

BI/CNS 150 MIDTERM EXAMINATION, 2013
You may read only this page before beginning the exam.

This examination contributes *30 percent of your final course grade*. There are five questions in the exam; ANSWER FOUR. The exam will be graded out of 30 possible points, 7.5 points for each of the *four questions you answer*. Please indicate clearly in your answers, which questions you answered. This examination covers lectures and reading material through Monday, October 24, 2010. Each question has the statement, "end of Question n". Please make sure you have all the pages before you start.

Three and one-half hours are permitted for completion of the exam. Use the first half hour to read all of the questions calmly and to choose which four to answer. Use the next three hours to answer the four questions. If time expires, you may draw a line across the page, complete the exam, and indicate the amount of extra time you used. Credit *may* be given for answers completed in this manner. This will aid us in assessing the appropriateness of the time limit.

You may refer to lecture notes, this year's problem sets, and your textbook to complete this exam. You may also use a calculator or computer, if desired. You may not collaborate or discuss questions or answers with anyone else before the exam due date. You may not use the Internet while taking the exam.

If you feel that you need to make any further assumptions about the systems described in any of the problems, feel free to write your assumption by your answer and the reason that you needed to make that assumption; however, no further information should be necessary to answer the problems correctly.

You must *answer each question on a separate sheet of paper* and *write your name and the page number on each sheet of paper used*. This allows us to sort the questions for grading. Fill out this cover sheet and staple it to the front of your exam. Staple another cover sheet to the back of your exam.

Completed exams are due by *4:30 PM on Tuesday, November 1*. You may hand in your answers to any TA, or place them in the Bi 150 mailbox on the 3rd floor of Kerckhoff (In front of room 326). Late exams will be penalized. Corrected exams will be returned ASAP.

NAME: _____
Section: _____
Mail Code: _____
TA Name: _____
Date and Time turned in: _____
Number of Pages: _____

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

A) Membrane potential (5 points, as apportioned below):

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.

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

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

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

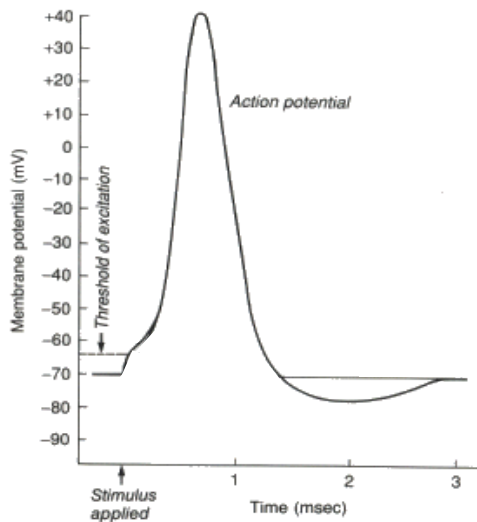
e) (1 point) Now that the chloride concentration is higher, do chloride ions flow in or out when chloride channels are opened? Which way does current flow?

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

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. $E_K < V_m < E_{\text{Na}}$



end of question 1

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).

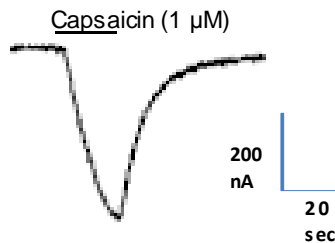


Figure 1. Black bar indicates the duration of capsaicin application to TrpV1

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 μ 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)**

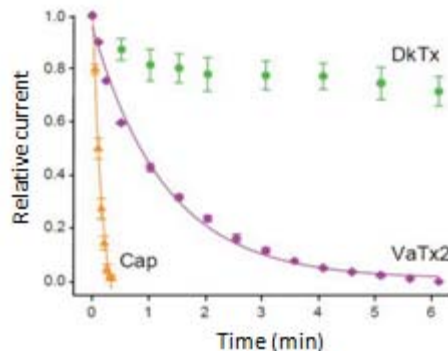


Figure 2. The measured current flow is plotted as the *relative current* (y-axis) compared to the current at time 0 min of the corresponding compound. Notice that the negative current of Figure 1 is now shown as a positive signal in Figure 2. The relative current at 0 minutes has the value of 1.0.

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 most likely cations? Then conclude whether each is, or is not, permeable in the channel (2 points).**

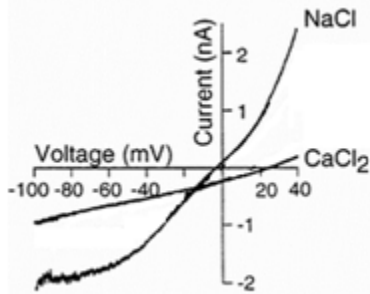


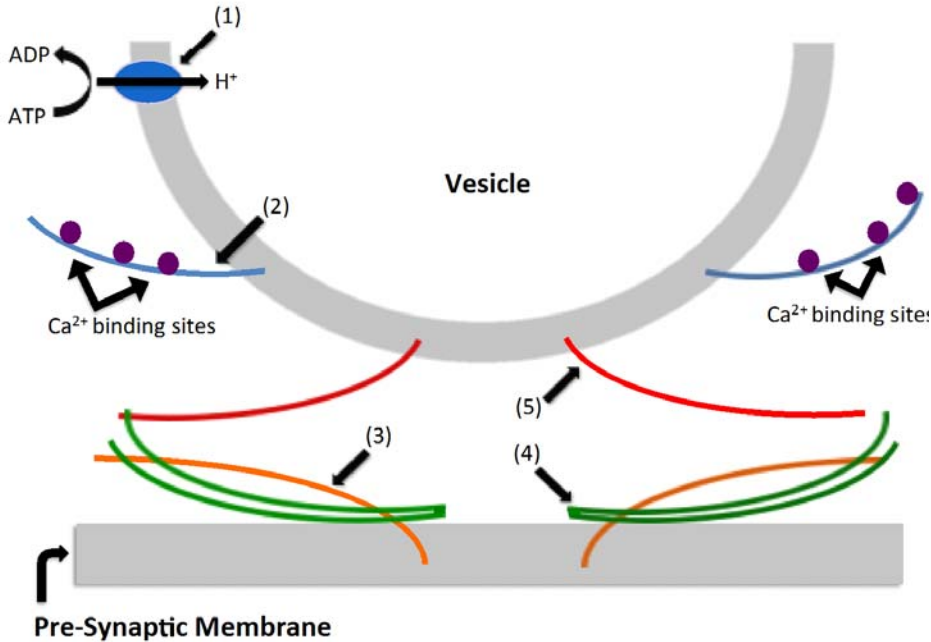
Figure 3. Current-voltage relation for the TrpV1 channel I in the presence of the usual NaCl-rich extracellular solution, as well as in a special external solution that contains only isotonic CaCl₂.

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)?**

end of question 2

Question 3: Synaptic Transmission (7.5 points)

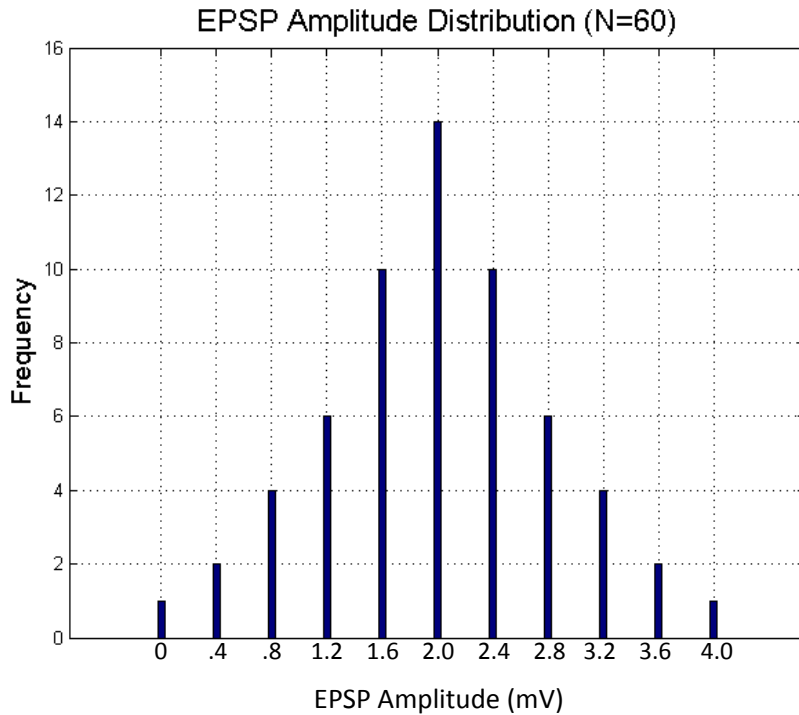
A. Vesicle Fusion (4 points, as apportioned below)



- a. (2 points) Provide the name for each of the 5 proteins or structures in the figure above.
- 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.
- 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.

B. Excitatory Post-Synaptic Potentials & Quantal Release (3.5 points, as apportioned below)

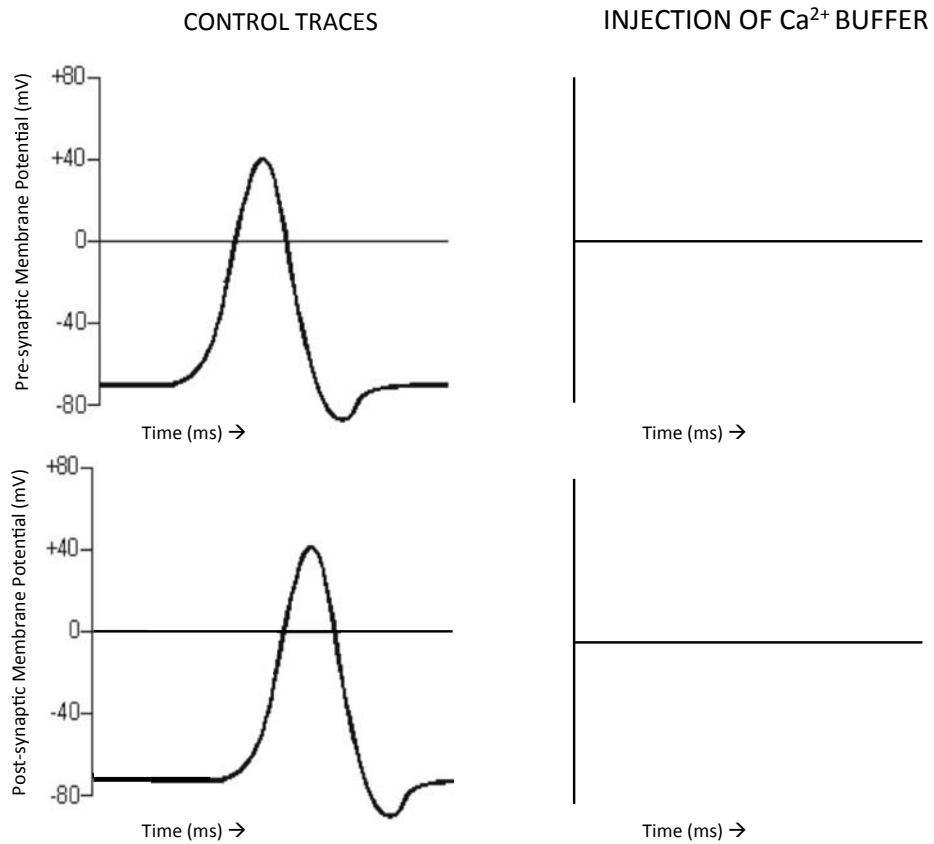
You stimulate the motor nerve; you record and measure the amplitude of 60 excitatory post-synaptic potentials (EPSPs) at the neuromuscular junction. The amplitude distribution of these 60 EPSPs is plotted below:



- a. (0.5 points) State the size of a quantum in mV.
- b. (0.5 points) The voltage change produced by a single channel is $0.2 \mu\text{V}$. How many channels are opened by the quantum?
- c. (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.
- d. (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.

C. (1 point) On the heels of your heuserin success, you begin a new experiment to study the role of calcium in synaptic transmission. You choose a neuronal synapse where you can record intracellularly from both the presynaptic terminal and the postsynaptic neurons. You simulate the presynaptic neuron. The pre- and postsynaptic membrane potentials are shown in the control traces below. Next, you inject the presynaptic terminal with a Ca^{2+} buffer that maintains $[\text{Ca}^{2+}]$ 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.

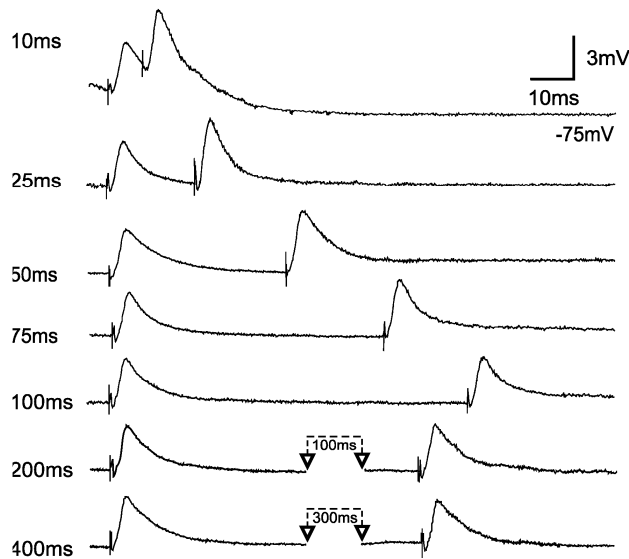


end of question 3

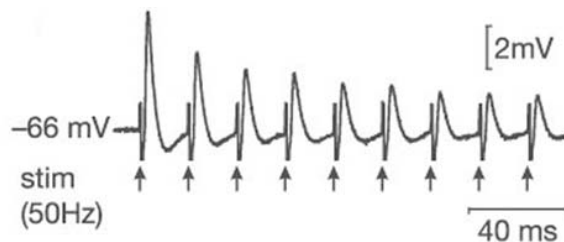
Question 4: Synaptic Plasticity (7.5 points)

A. 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)**



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)**



B. Long-term synaptic plasticity.

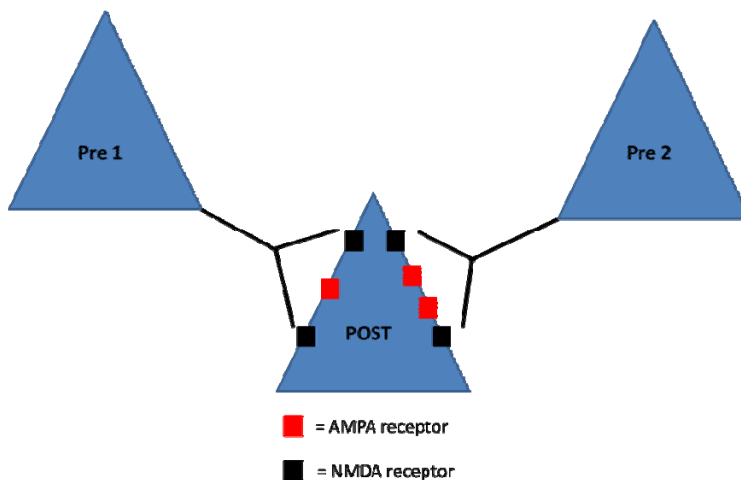
a. Below is an *unordered* list of steps thought to produce one form of frequency-dependent long-term potentiation at glutamatergic synapses in the hippocampus 30 minutes following high-frequency stimulation of a presynaptic neuron. **Write the events in the correct order. If any events overlap with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

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

C. Coincidence detection.

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

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 → POST is initially quite weak, and is unable to produce any appreciable depolarization of the postsynaptic neuron. The synapse between Pre 2 → POST is quite strong, and is able to produce a large amplitude EPSP in the postsynaptic neuron, which evokes an action potential. **Explain how the properties of NMDA receptors may allow for long-term potentiation between Pre 1 → POST when a high-frequency train of action potentials is evoked in Pre 1 and Pre 2 (1.5 Points).**



end of question 4

Question 5. Overview of neural control (7.5 points).

Acetylcholine, liberated from the terminals of spinal motor neurons, activates skeletal muscle.

Acetylcholine, liberated from the terminals of vagal nerves, slows the heartbeat.

The mechanisms of transmitter release are quite similar at these two synapses, but quite different events then occur in the postsynaptic cell.

At 1.5 points per question, contrast these subsequent events at the two synapses. Include the following points:

- (A) Receptors activated (general family, and pharmacological classification).**
- (B) Details of the activation (direct vs indirect; molecular components of the pathway).**
- (C) Time course of the overall action.**
- (D) Type and ionic selectivity of the channel eventually activated.**
- (E) Ultimate effect on action potential frequencies.**

end of question 5