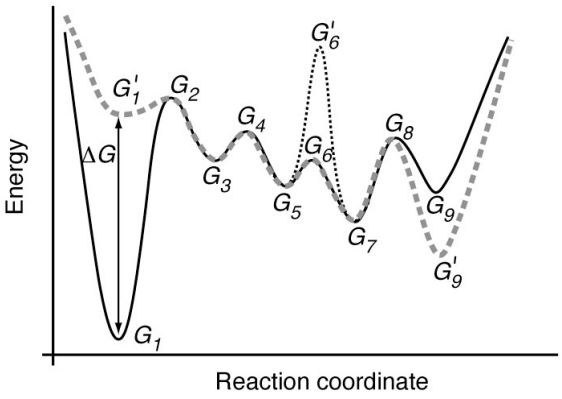
580.439/639 Final Exam 2010

3 hours, open book except for allowed cheat sheets. Do all problems

Problem 1



Consider the diagram at right which shows the energy landscape of a receptor molecule, like rhodopsin, in the absence of activating energy (solid black line) and after activation by absorbing a photon (dashed gray line). This is a simplified version of the steps in visual transduction (based on Deupi and Kobilka *Physiology* 25:293, 2010). The diagram shows the relative energy of various conformations of the receptor molecule. This is different from the assumption made in analyzing ion channels in which the reaction coordinate was the position of an ion within the channel molecule, whose conformation was assumed fixed. In this case, the reaction coordinate corresponds to various conformations of the molecule itself and there is no mobile ion involved. The molecule can move among the various states indicated on the energy diagram according to kinetics like those used to model ion movements. For simplicity, we ignore movements of charges within the molecule as it changes conformations.

When the molecule absorbs a photon, its energy diagram changes (assumed instantaneously) from the solid line to the dashed line, with a change in the energy level of the G_1 state to G_1' and the G_9 state to G_9'. The other energy levels do not change. (Ignore the dotted lines and the G_6' state for parts a) through c) below.)

Part a) Assuming that the system is in steady state, compute the fraction of receptor molecules in each of the various conformations (G_1, G_3, G_5, G_7, and G_9) of the unactivated molecule. Repeat this calculation for the activated molecule (for states G_1', G_3, G_5, G_7, and G_9'), again assuming a steady state. (The answer is long-winded, so for the repeat, just state how the equations change for the activated state without rewriting the whole thing.) It may be useful to define rate constants for transitions along the reaction coordinate, which will be needed later.

Part b) Based on the calculations of part a), explain qualitatively what happens to the conformation of the molecule when it absorbs a photon. The energy difference ΔG is large compared to RT.

Part c) What determines the maximum possible change in energy ΔG of the G_1 state (a question from freshman physics)?

Part d) Two levels of the G_6 peak are shown in the diagram. What would be the difference in the behavior of molecules with the two G_6 levels when activated by a photon? That is, what aspect

of the flux of molecules between the two steady-state conformation distributions in part a) would be affected by the level of G_6?

Part e) Write differential equations to model the flux of molecules through the energy barrier system immediately after absorbing a photon. Assume a fixed total number of molecules and assume that at time 0 (when the photon is absorbed), all the molecules are in the G_1' state. This will require defining appropriate rate constants (just define them, don't write out their values in terms of the energy levels). The differential equations will describe the time derivatives of the state variables x_1, x_3, x_5, x_7, and x_9, which are the fraction of molecules in each of the corresponding states. Writing this in the naïve and straightforward way gives a system like $d\vec{x}/dt = \mathbf{M}\vec{x}$, with a singular 5x5 matrix **M**. Rewrite the system in the same form with a 4x4 matrix that is not (obviously) singular.

Part f) (DO THIS LAST) In the barrier models for ion channels considered in class, the highest barrier in the system dominated the flux. That will be true for this system as well, for the energy diagram with the high G_6' barrier. Assume that states x_1, x_3, and x_5 are in equilibrium because their interstate barriers are low so their rate constants are (relatively) large. Assume also that x_7 and x_9 are at equilibrium. Then write a single differential equation for the conformational changes of the channel that would allow the shift of channel conformation from state x_1 to state x_9 to be computed. This equation will describe the flux over barrier G_6'. You may have to change the initial condition for this system to make it consistent with the other assumptions. Solve the differential equation for the fraction of molecule in the G_9 state.

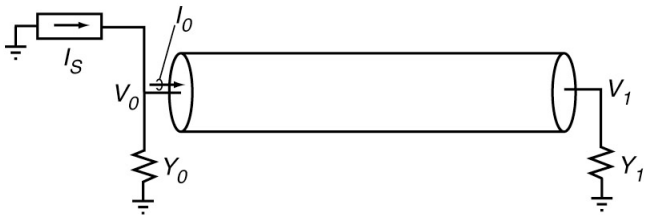
Problem 2

Consider the effect of activating a synapse on the input resistance of a neuron, measured at the soma. The input resistance R_{in} is defined as it is measured, as the ratio $\Delta V_S/I_S$, where ΔV_S is the change in somatic potential produced by injection of a (DC) current I_S into the soma. The membrane potential is defined to be 0 at the resting potential. The change in input resistance is the difference between $\Delta V_S/I_S$ with and without synaptic activation. In this problem assume the synapse is a fixed conductance that is off or on, i.e. a DC steady state.

Part a) Suppose the synapse is located on the soma of the cell. The soma membrane is linear, i.e. a fixed conductance R_S in parallel with a capacitance C_S and the synapse is a conductance g_{syn} in series with reversal potential E_{syn}. The cell also has dendrites with total input conductance G_{dend} attached to the soma. Compute the change in input resistance due to activation of the synapse measured as above. (This is easy, gives the expected result, and is here for practice.)

Part b) Now suppose that the synapse is located in the dendritic tree (at site i) at some distance from the soma. What is the change in conductance measured at the soma when the synapse is activated, computed as above. It will help to use the transfer impedance K_{is} and input impedances K_{SS} and K_{ii} in this problem. Again, assume DC steady state (q = 1). From what you know about transfer and input impedances, what does this result mean qualitatively about the ability to measure synaptic conductance changes from the soma for synapses at different distances along the dendrites?

Part c) When they interpreted the results of the calculation in part b), the original authors (Koch et al. *J. Neurosci.*



10:1728, 1990) used the fact that transfer impedances (K_{is}) are smaller than input impedances (K_{ii}). Show that this is so for the single cable in the figure at right above; that is, show that $K_{00} > K_{01}$, where $K_{00} = V_0/I_0$ and $K_{01} = V_1/I_S$. For simplicity, do the calculation in the DC steady state, so that Y_0, Y_1, K_{00}, and K_{01} are real numbers. Do not assume that $Y_1 = 0$!

Problem 3

This problem concerns calcium fluxes in the dendritic trees of neurons in thalamus. It is based on data in a recent paper (Crandall et al. *J. Neurosci*. 30:15419, 2010). To remind you, Figure 1 shows Hodgkin Huxley parameters for sodium (*Na*), L-type calcium (Ca_L) and T-type calcium (Ca_T) channels in cells of this type.

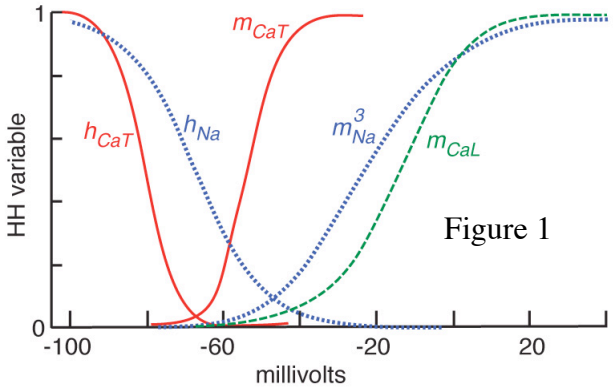


Figure 1

Part a) Figure 2A shows action potentials recorded in the cell body of a neuron. In Ai, the resting potential was -80 mV, in Aii and Aiii the resting potentials were -60 mV (adjusted by steady current injected in the soma). The action potentials were stimulated by brief currents injected into the soma, shown by the gray traces below the action potentials.

Explain the differences in the action potentials as the resting potential was changed (i.e. Ai versus Aii and Aiii). Refer, in your explanation, to the action potentials shown in B which are a control case (black, at top, produced in the same way as in Ai) and a case in which the same stimulus was provided in the presence of TTX (which blocks Na channels; red, below).

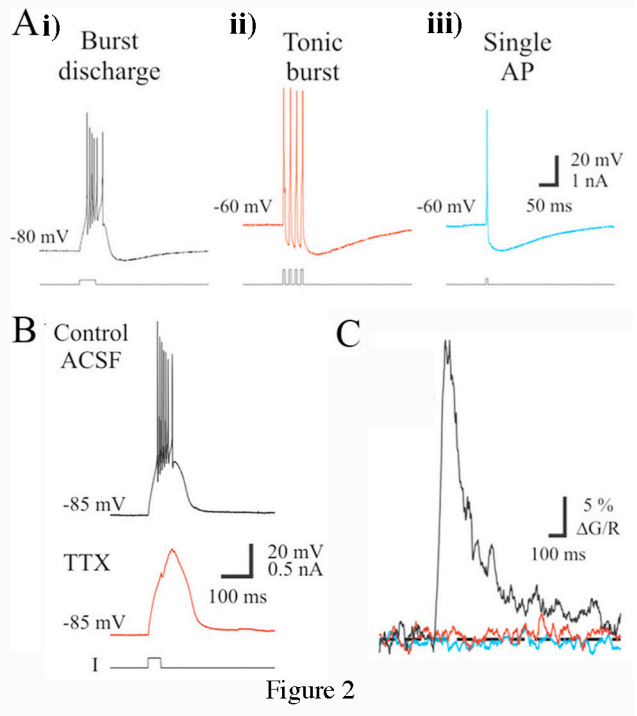


Figure 2

Part b) Figure 2C shows traces of the calcium concentration at a point in the dendritic tree of the same neuron. The traces are color coded to show their correspondence to the (simultaneously obtained) soma voltage traces in Fig. 2A. Notice the large increase in [Ca^{++}] during the burst discharge in Fig. Ai (black) and the lack of calcium signal during the tonic burst and single AP (red and blue signals). Similar behavior was observed throughout the dendritic tree. Explain why this might occur, i.e. why was there calcium entry in one case but not the other two. Assume that there are both L- and T-type calcium channels throughout the dendrites. Explain how calcium channels in the dendrites are activated by the spikes occurring in the cell body.

The calcium signals observed during the two action potentials in Fig 2B were nearly identical and similar to the one observed during the burst in Fig. Ai. Explain why. What do these results suggest about the relative importance of L- and T-type calcium channels in supporting calcium entry in these experiments?

Part c) The size of the calcium signal, i.e. the amplitude of the calcium transient in Fig. 2C, decreased as the recording point in the dendrites moved away from the cell body. One possibility to explain this is that the calcium signal should be stronger in smaller dendrites further from the cell body because of the changing surface to volume ratio. To think about this proposal, write a differential equation for the calcium concentration in a compartment consisting of a cylinder of radius a and length l. The compartment has calcium channels in its membrane that produce a calcium current I_{Ca} with units Amp/m^2 and an active transport system that removes calcium from the cytoplasm at rate $P[Ca]$ (P a constant) with units moles/s m^2. The differential equation should be of the form $d[Ca]/dt = F(I_{Ca},[Ca])$, where $[Ca]$ is the average calcium concentration in the dendritic cylinder. DON'T GET FANCY: MAKE A SIMPLE ONE-COMPARTMENT MODEL! MAKE THE UNITS CORRECT!

Using the differential equation, argue that smaller dendrites should indeed have larger calcium signals under certain assumptions that are clear from the terms in the differential equation. State those assumptions.

Part d) A careful examination of Fig. 2 shows that the calcium transients are very slow compared to the electrical signals. Explain how this could happen in your differential equation. In fact there are probably additional factors not accounted for in the differential equation, like buffering of the calcium by the dye or by intrinsic cell calcium buffers.

Part e) Figure 3 shows calcium signals produced by synaptic activation at glutamatergic synapses in the dendrites. For comparison, the black trace at right shows the results of electrical stimulation in the soma (as above) and the red curve at left shows the effect of sudden application of a pulse of glutamate to the dendrites. Note that the two modes of activation give similar results. The blue and green curves at left show calcium transients in the presence of two glutamate receptor antagonists. CPP is a pharmacological agent that blocks NMDA receptors and DNQX blocks AMPA receptors. Provide an explanation for the amplitudes of the blue and green curves in the left plot, especially for the slight difference between the blue and red curves and the large difference between the green and red curves.

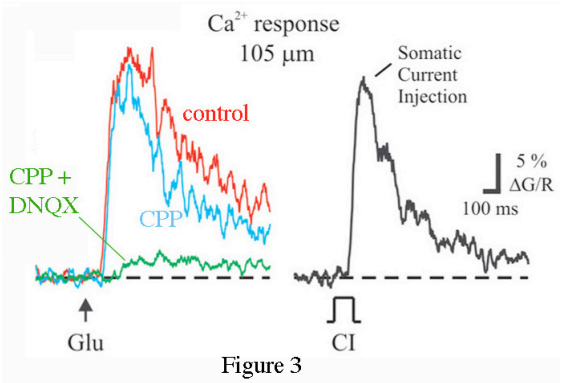


Figure 3