

Question 1: Membrane Potential, Action Potentials (7.5 points)

A) Membrane potential (5 points):

You are interested in studying the role of inhibition in cellular signaling. In order to understand the role of chloride flux, you decide to investigate the role of various chloride concentrations.

Ion	Internal Concentration (mM)	External Concentration (mM)	Conductance (mS/cm ²)
Na ⁺	15	140	0.05
K ⁺	140	5	1
Cl ⁻	4	110	0.01

$$R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1} \quad F = 9.65 \times 10^4 \text{ C mol}^{-1}$$

a) (1.5 points) Given the concentrations above, calculate the Nernst potential of each ion, and the resting membrane potential.

	Intracellular (mM)	Extracellular (mM)	G _x	Nernst (V)	
Na ⁺	15	140	0.05	0.057	
K ⁺	140	5	1	-0.085	Membrane Potential (V)
Cl ⁻	4	110	0.01	-0.085	-0.078

These are the calculations assuming T=298K, any temperature assumed was accepted.

b) (0.5 points) Do chloride ions flow in or out when chloride channels are opened? What is the approximate driving force?

Chloride ions will flow in, the driving force is relatively small. $ECDF_{Cl} = -78 - (-85) = +7 \text{ mV}$

c) (1 point) Now, you exchange the internal solution and raise the internal chloride concentration to 40 mM (The concentration of other ions is unaffected). Calculate the new Nernst potential for chloride, and the new membrane potential.

	Intracellular (mM)	Extracellular (mM)	G _x	Nernst (V)	
Na ⁺	15	140	0.05	0.057	
K ⁺	140	5	1	-0.085	Membrane Potential (V)
Cl ⁻	40	110	0.01	-0.025	-0.078

d) (0.5 points) Compare the membrane potential calculated in part Aa versus the one calculated in part Ac. Explain.

The membrane potential does not change because the conductance for chloride at rest is very small.

e) (1 point) Now that the chloride concentration is higher do chloride ions flow in or out when chloride channels are opened? Which way does current flow?

The membrane potential is depolarized from -78mV towards -25mV . Current flows in, chloride ions flow out. New $ECDF_{Cl} = -78 - (-25) = -53 \text{ mV}$.

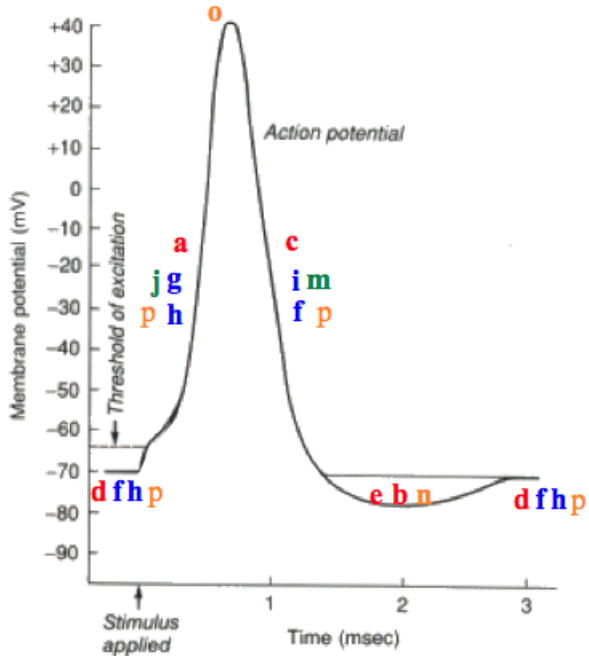
f) (0.5points) Name one type (including subtype) of ion channel that allows flux of chloride ions.
 $GABA_A$

B) Action potentials (2.5 points)

Use the following list to label the action potential shown below. Note some events may be used more than once or not at all.

- a. Depolarizing phase
- b. Hyperpolarizing phase
- c. Repolarizing phase
- d. Resting state
- e. Refractory period
- f. low voltage-gated Na^+ channel conductance
- g. elevated voltage-gated Na^+ channel conductance
- h. low voltage-gated K^+ channel conductance
- i. elevated voltage-gated K^+ channel conductance
- j. Na^+ inward
- k. Na^+ outward
- l. K^+ inward
- m. K^+ outward
- n. $V_m \approx E_K$
- o. $V_m \approx E_{\text{Na}}$
- p. $E_K < V_m < E_{\text{Na}}$

Only the ionic flows due to the AP were considered; Na⁺/K⁺ pump flows were ignored.



Question 2. Channel Modulation (7.5 points)

TrpV1, a cation channel, is activated by the main pungent ingredient in hot chili peppers, *capsaicin*. This produces the burning sensation in humans, and presumably in other animals that express this channel in sensory neurons.

- A. Ruthenium red dye (RR) is an antagonist of TrpV1. Below is the I_m vs time plot of TrpV1 when capsaicin binds to it. **Draw and label the expected traces of I_m vs time for the capsaicin application to TrpV1**
- in the absence of RR (as in the figure),
 - in the presence of RR, and
 - after RR is completely washed off (2 pt).

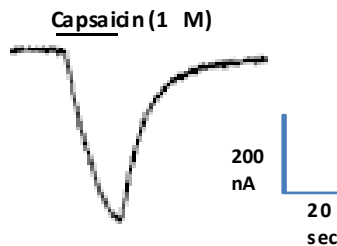
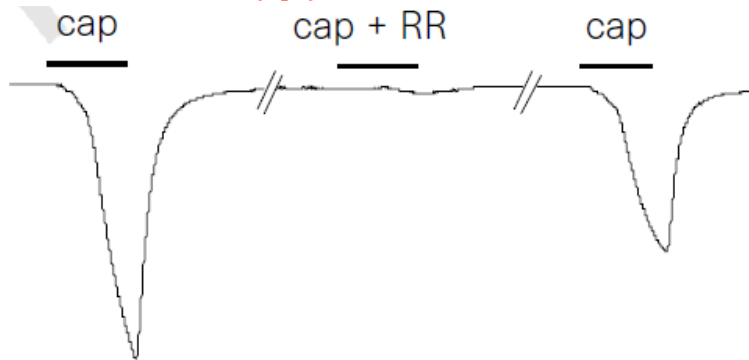


Figure 1. Black bar indicates the duration of capsaicin application to TrpV1

It should show there is no current when RR is present (1 pt), and current is restored when RR is washed out (1pt).



- B. In addition to capsaicin, VaTx2 (found in a West Indies tarantula) and DkTx (found in the earth tiger tarantula) bind to TrpV1 and activate it. To study the binding of each toxin to TrpV1 relative to capsaicin, you incubate TrpV1 with the corresponding compound at a concentration of $1 \mu\text{M}$. All TrpV1 channels are activated by each compound and conduct current. This is recorded as current at time 0 min. Next, you wash out the compound over time and measure the TrpV1 current at 0.5 min intervals. See Figure 2. **Which compound binds for the longest**

time to the channel? Which is the briefest binder? Provide your reasoning based on Figure 2. (1.5 pt)

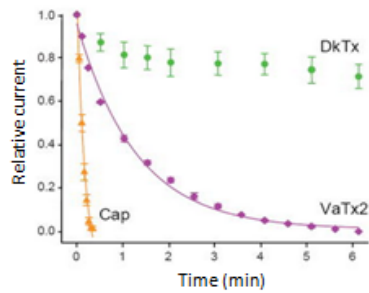


Figure 2. The measured current flow is plotted as the *relative current* (y-axis) compared to the current at time 0 min of the corresponding compound. Notice that the negative current of Figure 1 is now shown as a positive signal in Figure 2. The relative current at 0 minutes has the value of 1.0.

DkTx binds to TrpV1 for the longest time and causes it to conduct current after prolonged period of wash out. Capsaicin is the briefest binder (0.5 pt).

(1 pt for explanation relating the answer to Figure 2).

C. To investigate the permeability of TrpV1, you perform voltage clamp experiments as in Figure 3. This experiment informs us about the channel's permeability to three cations. **Which are the cations, and what can we conclude about their permeability in the channel (2 points)?**

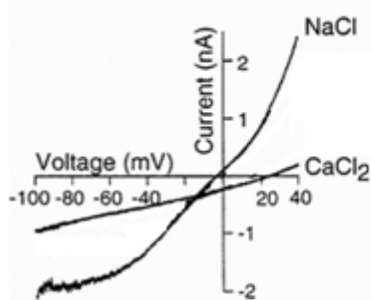


Figure 3. Current-voltage relation for the TrpV1 channel l in the presence of the usual NaCl-rich extracellular solution, as well as in a special external solution that contains only isotonic CaCl₂.

The obvious: Na⁺ & Ca²⁺. (0.5 pt), K⁺ (0.5pt). Figure 3 shows the reversal potential for Na⁺ and Ca²⁺ are not the same as the Nernst potential of the individual cation. This implies that there must be outward cation flow that causes the reversal potential of Na⁺ and Ca²⁺ to be

more negative than their corresponding Nernst potential. Since the cells have high concentration of K^+ , we can conclude that TrpV1 is also permeable to K^+ . (1 pt)

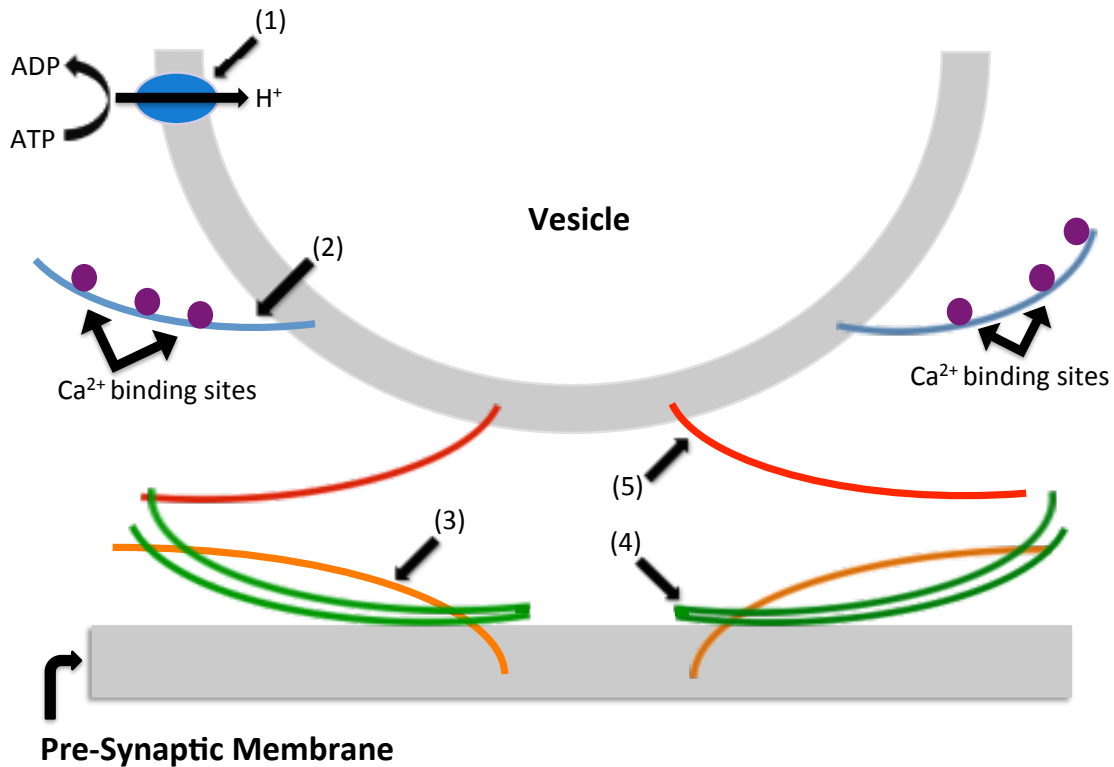
D. Re-read this question's first paragraph. A local anesthetic such a procaine can suppress pain caused by the tarantula toxins. Yet procaine does not directly block TrpV1. **Which channel(s) do local anesthetics block, and how does this suppress the pain (2 points)?**

Procaine blocks voltage-gated Na^+ channel (1 pt). This suppresses the propagation of pain stimulus from sensory neurons to central nervous system. (1 pt)

end of question 2

Question 3: Synaptic Transmission (7.5 points)

1. Vesicle Fusion (4 points)



A. **(2 points)** Provide the name for each of the proteins or structures in the figure above (.4 for each correctly labeled answer)

(1) ATP-driven proton pump

(2) Synaptotagmin

(3) Syntaxin (.2 points for saying only T-SNARE)

(4) SNAP-25 (.2 points for saying only T-SNARE)

(5) VAMP/Synaptobrevin (.2 points for saying only V-SNARE)

B. **(1 point)** Botulinum toxin type A is a naturally occurring toxin that specifically cleaves SNAP-25 proteins. State the direction of its effect on transmitter release from the pre-synaptic terminal (increase, decrease, or no effect) and its mechanism of action.

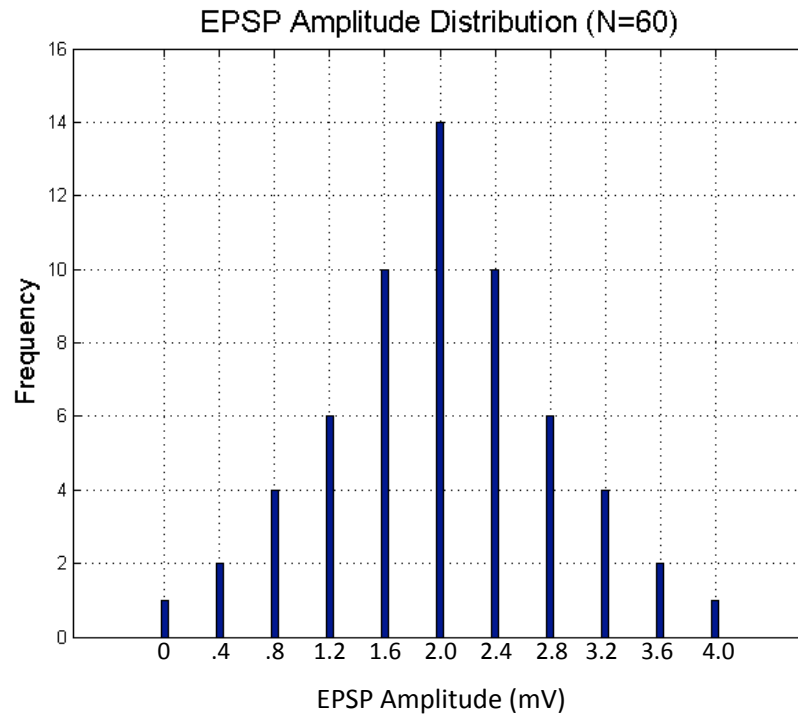
There would be a decrease in transmitter release. By cleaving SNAP-25, the ternary SNARE complex is unable to properly form, which would prevent vesicle docking and transmitter release.

- C. **(1 point)** While examining the relationship of synaptic vesicle exocytosis and quantal transmitter release in the neuromuscular junction, you discover a drug, heuserin, that can prolong the duration of the presynaptic action potential. Would heuserin affect the rate of vesicle fusion with the pre-synaptic membrane? Briefly explain why or why not.

A longer pre-synaptic action potential would prolong the duration of calcium channel opening, increasing intracellular calcium. An increase in calcium concentration would increase the number of vesicles that fuse with the presynaptic membrane by binding to synaptotagmin more rapidly. (Increasing intracellular calcium increases the probability/rate of synaptotagmin binding Ca^{2+})

2. Excitatory Post-Synaptic Potentials & Quantal Release (3.5 points)

- A. You record and measure the amplitude of 60 Excitatory Post-Synaptic Potentials (EPSPs) in the neuromuscular junction. The amplitude distribution of these 60 EPSPs is plotted below:



1. **(0.5 points)** State the size of a quantum in mV.

0.4 mV –the smallest amplitude EPSP that occurs without failure

2. **(0.5 points)** The voltage change produced by a single channel is 0.2 μV . How many channels are opened by the quantum?

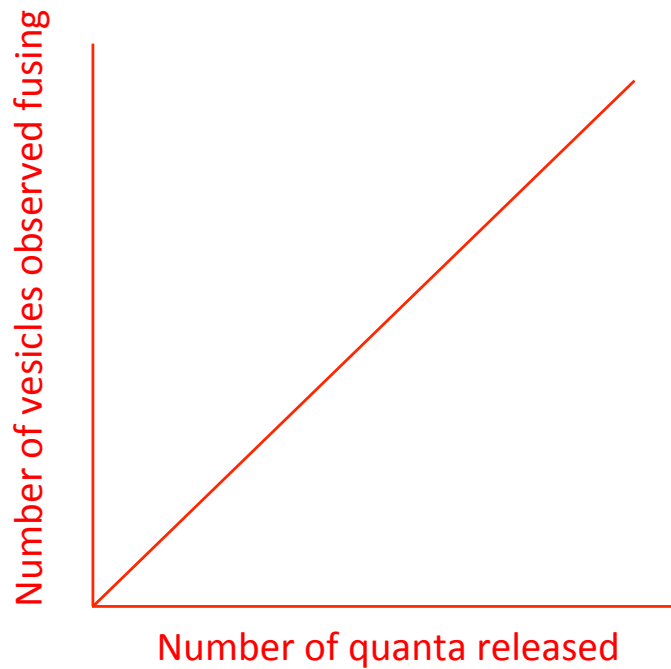
$0.2 \mu\text{V}/\text{channel}) \times (\text{number of channels}) = .4 \text{ mV}$ or 400 μV

$400 \mu\text{V} / (0.2 \mu\text{V}/\text{channel}) = 2,000$ channels

3. **(1 point)** Assuming every molecule released by a vesicle into the synaptic cleft binds to a receptor on the post-synaptic membrane, at least how many neurotransmitter molecules must be in each synaptic vesicle? Explain your reasoning.

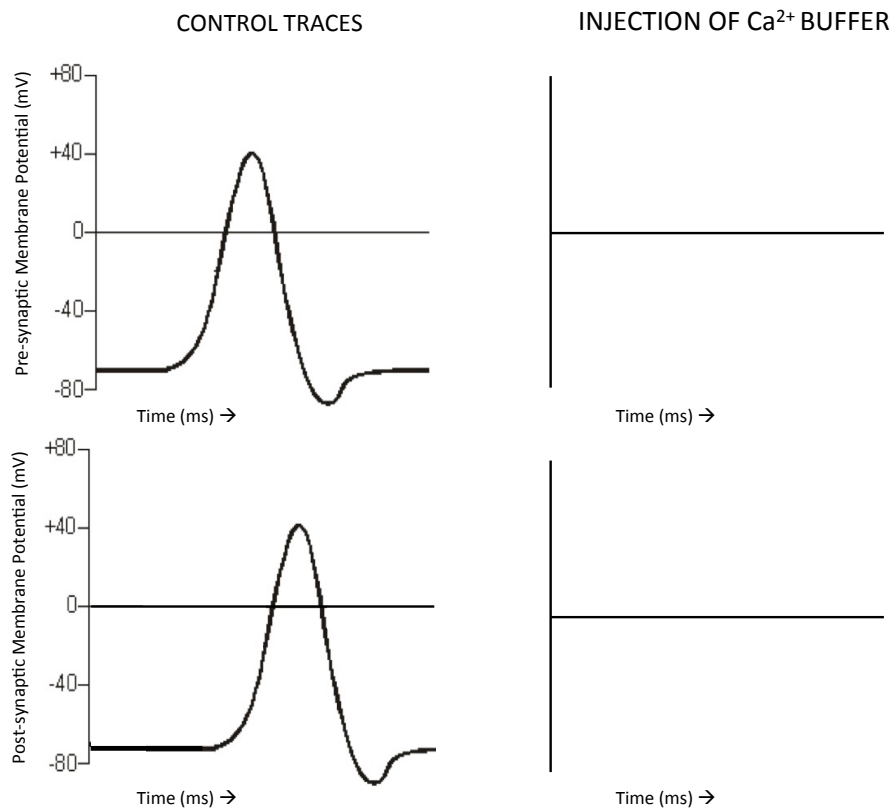
2 Ach molecules need to bind to each nACh receptor to open the channel.
2,000 channels are opened by a quantum, so at least 4,000 molecules must be in the vesicle to open 2,000 channels.

4. **(0.5 points)** Draw a graph showing the number of vesicles observed fusing on the y-axis and the number of quanta released on the x-axis. Describe the relationship between the number of vesicles that fuse with the pre-synaptic membrane and the number of quanta released.



One quanta is released by one vesicle fusing, so the relationship should be linear and increasing.

- B. **(1 point)** On the heels of your heuserin success, you begin a new experiment to study the role of calcium in synaptic transmission. You start by recording the membrane potential of the presynaptic and postsynaptic membranes, shown in the control traces below. Next, you inject the presynaptic terminal with a Ca^{2+} buffer that binds Ca^{2+} and maintains its concentration at a very low level. Draw the waveforms for the pre-synaptic membrane potential and post-synaptic membrane potential in the axes provided to the right of the control traces. Briefly state how the pre-synaptic potential and post-synaptic potential are affected by the injection of Ca^{2+} buffer.

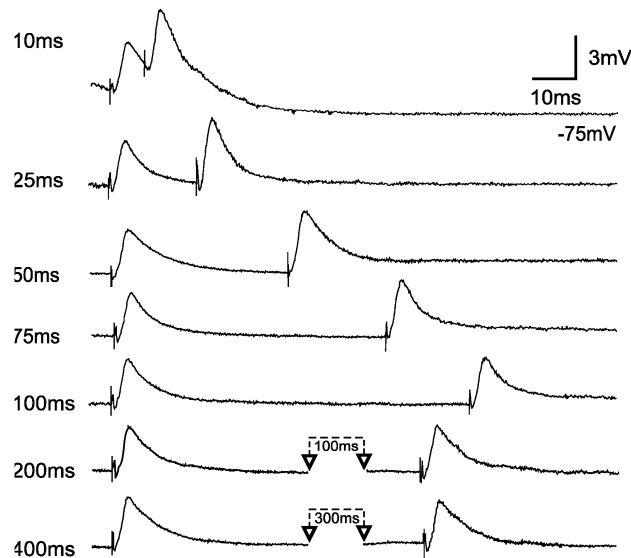


After injection of the calcium buffer, the presynaptic potential will be similar to the control trace because calcium does not contribute significantly to the membrane potential of the presynaptic cell. The postsynaptic membrane potential will remain close to, or at, the resting membrane potential because very few vesicles will fuse with the pre-synaptic membrane, preventing transmitter from being released. With little or no transmitter release, few post-synaptic ion channels will open and the membrane potential will remain relatively unchanged.

Question 4: Synaptic Plasticity

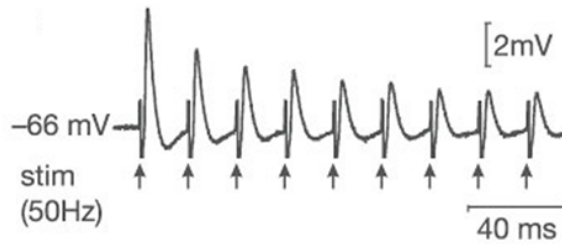
1. Short term synaptic plasticity.

A. The figure below shows the results of an experiment demonstrating the existence of *paired pulse facilitation*. In this experiment, two action potentials are evoked in a presynaptic neuron at various inter-stimulus intervals (10 – 400 ms), while excitatory postsynaptic potentials (EPSPs) are recorded (displayed below) from a downstream, postsynaptic neuron. **Explain the phenomenon of *paired pulse facilitation* by referring to the figure below. Which mechanism is thought to underlie *paired pulse facilitation*? (1.5 Points)**



As shown in the figure, at low inter-stimulus intervals (less than ~100 ms) the second EPSP is larger in amplitude than the first EPSP. This is the phenomenon of *paired pulse facilitation* (0.75 Points). Paired pulse facilitation results from an increase in the intracellular calcium concentration in the presynaptic terminal following the first action potential. This calcium, which enters through voltage-gated calcium channels, increases the probability of vesicle release in response to the second evoked action potential, thereby facilitating vesicle fusion and resulting in a larger amplitude EPSP (0.75 Points).

B. The figure below shows the results of an experiment demonstrating the existence of *short-term synaptic depression*. In this experiment, 9 action potentials (arrows) are evoked in a presynaptic neuron at a rate of 50 Hz, while EPSPs are recorded (displayed below) from a downstream, postsynaptic neuron. **Explain the phenomenon of *short-term synaptic depression* by referring to the figure below. Which mechanism is thought to underlie *short-term synaptic depression*? (1.5 Points)**



As shown in the figure, the amplitude of EPSPs evoked by a train of action potentials in the presynaptic neuron shows a gradual decline with each successive action potential. This is the phenomenon of *short-term synaptic depression* (0.75 Points). Short-term synaptic depression results from a depletion in the number of readily releasable vesicles in the presynaptic neuron as a result of the high-frequency action potential train (0.75 Points)

2. Long-term synaptic plasticity.

A. Below is an *unordered* list of steps thought to produce one form of frequency-dependent long-term potentiation at glutamatergic synapses in the hippocampus 30 minutes following high-frequency stimulation of a presynaptic neuron. **Write the events in the correct order. If any events overlap with each other, indicate where this occurs. (2 Points)**

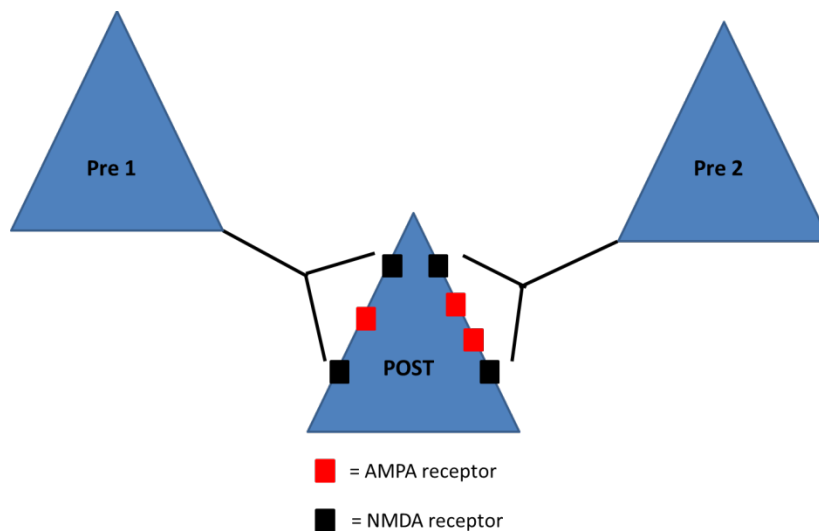
- 1) CamKII performs autophosphorylation. (10)
- 2) Mg^{2+} is expelled from the pore of NMDA receptors. (6)
- 3) Glutamate is released by the presynaptic neuron. (2)
- 4) Glutamate binds to NMDA receptors. (3-4)
- 5) The open-state conductance of AMPA receptors increases. (12)
- 6) The membrane potential is driven to the reversal potential for AMPA receptors. (5)
- 7) Several action potentials arrive at the presynaptic terminal. (1)
- 8) Ca^{2+} enters through NMDA receptors. (7)
- 9) The kinase CaMKII becomes activated. (9)
- 10) AMPA receptors become phosphorylated. (11)
- 11) Glutamate binds to AMPA receptors. (3-4)
- 12) Calmodulin binds Ca^{2+} ions. (8)

3. Coincidence detection.

A. NMDA receptors are thought to act as coincidence detectors. **What two events are they detecting the coincidence of, and how does the channel accomplish this? (1 point).**

NMDA receptors are able to detect the coincidence of synaptic input, signaled by the presence of glutamate, and depolarization of the postsynaptic membrane (0.5 Points). The channel is able to accomplish this because its gating depends both on the presence of its ligand (glutamate) and depolarization in the postsynaptic membrane, which expels the Mg^{2+} ion normally blocking the channel near resting membrane potential (0.5 Points).

B. The schematic below shows two glutamatergic neurons (Pre 1 and Pre 2) making synapses onto a postsynaptic neuron (POST). AMPA and NMDA receptors are present at both synapses. The synapse between Pre 1 \rightarrow POST is initially quite weak, and is unable to produce any appreciable depolarization of the postsynaptic neuron. The synapse between Pre 2 \rightarrow POST is quite strong, and is able to produce a large amplitude EPSP in the postsynaptic neuron, which cause it to fire an action potential. **Explain how the properties of NMDA receptors may allow for long-term potentiation between Pre 1 \rightarrow POST when a high-frequency train of action potentials is evoked in Pre 1 and Pre 2 (1.5 Points).**



Because the synapse between Pre 1 \rightarrow POST is initially quite weak, high frequency activation of this pathway will not produce enough depolarization in POST to expel the Mg^{2+} blocking the NMDA receptor's pore, so no Ca^{2+} can enter the postsynaptic neuron, which is a prerequisite for long-term potentiation. However, when both Pre 1 and Pre 2 produce a high-frequency train of action potentials, the NMDA receptors at the Pre1 \rightarrow POST synapse are able to open. Glutamate is released from Pre 1, while the postsynaptic depolarization necessary to expel the Mg^{2+} is provided by the large depolarizing current generated by AMPA receptors at the Pre 2 \rightarrow POST synapse. Opening of NMDA receptors at the Pre 1 \rightarrow POST synapse allows calcium to enter the postsynaptic cell, which, through its action on downstream messengers, produces long-term potentiation by an increase in the number and conductance of AMPA receptors (1.5 Points).

Question 5:

Key will be posted shortly.