential?)



Observation

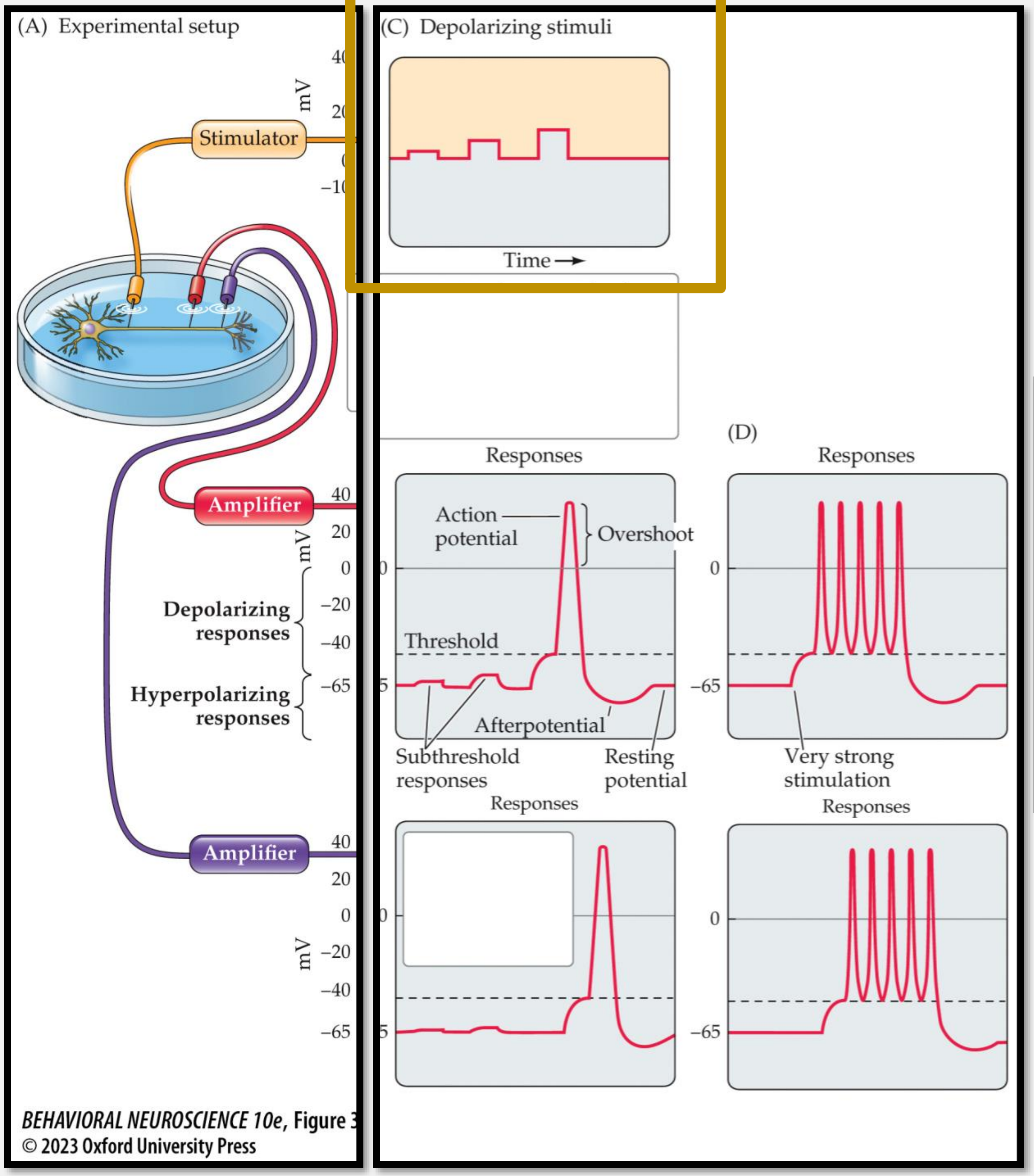
Depolarizing stimulus

Question:

What happens if we depolarize the cell, i.e., decrease the membrane potential?

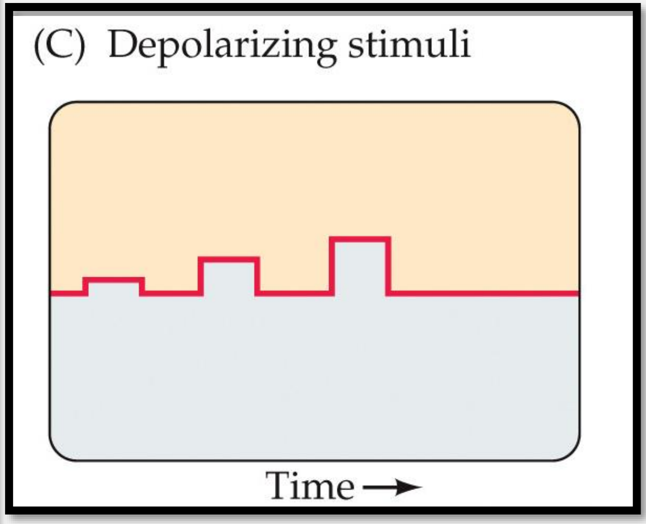
Step 1:

Provide depolarizing stimuli of increasing strength over time



Step 1:

Provide depolarizing stimuli of increasing strength over time



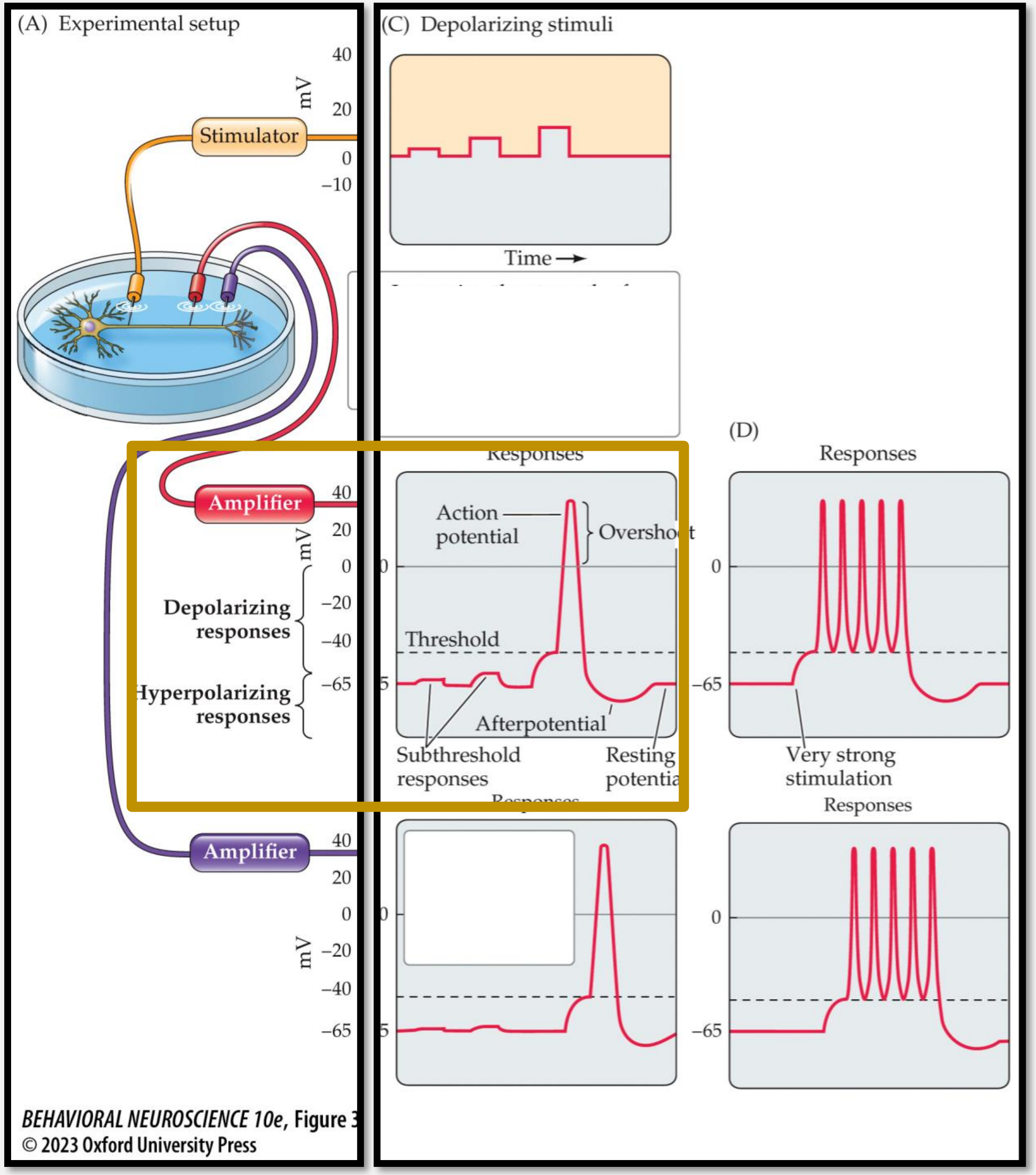
Depolarizing stimulus

Question:

What happens if we depolarize the cell, i.e., decrease the membrane potential?

Step 2:

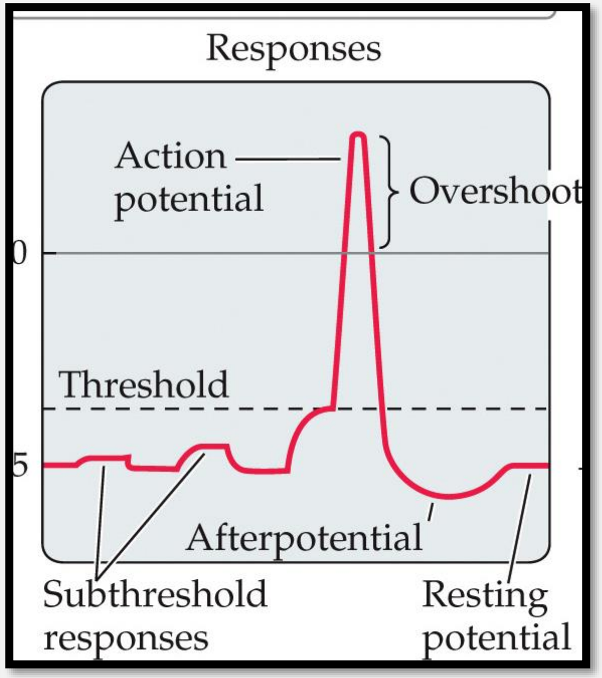
Observe adjacent aspect of axon for changes in membrane potential



BEHAVIORAL NEUROSCIENCE 10e, Figure 3

Step 2:

Observe adjacent aspect of axon for changes in membrane potential



Observation

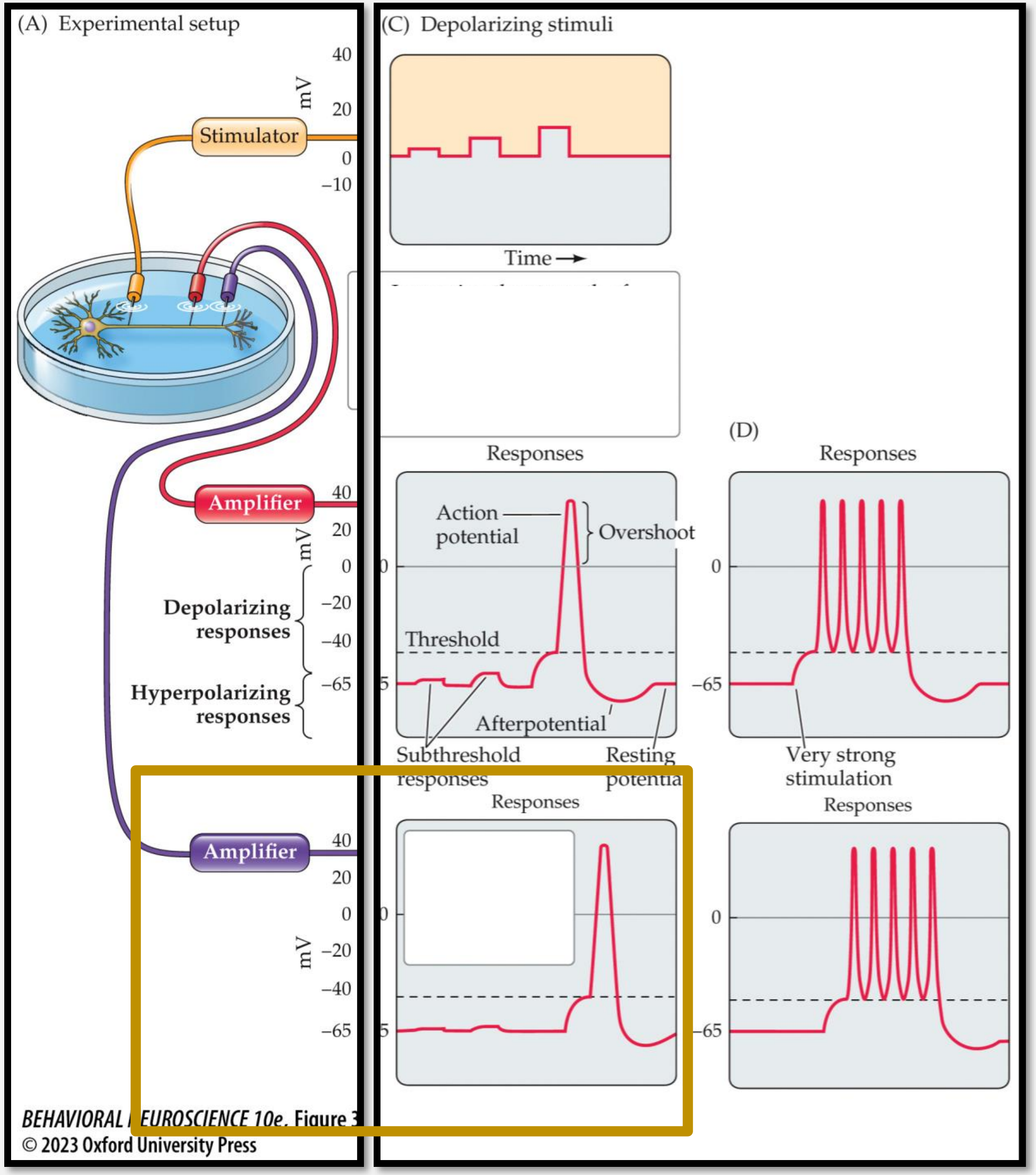
Depolarizing stimulus

Question:

What happens if we depolarize the cell, i.e., decrease the membrane potential?

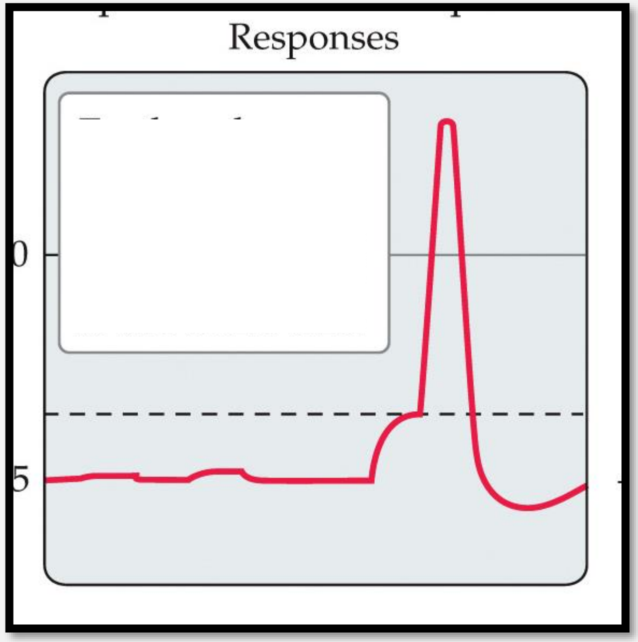
Step 3:

Observe 2nd adjacent aspect of axon for changes in membrane potential



Step 3:

Observe 2nd adjacent aspect of axon for changes in membrane potential



Observation

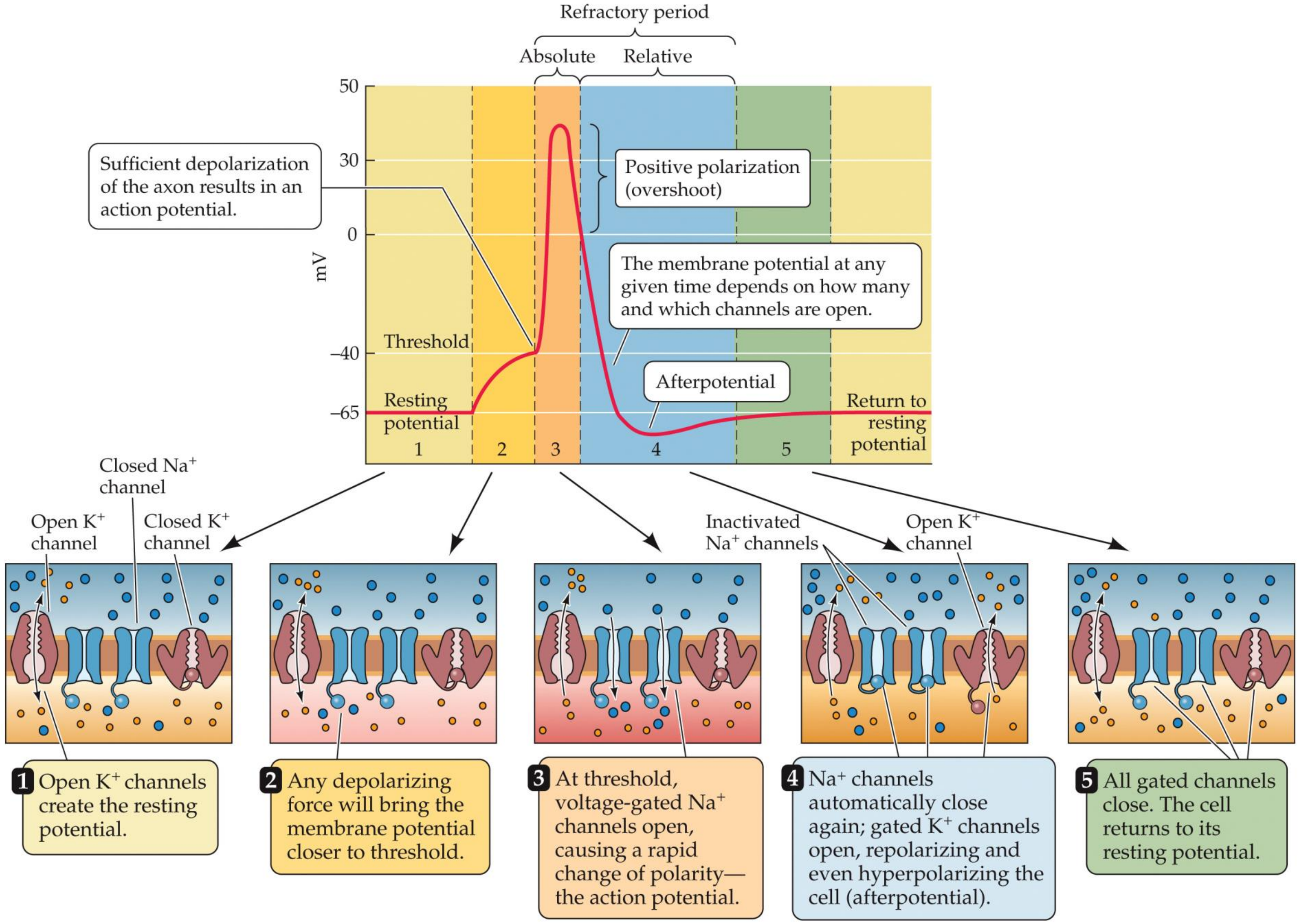
AP – Key Characteristics

Summary

- Action potentials are produced by movement of Na^+ ions into the cell.
- At the peak of an action potential, the concentration gradient pushing Na^+ ions into the cell equals the positive charge driving them out.
- Membrane shifts briefly from a resting state to an active state and back (i.e., the membrane suddenly and briefly becomes permeable to Na^+ ions).

Steps

- Voltage-gated Na^+ channels open in response to depolarization, and Na^+ ions enter; more channels open and more Na^+ enters;
- Continues until membrane potential reaches the Na^+ equilibrium potential of +40 mV.
- As cell interior becomes more positive, voltage-gated K^+ channels open.
- K^+ moves out and the resting potential is restored.

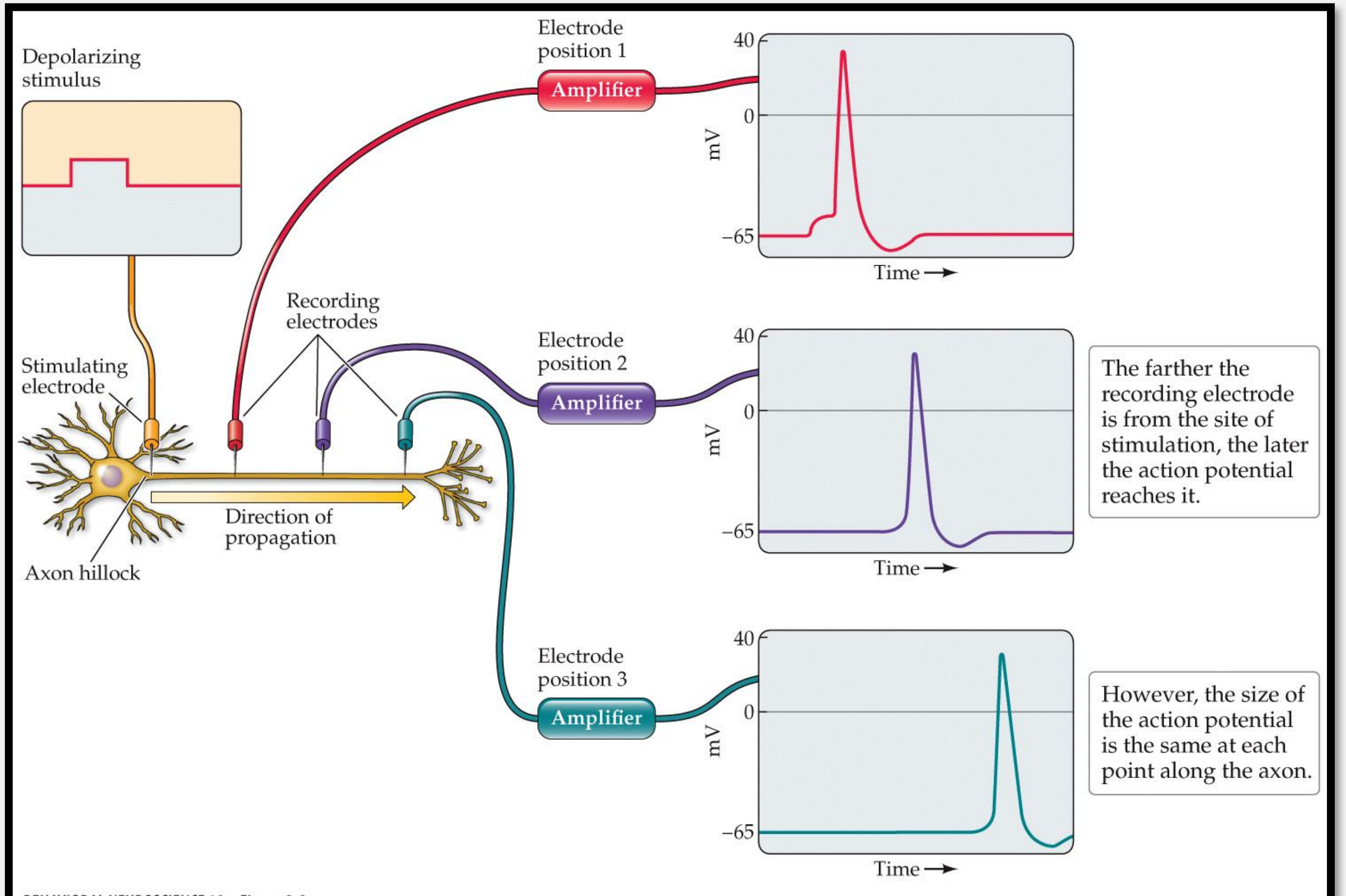


BEHAVIORAL NEUROSCIENCE 10e, Figure 3.7

AP conduction down the axon

AP transmission

- One way train
- AP regenerates at the same strength every time – All or none



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.8

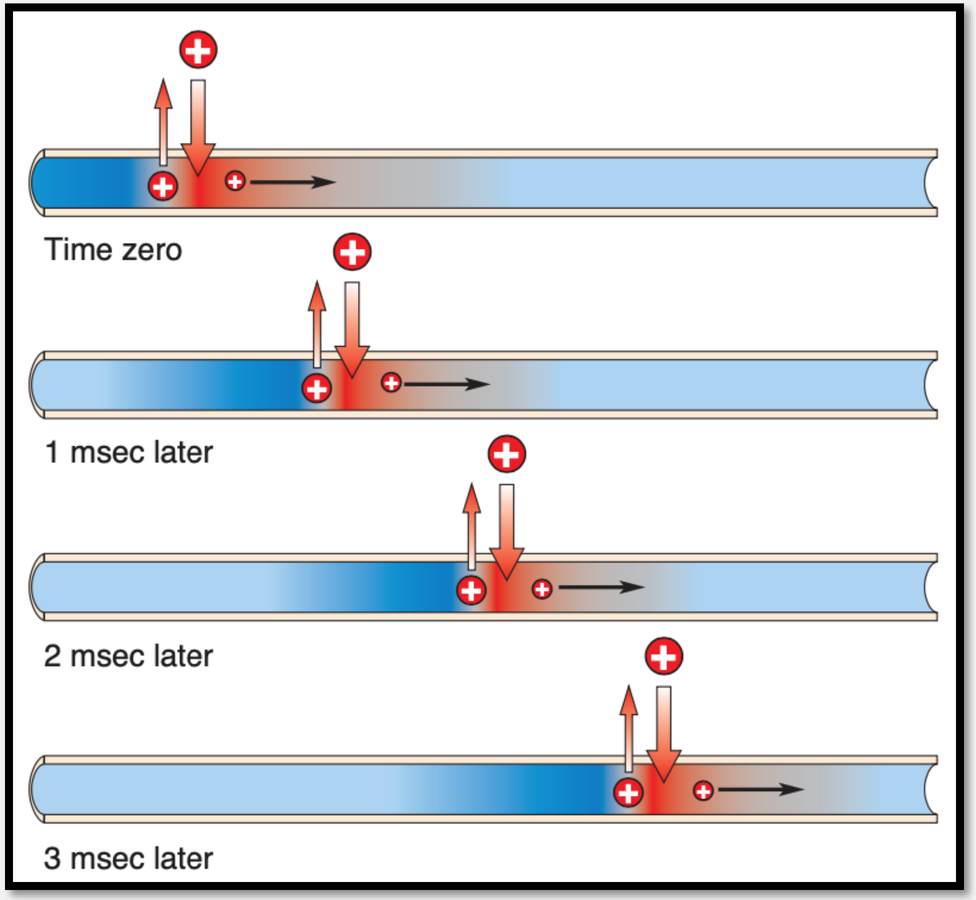
AP conduction down the Axon

Absolute refractory period

- Na+ inactivation gate // hyperpolarization via K+ voltage gated channels
- Cannot elicit a 2nd AP

Relative refractory period

- Some Na+ voltage gated channels have returned to rest
- Sufficiently strong stimulus can elicit another action potential



AP – Key Characteristics

Summary

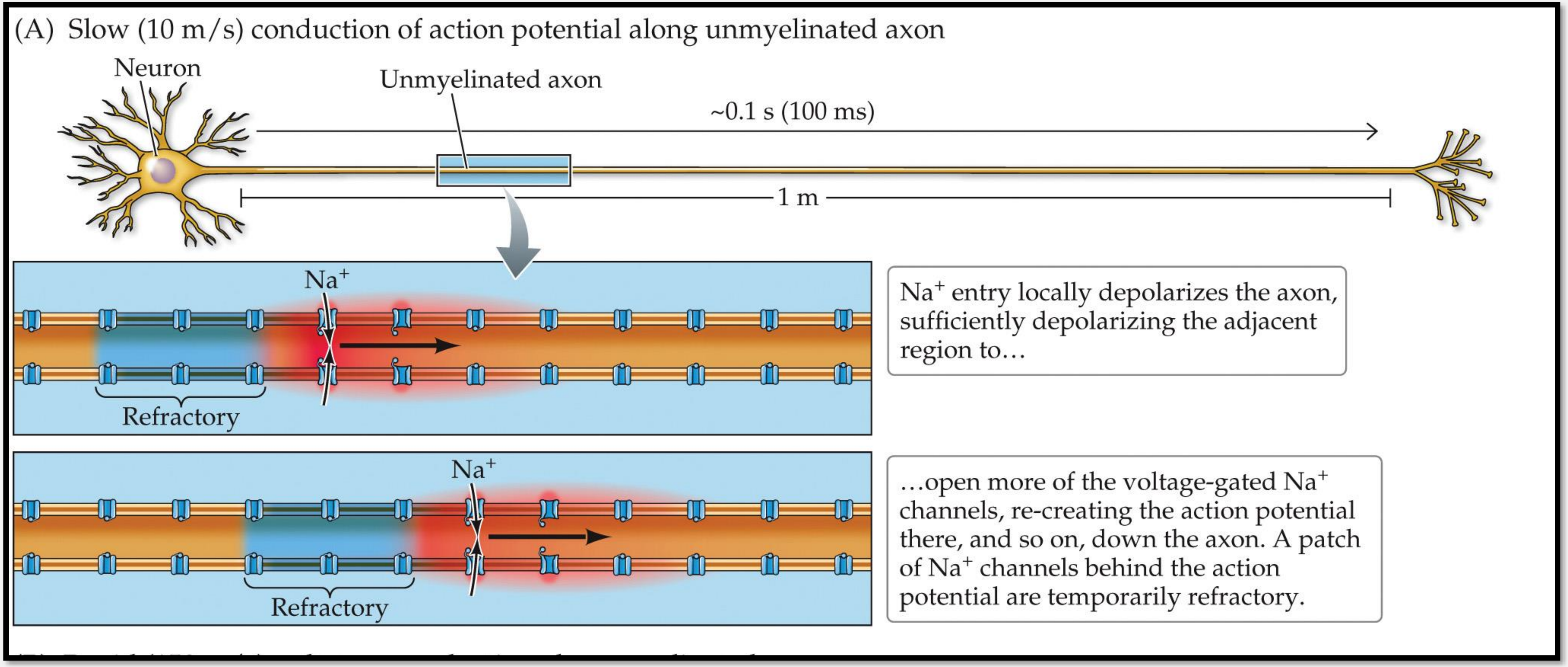
- Action potentials are regenerated along the axon—each adjacent section is depolarized and a new action potential occurs.
- Action potentials travel in one direction because of the refractory state of the membrane after a depolarization.
- Action potentials are an all or none process – when threshold is reached an action potential will occur at the same strength every time.

Conduction velocity

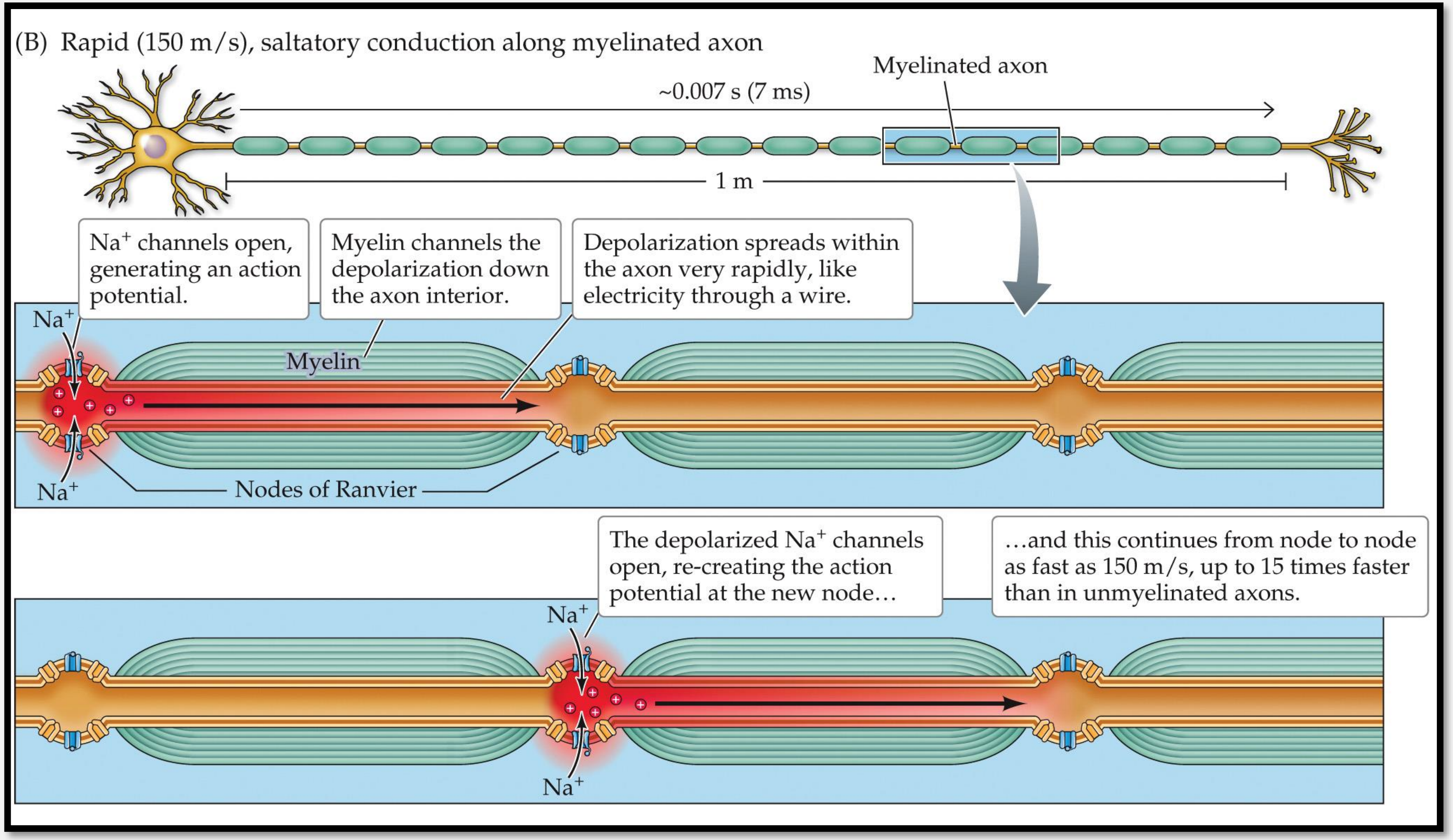
How can we increase the speed of propagation of action potentials?

Hint: Think of Ohm’s law V = IR, or about electricity in wires.

AP conduction



Myelination



Insulation

Increases resistance around the membrane (prevents leaks and interference)

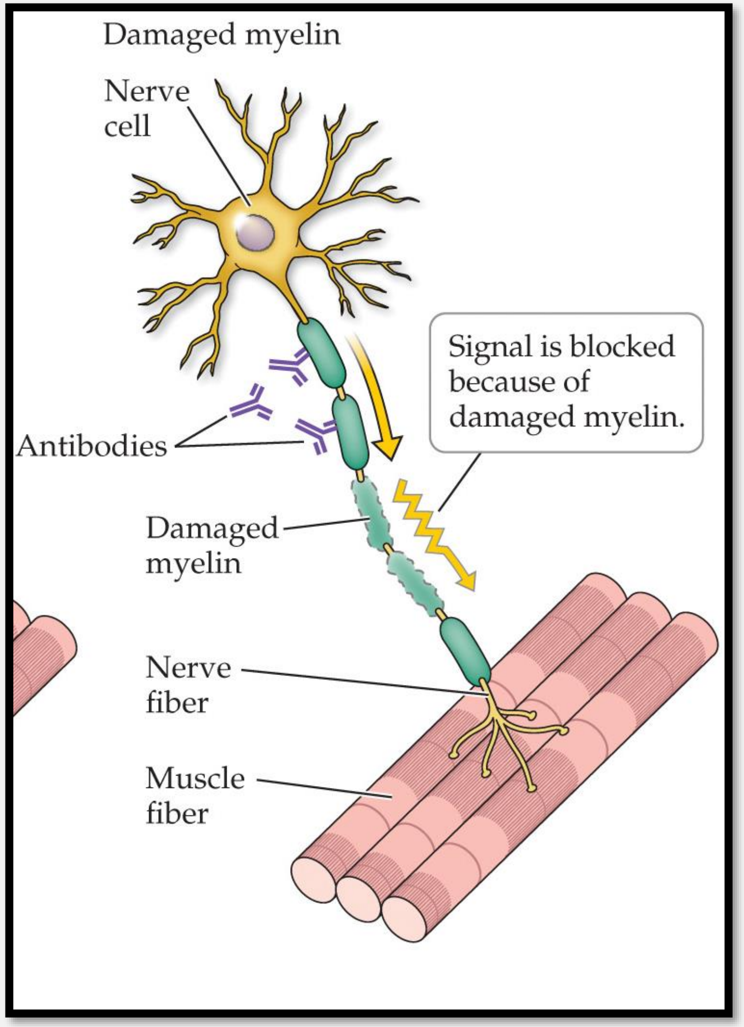
Creates a path of least resistance for current (+ ion flow)

Signal regenerates at each Node of Ranvier

Importance of myelin

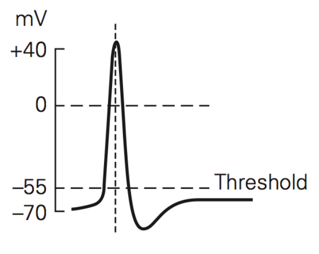
Multiple Sclerosis (MS)

- disorder that occurs when body's immune system produces antibodies that attack myelin, and thus conduction of action potentials.
- Wide variety of symptoms that affect sensory and/or motor systems, depending on which axons are attacked.

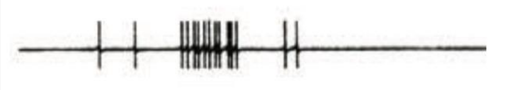


Methods for recording Action Potentials

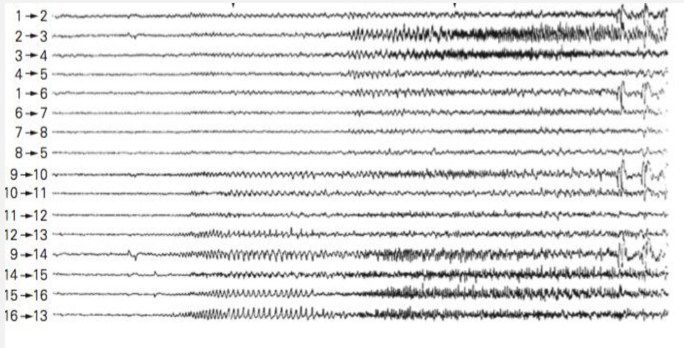
intracellular electrophysiology



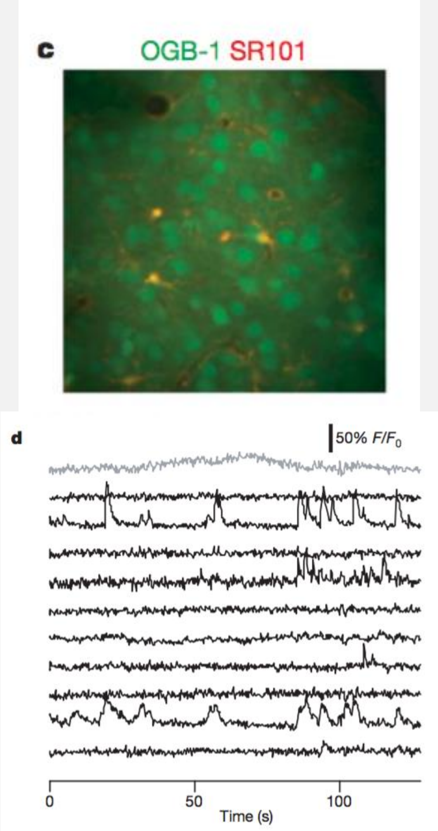
extracellular electrophysiology



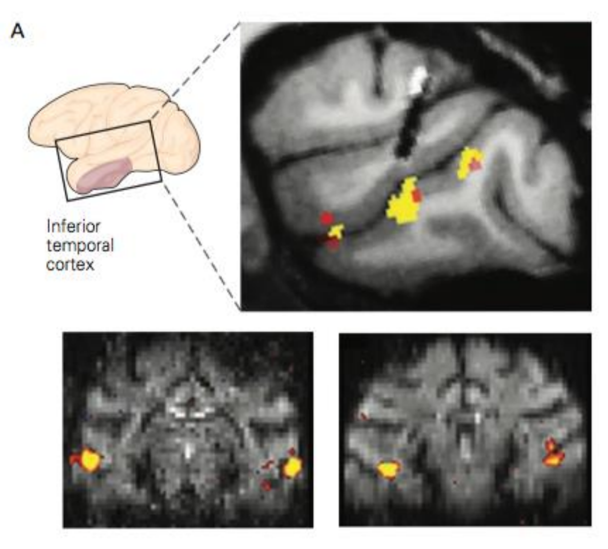
EEG



calcium imaging

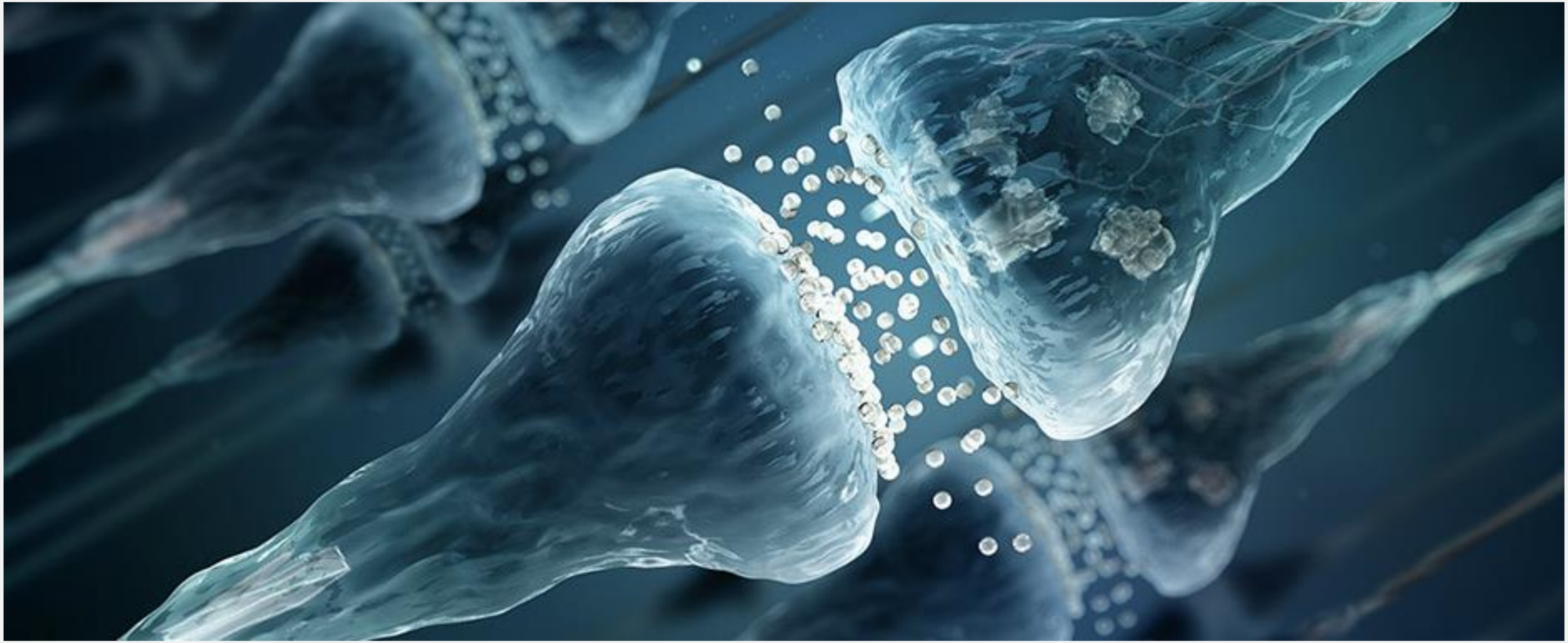


functional imaging (eg fMRI)



increasingly indirect reflections of APs

Synaptic Transmission



Learning Objectives

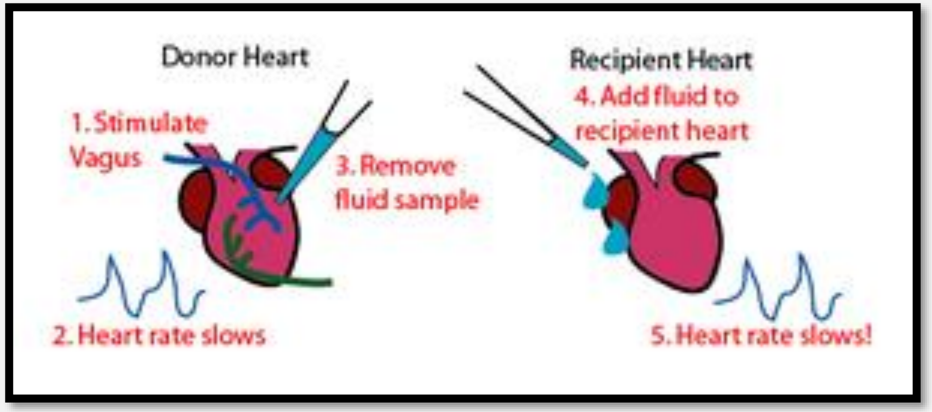
By the end of this lesson, you will…

1. Be able to list the stages of chemical synaptic transmission
2. Be able to describe the mechanism by which excitatory and inhibitory neurotransmitters function
3. Distinguish between action potentials and post synaptic potentials.
4. Distinguish between temporal and spatial summation.
5. Apply basic neurophysiology principles to the knee jerk reflex neural chain.
6. Describe the strengths and weaknesses of two visualization techniques for viewings neuronal circuits.

Synaptic transmission

How do neurons communicate?

Thomas Renton Elliot (1904)
- Adrenaline (EPI)



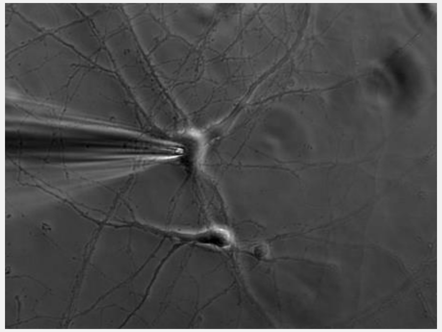
John Eccles (1951)
- Eccles, coombs and brock

Charles Scott Sherringham (1904)
- The Synapse

"Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery"

Henry Dale (1914/1933)
- Acetylcholine (Ach)

Otto Loewi (1921)
- Vagusstoff
- Accelerans-stoff



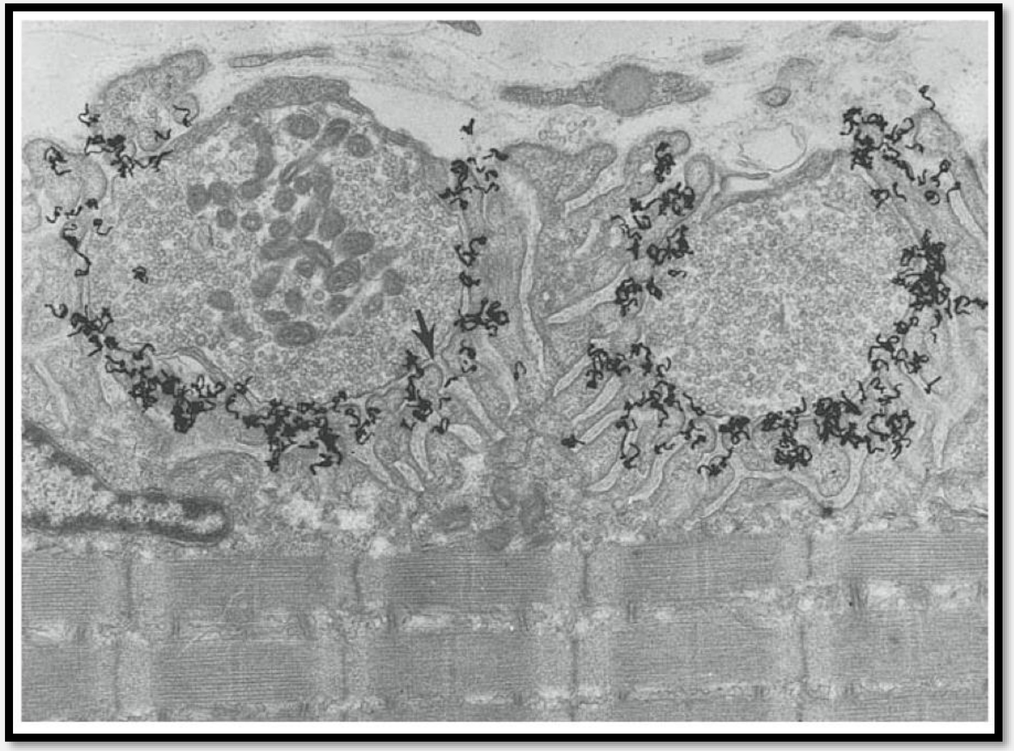
Visualizing vesicles and receptors with EM

Typical CNS synapse

- vesicles, mitochondria, 2 active zones (dark fuzzy bands indicated by arrows = regions of dense synaptic machinery)

The neuromuscular junction

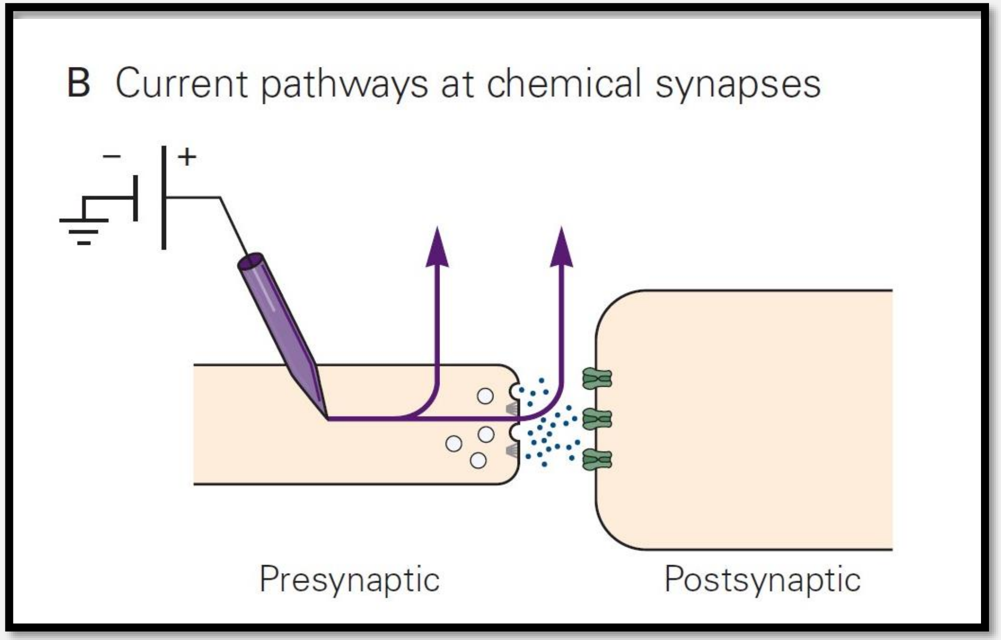
- Acetylcholine receptors darkly labelled, seen at postsynaptic membrane immediately across from synaptic vesicles.
- Note the insane number of vesicles.



The Synapse

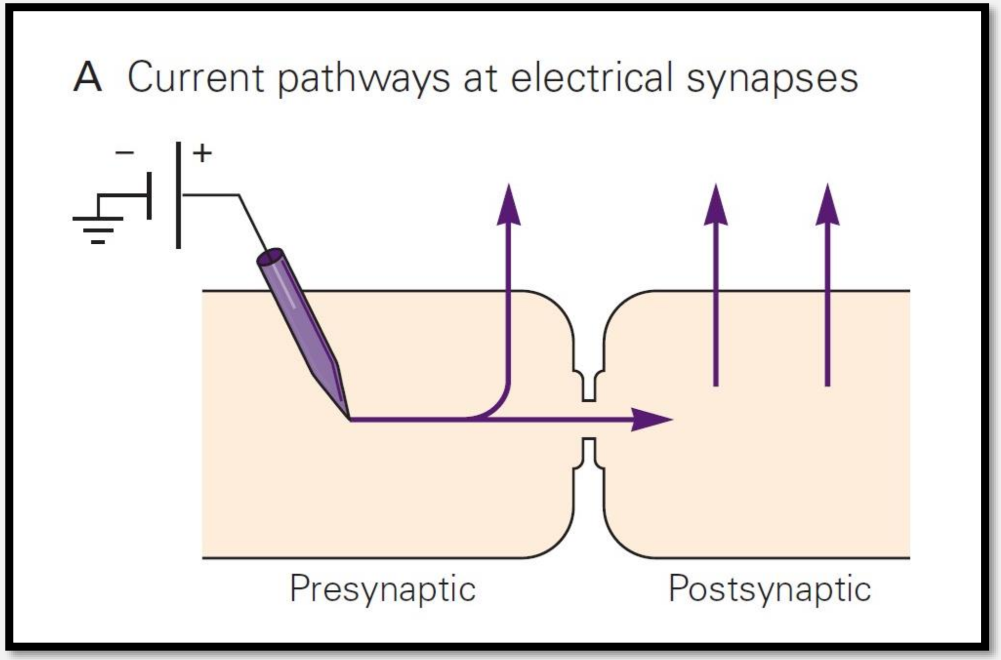
Chemical synapses

- Injected current causes an action potential in the presynaptic cell, release of neurotransmitters and a change in potential of the postsynaptic cell



Electrical Synapses

- Transmit signals between neurons
- Current enters the post synaptic cell through a gap junction channel

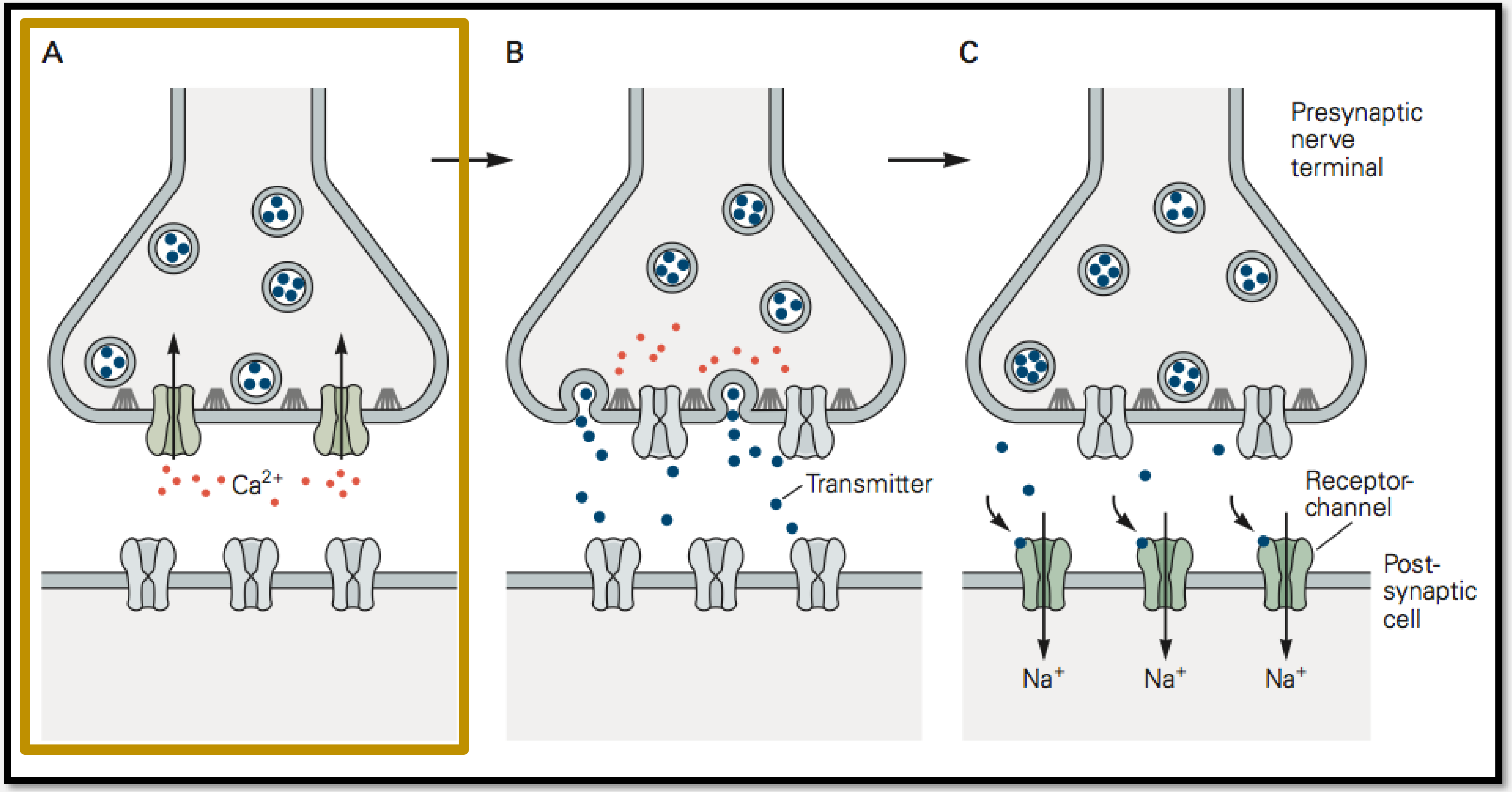


Kandel Fig 8-1

Synaptic Transmission

A

- Vesicles dock near the plasma membrane
- Presynaptic terminal depolarization
- Arriving AP causes opening of VG Ca2+ channels

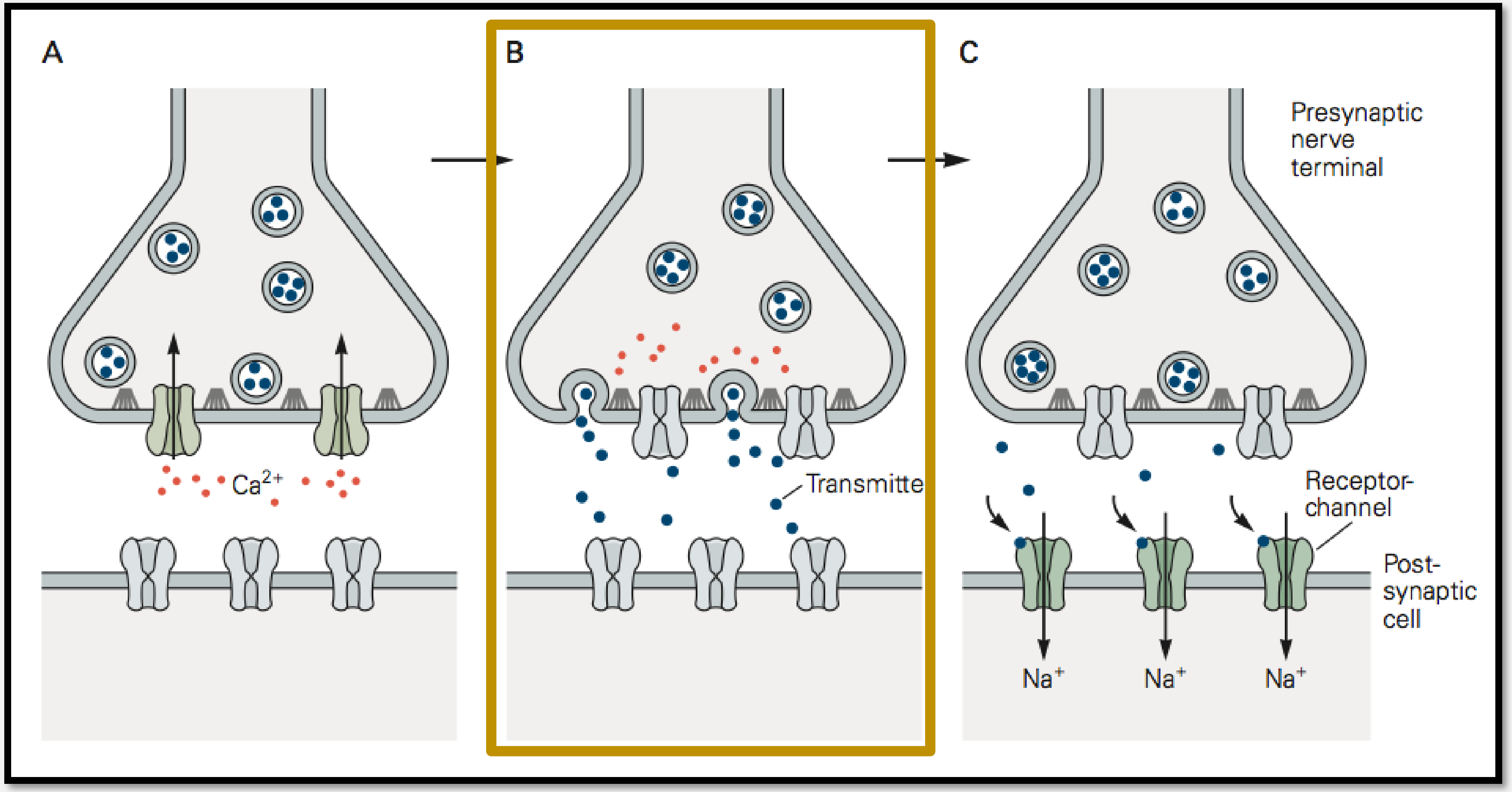


Kandel Fig 8–8

Synaptic Transmission

B

- Ca2+ entry triggers vesicle fusion with presynaptic membrane
- Neurotransmitter (NT) ligands spills into synaptic cleft
- **Ligands –** Bind specific receptors

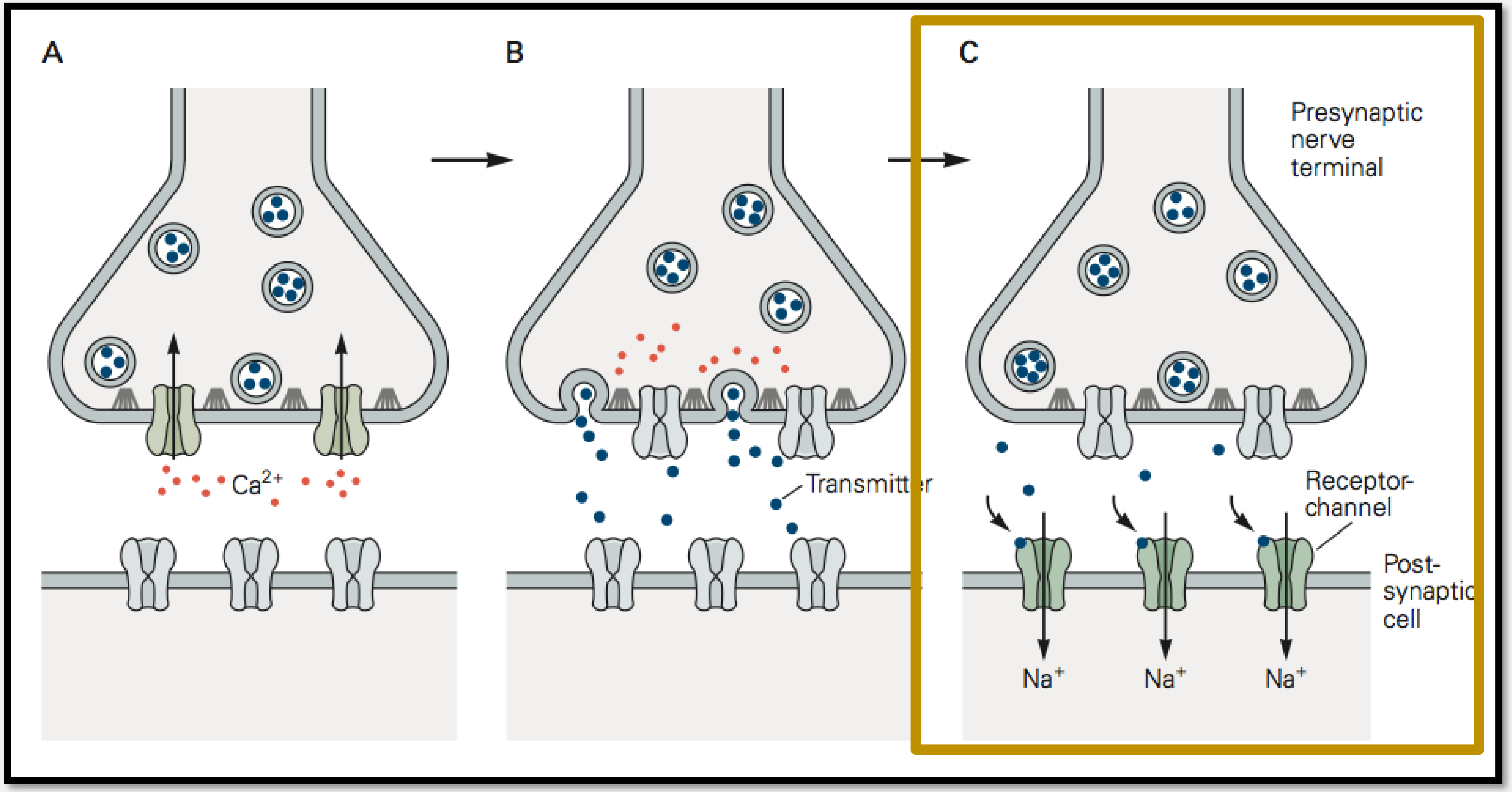


Kandel Fig 8–8

Synaptic Transmission

C

- NT's bind to post synaptic membrane receptors
- **Ionotropic receptors** have associated ion channels that open when bound
- Depending on receptor – EPSP or IPSP



Kandel Fig 8–8

Terminology

Post synaptic potential

- A brief change from resting potential in the post synaptic cell

Excitatory Post synaptic potential (EPSP)

- When the post synaptic cell becomes depolarized (excited) and therefore more likely to fire an action potential

Inhibitory Post synaptic potential (EPSP)

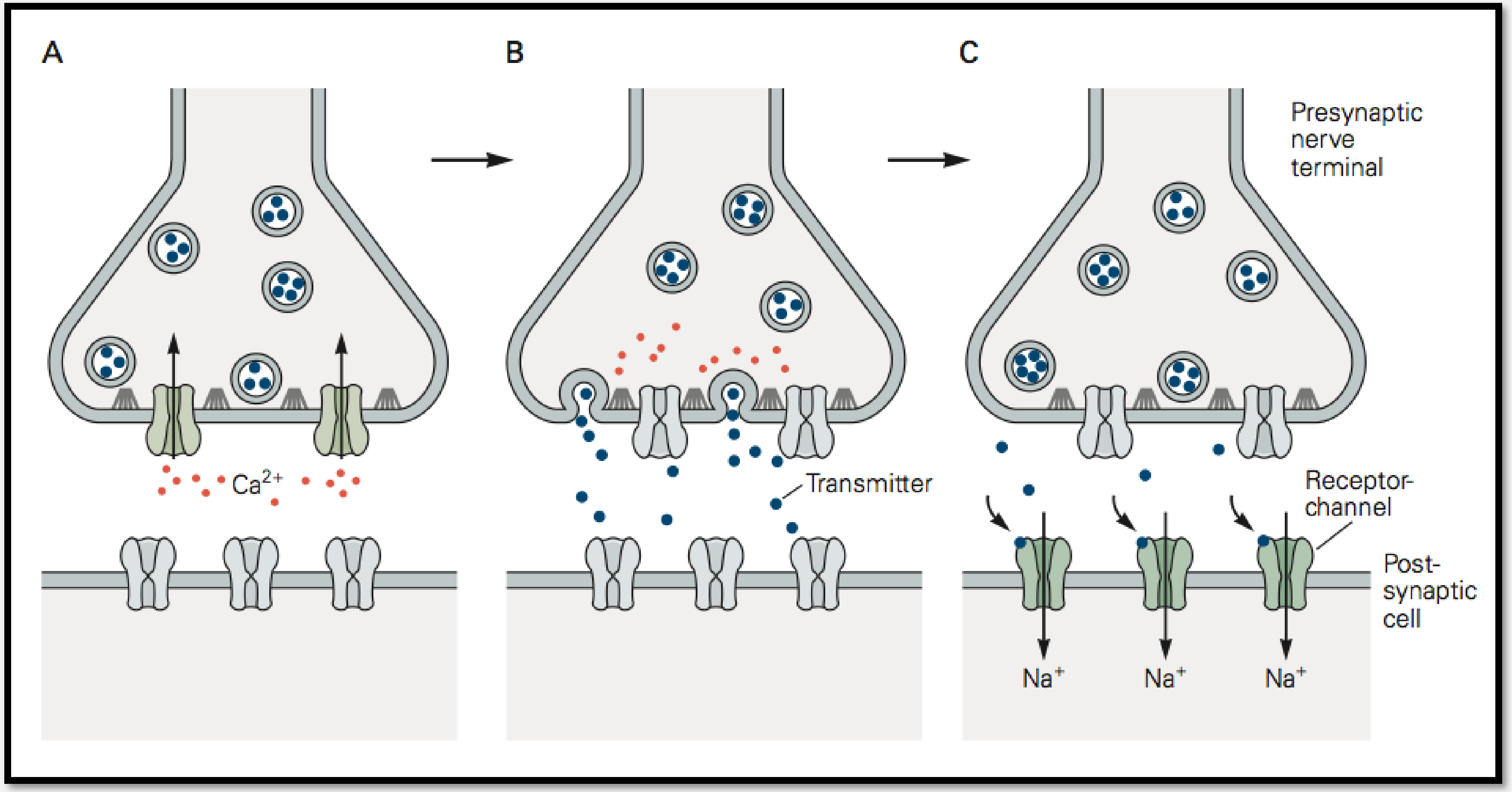
- When the post synaptic cell becomes hyperpolarized and therefore less likely to fire action potential (i.e., the AP is inhibited)

Synaptic delay

- Delay between the presynaptic AP reaching the axon terminal and creating a post synaptic potential

Test your knowledge

In our example… would an EPSP or IPSP occur here?



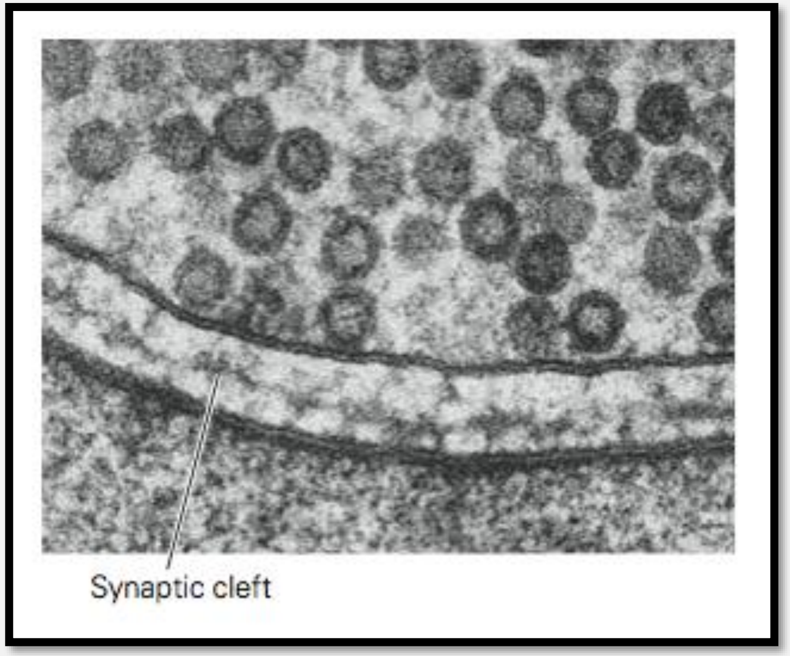
Visualizing vesicles during synaptic transmission

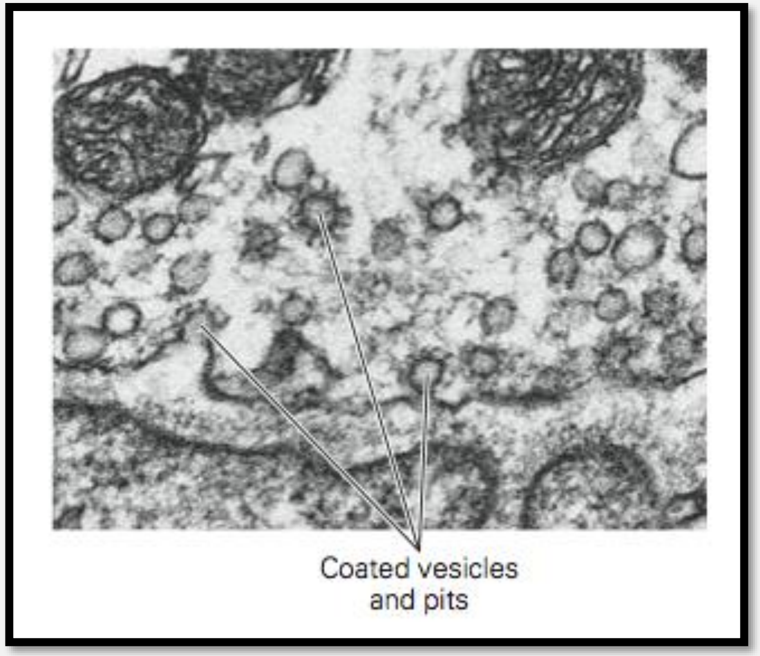
At Rest

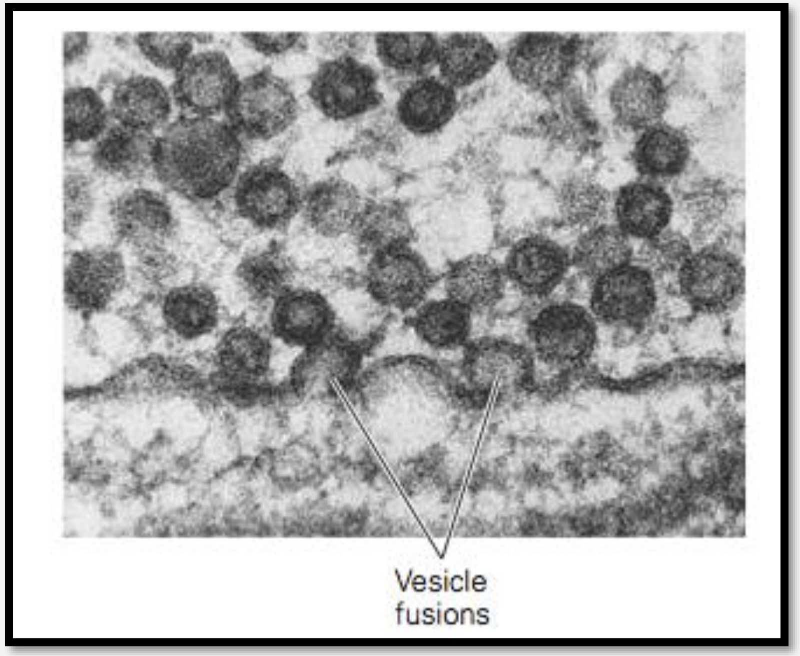
- Vesicles docked and ready to be released
- Stimulate a depolarization and freeze the tissue

Exo and endocytosis

- 5 ms later – can see fusion with the presynaptic membrane
- 10 sec later – endocytosis of vesicles is visible.







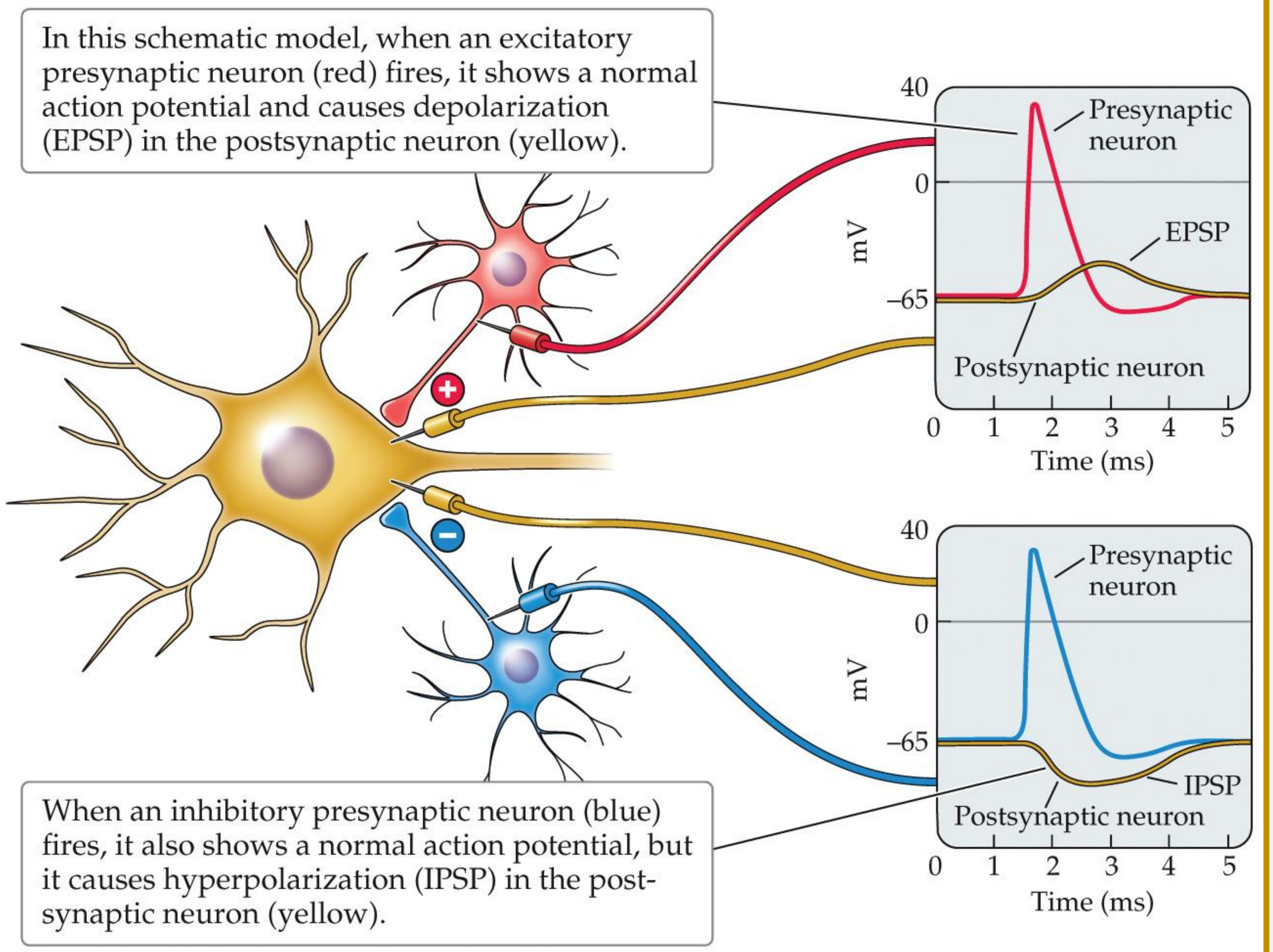
Recording post synaptic potentials

In red

- Excitatory connection

In Blue

- inhibitory connection



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.11

Measure AP on pr

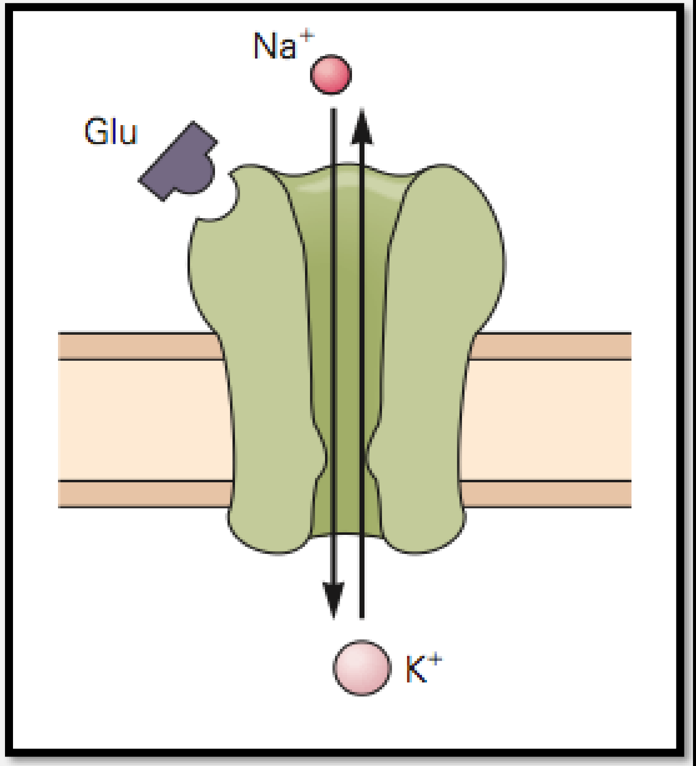
Excitatory Neurotransmitters

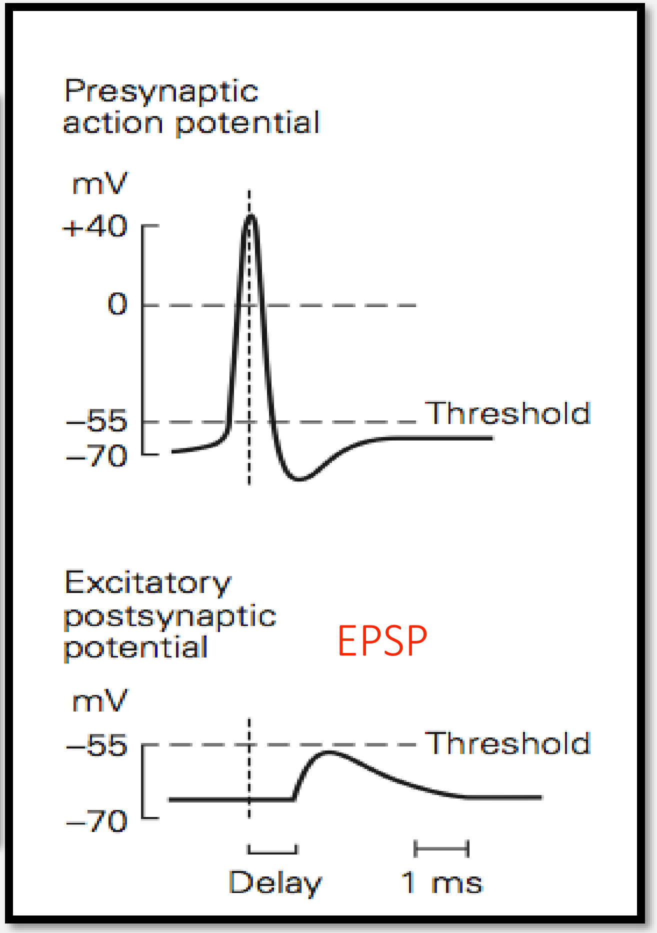
Glutamate

- Primary excitatory NT in the brain

AMPA Receptors

- Glutamate ionotropic receptors
- Ligand-gated
- Depolarizing
- Non-selective cation channel
- Na+ enters, K+ exits

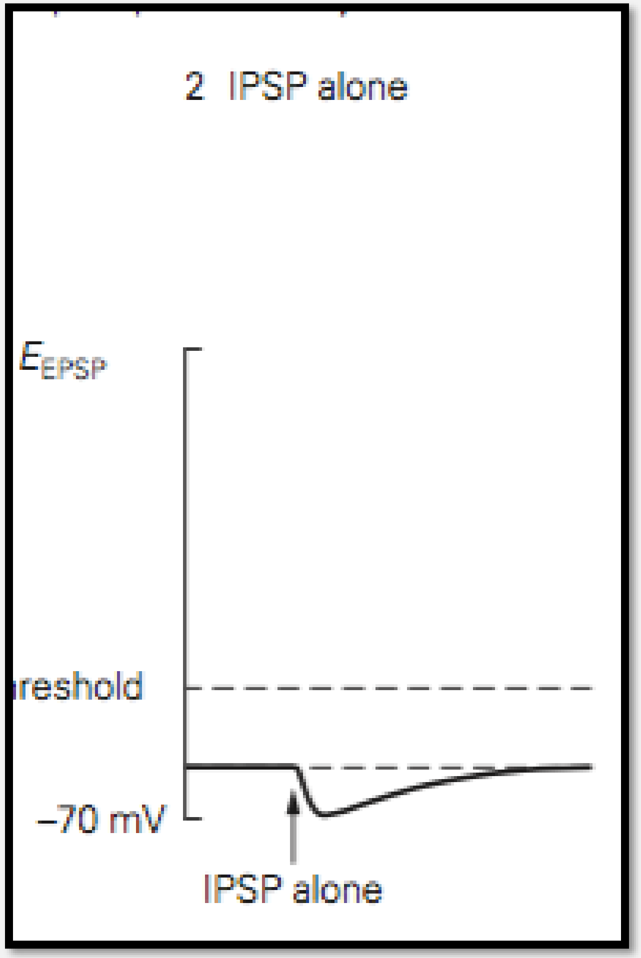




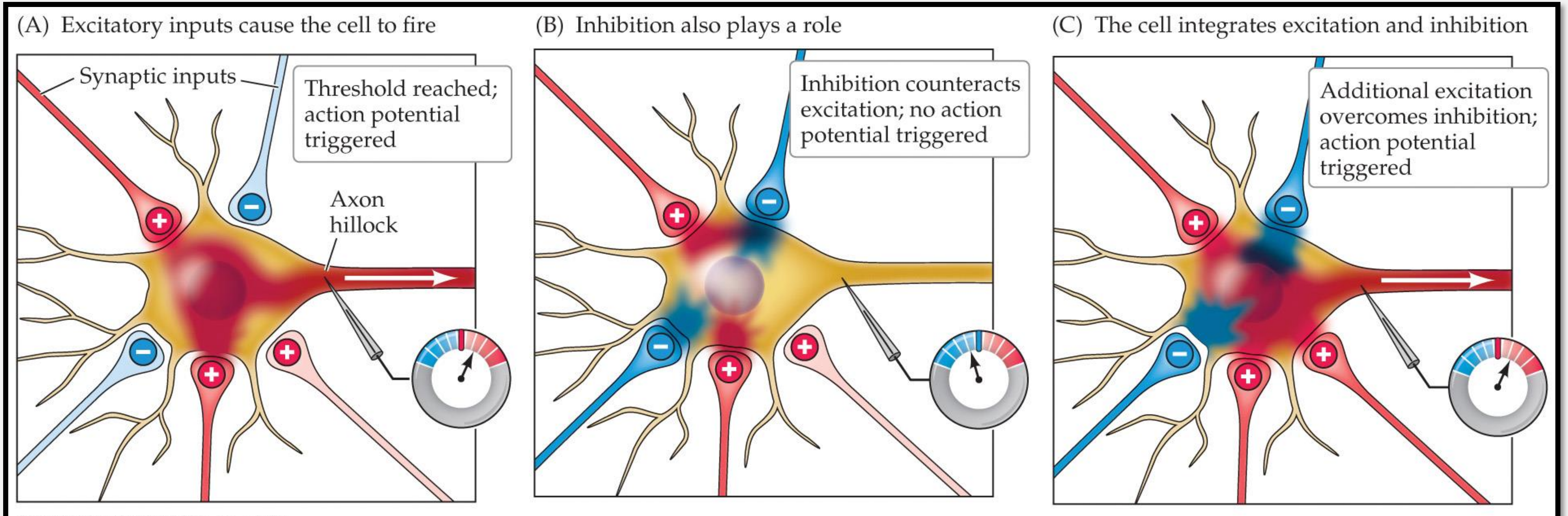
Ionotropic inhibitory NT receptors

GABA

- Primary fast inhibitory NT
- Activates Cl- receptor channels ($GABA_A$)
- Post synaptic hyperpolarization
- "Clamps" membrane at -70mV
- Prevent depolarization + APs
- Alter firing rate of neurons (provides information!)



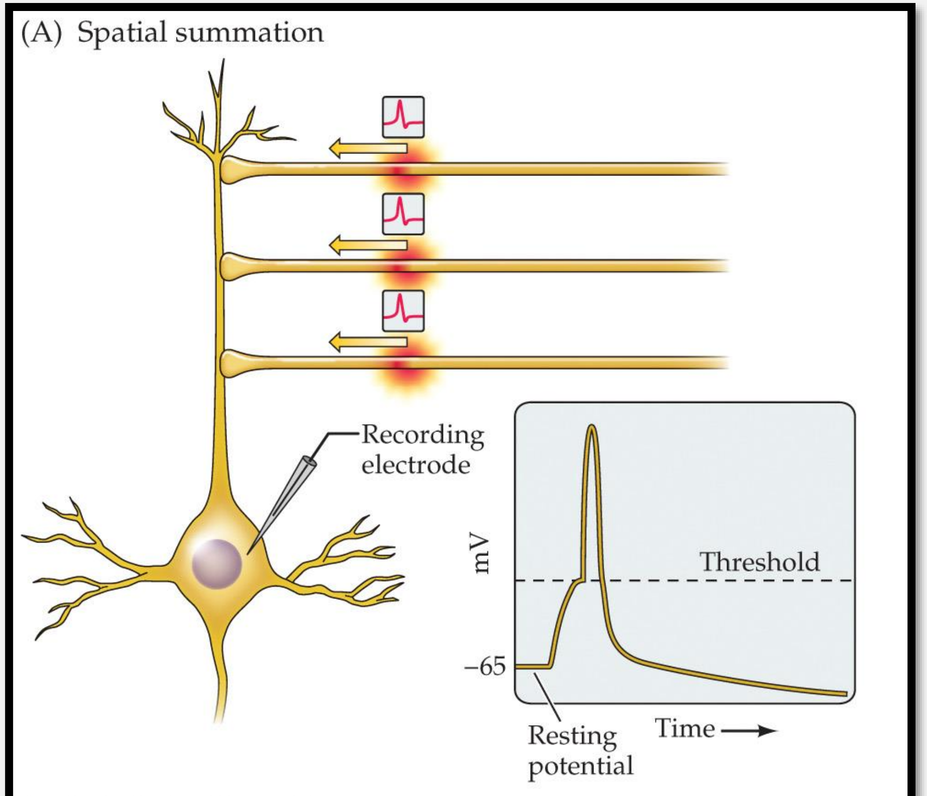
Integration of inputs



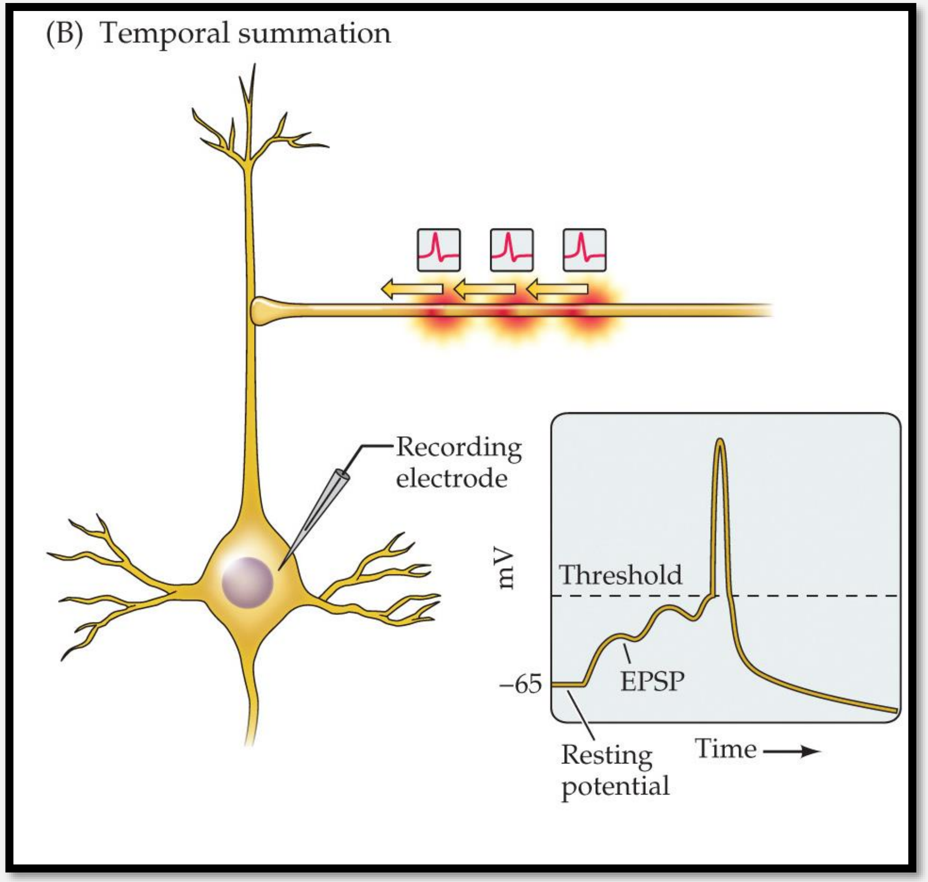
BEHAVIORAL NEUROSCIENCE 10e, Figure 3.12

Spatial versus Temporal Summation

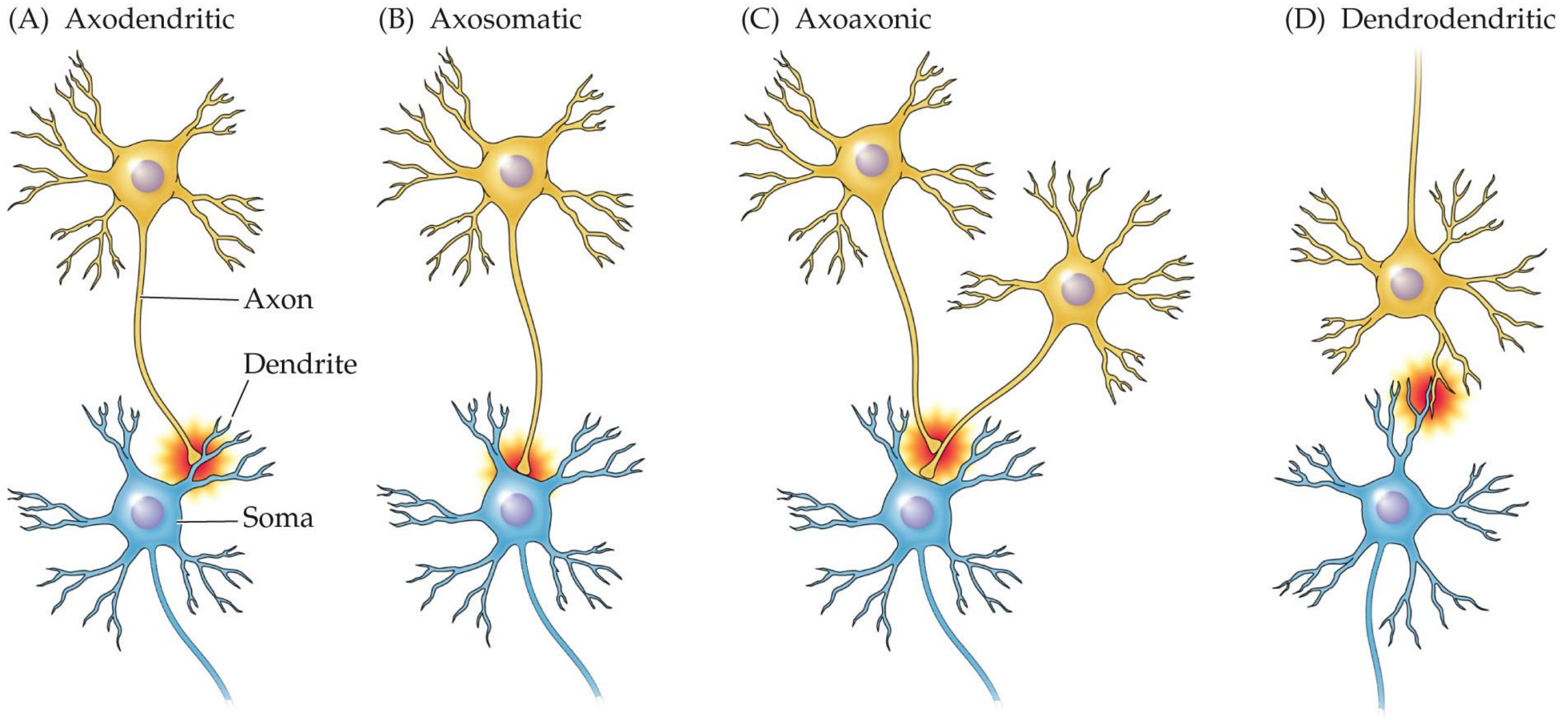
Spatial Summation (A)



Temporal summation (B)



Types of synapses



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.20

Neuronal Circuitry

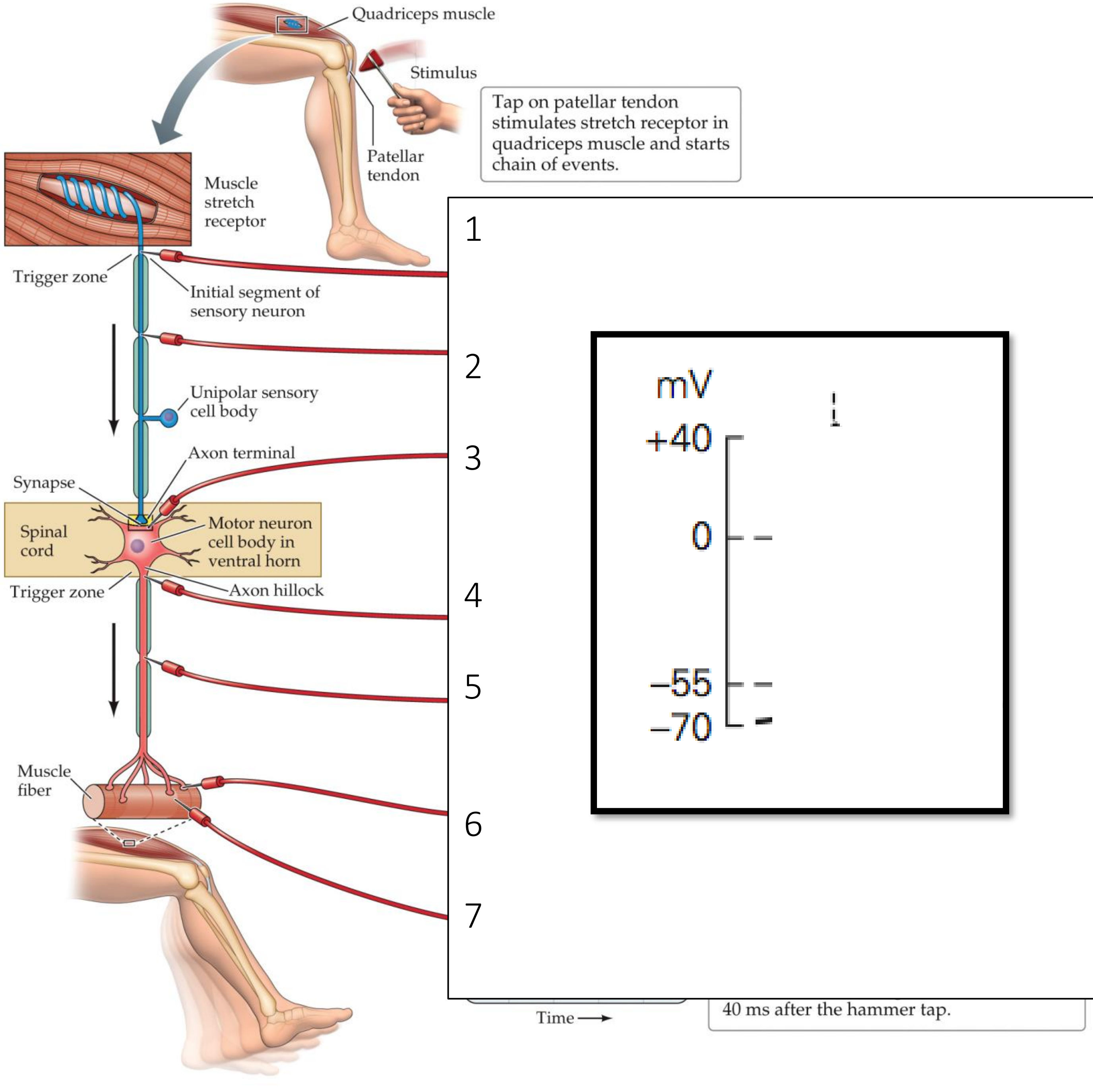
Neurons and synapses combine to make circuits.

Neural chain:

- simple series of neurons (e.g., the **knee-jerk reflex** consists of a sensory neuron, a synapse, and a motor neuron).
- Extremely fast: large myelinated axons, sensory cells synapse directly onto motor neurons, ionotropic synapses.

Knee Jerk Activity

At each recording point, draw out the potential changes. You can assume that an AP is initiated at each trigger zone



BEHAVIORAL NEUROSCIENCE 10e, Figure 3.22

Visualizing circuits

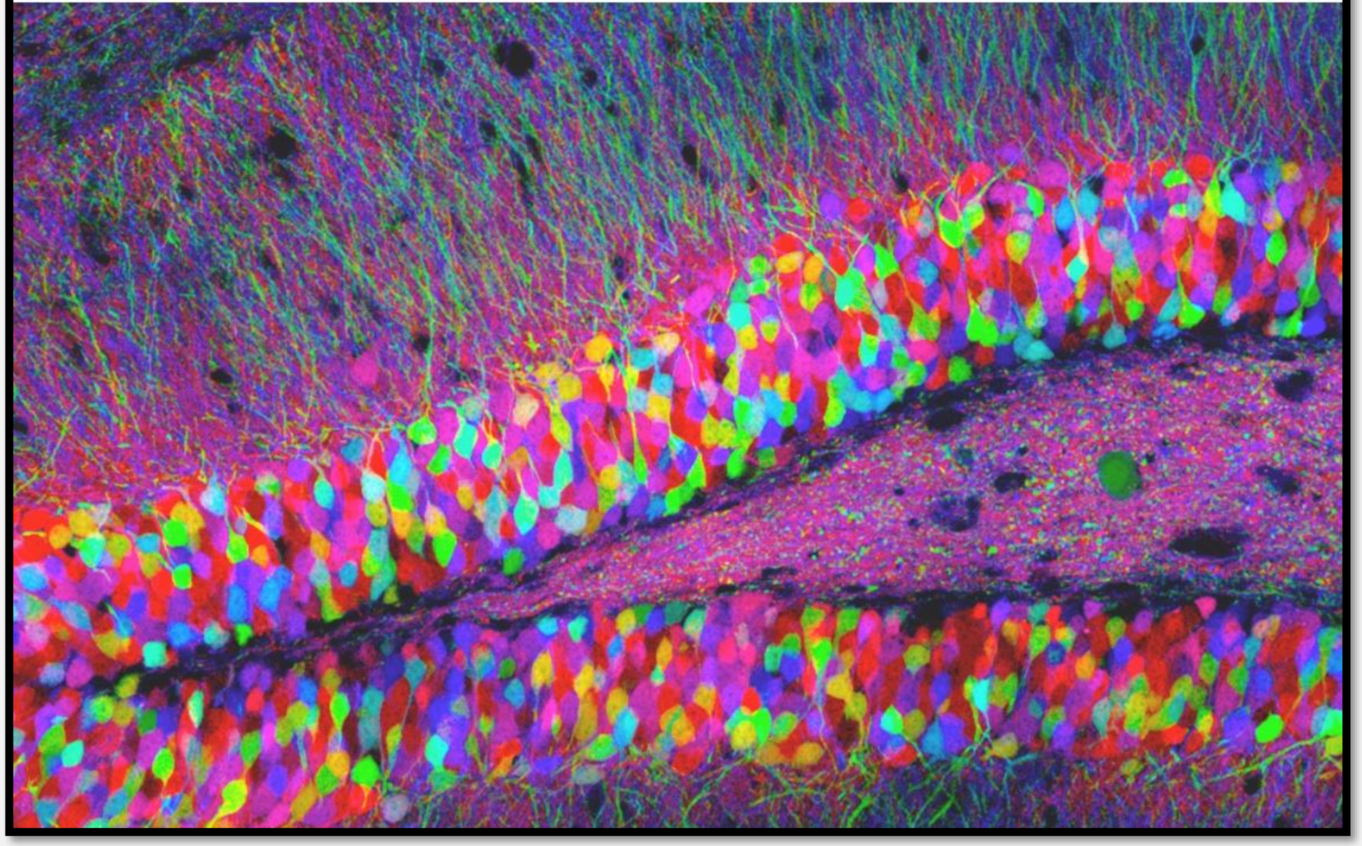
Mapping circuit connectivity with Brainbow

Expression of multiple different coloured FPs

- Randomly expressed FPs (red, blue, green) combine to create unique colours in different neurons
- Goal: trace dendrites and axons of many or all neurons – see how they connect and form circuits

Brainbow in the dentate gyrus region of the hippocampus

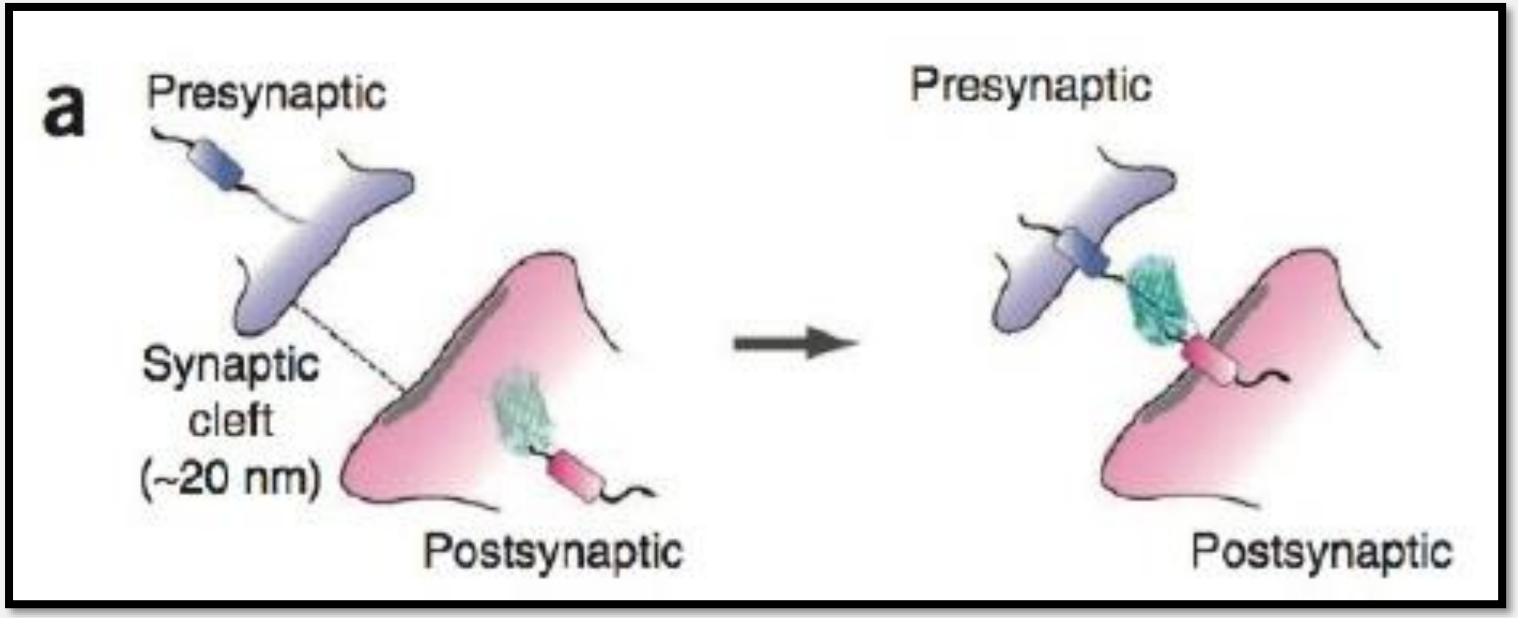
Easy to map this connectome?



Mapping connectivity with GRASP

GFP reconstitution across synaptic partners

- Fluorescent labelling of “synaptically-connected” neurons
- Based on spatial proximity
- Promotors are key!
  - Label pre - machinery/post synaptic machinery with complimenting “halves” of GFP



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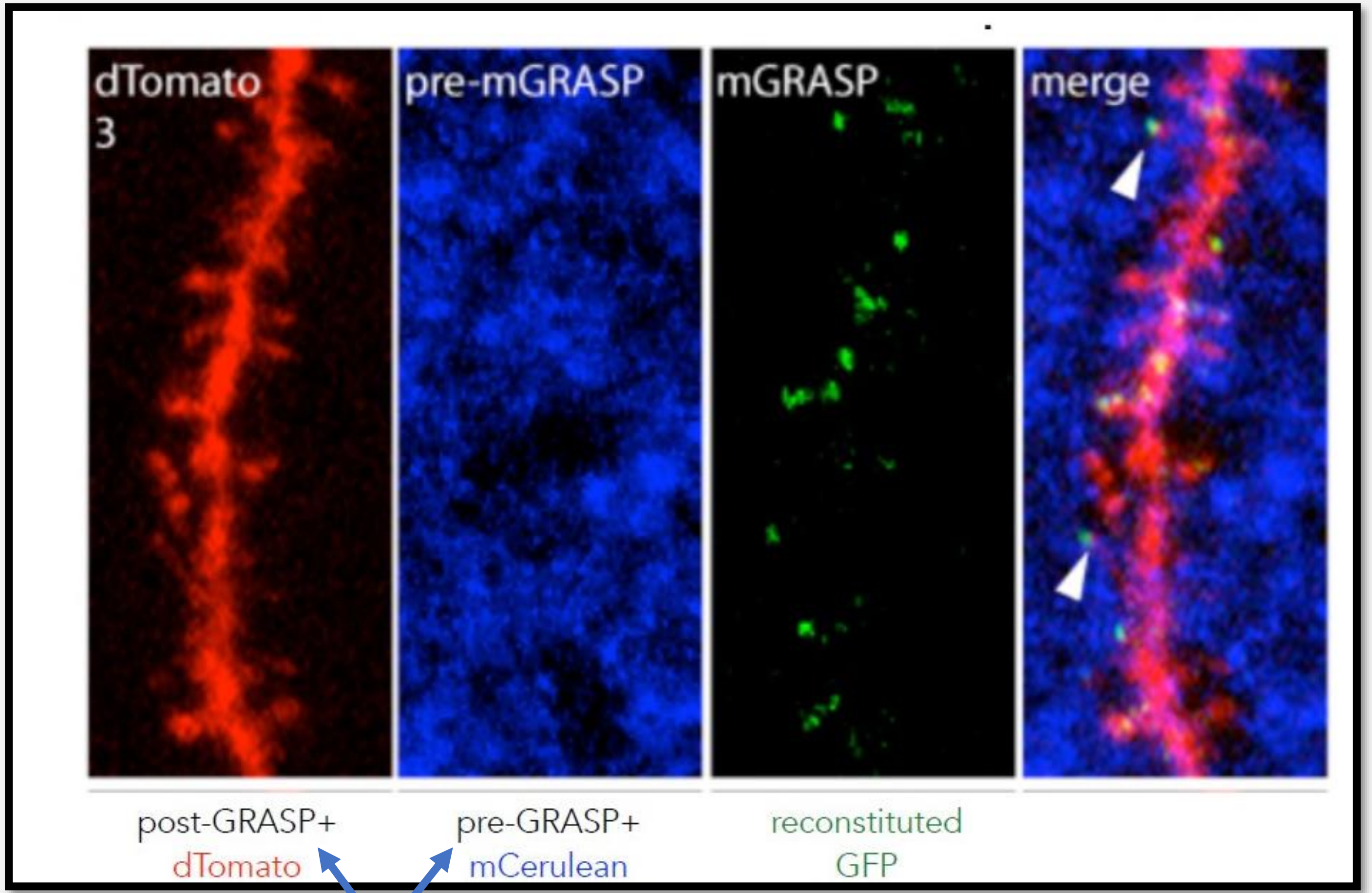
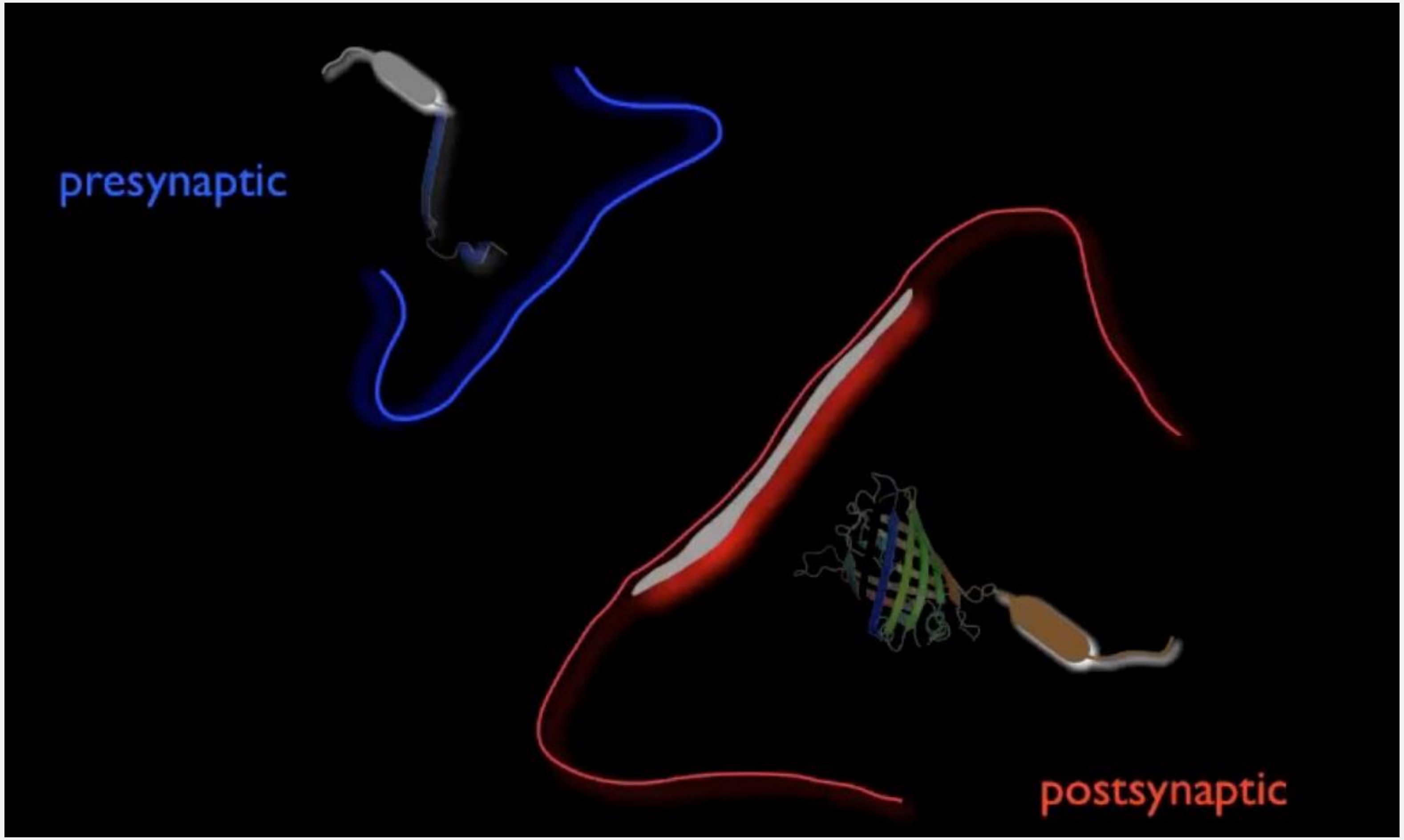


Reporters to see where pre and post molecules are

mGRASP

Mammalian GFP Reconstitution Across Synaptic Partners

- Video showing an application of mGRASP combined with 3D modeling imaging in the hippocampus



https://www.youtube.com/watch?v=5ftqp6CLTgU