

## 580.439/639, Solutions to Final Exam 2010

### Problem 1

The figure at right shows definitions of the rate constants for use in this problem.

**Part a)** Steady state means the fraction of channel in each state is fixed in time. As argued in class, that means the net transitions across each energy peak are zero, so that

$$J_{i \rightarrow i+1} = k_i x_i - k_{i+1} x_{i+1} = 0,$$

where  $J_{i \rightarrow i+1}$  is the net flux from energy well  $i$  to well  $i+1$  and  $x_i$  is the fraction of the channel in the  $i^{\text{th}}$  well. Note that  $x_1 + x_3 + x_5 + x_7 + x_9 = 1$ . Writing a similar flux equation for each energy peak and applying the zero-flux condition gives the following ratios

$$\frac{x_1}{x_3} = \frac{k_2}{k_1} = \frac{\text{const } e^{-(G_2-G_3)/RT}}{\text{const } e^{-(G_2-G_1)/RT}} = e^{-(G_1-G_3)/RT} \gg 1,$$

and

$$\frac{x_3}{x_5} = \frac{k_4}{k_3} = e^{-(G_3-G_5)/RT} < 1, \quad \frac{x_5}{x_7} = \frac{k_6}{k_5} = e^{-(G_5-G_7)/RT} < 1, \quad \frac{x_7}{x_9} = \frac{k_8}{k_7} = e^{-(G_7-G_9)/RT} > 1,$$

where the relative sizes of the ratios follow from the energy differences in the diagram.

The fractions can be obtained by solving successively, using the fact that the  $x_i$ 's sum to 1:

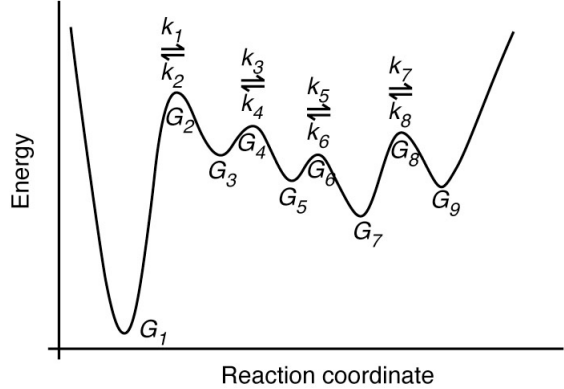
$$\begin{aligned} 1 &= x_1 + x_3 + x_5 + x_7 + x_9 \\ &= \left[ \frac{k_2}{k_1} \frac{k_4}{k_3} \frac{k_6}{k_5} \frac{k_8}{k_7} + \frac{k_4}{k_3} \frac{k_6}{k_5} \frac{k_8}{k_7} + \frac{k_6}{k_5} \frac{k_8}{k_7} + \frac{k_8}{k_7} + 1 \right] x_9 \\ x_9 &= 1 / \left[ \frac{k_2}{k_1} \frac{k_4}{k_3} \frac{k_6}{k_5} \frac{k_8}{k_7} + \frac{k_4}{k_3} \frac{k_6}{k_5} \frac{k_8}{k_7} + \frac{k_6}{k_5} \frac{k_8}{k_7} + \frac{k_8}{k_7} + 1 \right] = 1 / Z \end{aligned}$$

and

$$x_7 = \frac{k_8}{k_7} / Z, \quad x_5 = \frac{k_6}{k_5} \frac{k_8}{k_7} / Z, \quad x_3 = \frac{k_4}{k_3} \frac{k_6}{k_5} \frac{k_8}{k_7} / Z, \quad x_1 = \frac{k_2}{k_1} \frac{k_4}{k_3} \frac{k_6}{k_5} \frac{k_8}{k_7} / Z.$$

Note that these simplify when written in terms of energies

$$x_9 = 1 / Z, \quad x_7 = e^{-(G_7-G_9)/RT} / Z, \quad x_5 = e^{-(G_5-G_9)/RT} / Z, \quad x_3 = e^{-(G_3-G_9)/RT} / Z, \quad x_1 = e^{-(G_1-G_9)/RT} / Z \quad (*)$$



$$Z = 1 + e^{-(G_7-G_9)/RT} + e^{-(G_5-G_9)/RT} + e^{-(G_3-G_9)/RT} + e^{-(G_1-G_9)/RT} .$$

For the activated state, the solution is the same except that  $G_1$  is replaced by  $G'_1$  and  $G_9$  is replaced by  $G'_9$ . Note that the relative sizes change so that  $x_1/x_3 < 1$  and  $x_7/x_9 < 1$ .

The same result could be obtained by assuming that the conformations are in thermodynamic equilibrium so that

$$G_i + RT \ln x_i = G_j + RT \ln x_j \quad \text{so that} \quad x_i = x_j e^{-(G_i-G_j)/RT} ,$$

and so on.

**Part b)** From the relative sizes given above, most of the unactivated molecule is in state 1 which has a much lower energy level than the other states. After the molecule is activated, state 1 becomes the highest energy state and the molecule changes conformation so that most of it ends up in state 9'. The photon absorption thus drives a change of state of the molecule.

**Part c)** The energy in the photon ( $n_A h\nu$  per mole) must exceed the energy change of the molecule when it is activated. Only two states ( $G_1$  and  $G_9$ ) change energies, so the average energy change is  $\Delta G e^{-(G_1-G_9)/RT} / Z + (G'_9 - G_9) / Z$ . Because most of the unactivated molecule is in state 1, the first term dominates and a good approximation is  $\Delta G \leq n_A h\nu$ . It turns out that these energies are close.

**Part d)** The rate of change of conformation from state 1 to state 9' will be slower because the forward rate  $k'_5$  over the elevated barrier will be slower than the unactivated rate  $k_5$ . Because none of the energy wells change, no change in the ultimate steady state distribution of the molecule will occur (Eqn. (\*) doesn't depend on peak energies).

**Part e)** Using the rate constants above, the differential equations for the conformations can be written as

$$\begin{aligned} \frac{dx_1}{dt} &= -k'_1 x_1 + k_2 x_3 \\ \frac{dx_3}{dt} &= k'_1 x_1 - (k_2 + k_3) x_3 + k_4 x_5 \\ \frac{dx_5}{dt} &= k_3 x_3 - (k_4 + k_5) x_5 + k_6 x_7 \\ \frac{dx_7}{dt} &= k_5 x_5 - (k_6 + k_7) x_7 + k'_8 x_9 \\ \frac{dx_9}{dt} &= k_7 x_7 - k'_8 x_9 \end{aligned} .$$

The primes mark the rate constants that change with activation. Notice that these equations are singular because any one can be obtained by adding and subtracting the others; for example  $dx_9/dt$  is the sum of the other equations.. Using the fact that  $x_1 + x_3 + x_5 + x_7 + x_9 = 1$  allows the last equation to be eliminated and the  $x_9$  term in the  $d/dt$  equation to be written as follows:

$$\begin{aligned}\frac{dx_1}{dt} &= -k'_1 x_1 + k_2 x_3 \\ \frac{dx_3}{dt} &= k'_1 x_1 - (k_2 + k_3) x_3 + k_4 x_5 \\ \frac{dx_5}{dt} &= k_3 x_3 - (k_4 + k_5) x_5 + k_6 x_7 \\ \frac{dx_7}{dt} &= -k'_8 x_1 - k'_8 x_3 + (k_5 - k'_8) x_5 - (k_6 + k_7 + k'_8) x_7 + k'_8\end{aligned}$$

The problem didn't ask for exact initial conditions, but these are given in Eqn. (\*).

**Part f)** Using the method in part a), the equilibrium fractions  $x_1$  and  $x_3$  can be written in terms of  $x_5$  as

$$x_1 = x_5 e^{-(G_1 - G_5)/RT} \quad x_3 = x_5 e^{-(G_3 - G_5)/RT} .$$

Similarly  $x_7$  and  $x_9$  are related as

$$x_9 = x_7 e^{-(G_9 - G_7)/RT} . \quad (\dagger)$$

The following differential equation models flux across the  $G'_6$  barrier

$$\frac{dx_5}{dt} = -k''_5 x_5 + k''_6 x_7 ,$$

where the double primes mark the rate constants across the  $G'_6$  barrier. To reduce this to one variable, use the fact that the total amount of molecule is fixed and eliminate all variables except  $x_5$  and  $x_7$  using the equilibrium condition:

$$\begin{aligned}1 &= x_1 + x_3 + x_5 + x_7 + x_9 \\ &= x_5 (e^{-(G_1 - G_5)/RT} + e^{-(G_3 - G_5)/RT} + 1) + x_7 (1 + e^{-(G_9 - G_7)/RT}) ,\end{aligned}$$

so that

$$x_7 = \frac{1 - x_5 (e^{-(G_1 - G_5)/RT} + e^{-(G_3 - G_5)/RT} + 1)}{1 + e^{-(G_7 - G_9)/RT}} = A - Bx_5 , \quad (\dagger\dagger)$$

and the differential equation becomes

$$\frac{dx_5}{dt} = -(k''_5 + k''_6 B)x_5 + k''_6 A . \quad (**)$$

The initial conditions are those given in Eqn. (\*), because the equilibrium among states is not affected by the energy peak. Thus  $x_5(0) = e^{-(G_5 - G_0)/RT} / Z$ . The steady-state value of  $x_5$  is found by setting  $dx_5/dt=0$  in Eqn. (\*\*).

$$x_5(t \rightarrow \infty) = x_5(\infty) = \frac{k_6'' A}{k_5'' + k_6'' B}.$$

The solution to (\*\*) is thus

$$x_5(t) = [x_5(0) - x_5(\infty)] e^{-(k_5'' + k_6'' B)t} + x_5(\infty),$$

and  $x_9(t)$  can be obtained by combining Eqns. (†) and (††).

$$x_9 = e^{-(G_9 - G_7)/RT} x_7 = e^{-(G_9 - G_7)/RT} (A - Bx_5).$$

## Problem 2

**Part a)** The total input conductance of the soma is  $G_T = G_{dend} + 1/R_S$ . With no synaptic activation, the voltage change in response to current  $I_S$  is  $\Delta V_S = I_S/G_T$ . Thus the input resistance with no synapse is  $\Delta V_S/I_S = 1/G_T$  as expected.

When the synapse is activated, Kirchoff's current law gives

$$I_S = G_T V_S + g_{syn} (V_S - E_{syn}).$$

Using this equation to compute the membrane potential with and without injected current but with the synapse on gives

$$I_S = 0: V_S = \frac{g_{syn} E_{syn}}{G_T + g_{syn}} \quad \text{and} \quad I_S \neq 0: V_S = \frac{I_S + g_{syn} E_{syn}}{G_T + g_{syn}},$$

so the input resistance is

$$\frac{\Delta V_S}{I_S} = \frac{\frac{I_S + g_{syn} E_{syn}}{G_T + g_{syn}} - \frac{g_{syn} E_{syn}}{G_T + g_{syn}}}{I_S} = \frac{1}{G_T + g_{syn}}.$$

This is the parallel combination of the synaptic, somatic, and dendritic resistances, as expected from circuit theory. The change of input resistance is

$$R_{in}(\text{with synapse}) - R_{in}(\text{w/o synapse}) = \frac{1}{G_T + g_{syn}} - \frac{1}{G_T} = \frac{-g_{syn}}{G_T (G_T + g_{syn})}.$$

This makes more sense as a change in conductance, just  $g_{syn}$ .

**Part b)** The potential produced at the soma by activating the synapse on the dendrites is  $K_{is} I_i$  where  $I_i$  is the synaptic current into the dendrite. At the same time, the current injected in the soma produces a potential  $K_{is} I_S$  at the synapse (recall that  $K_{is} = K_{Si}$ )

The current  $I_i$  injected at the synapse is computed in the usual way, except that the effect of  $I_S$  at the synaptic site must be included:

$$\begin{aligned}
I_i &= g_{syn}(E_{syn} - V_i) \quad \text{and} \quad V_i = I_i K_{ii} + K_{iS} I_S \\
&= g_{syn}(E_{syn} - I_i K_{ii} - K_{iS} I_S) \\
&= \frac{g_{syn} E_{syn} - g_{syn} K_{iS} I_S}{1 + g_{syn} K_{ii}}
\end{aligned}$$

Now the potential in the soma can be written as

$$\begin{aligned}
V_S &= R_T I_S + K_{iS} I_i \\
&= R_T I_S + \frac{g_{syn} K_{iS} E_{syn}}{1 + g_{syn} K_{ii}} - \frac{g_{syn} K_{iS}^2 I_S}{1 + g_{syn} K_{ii}}
\end{aligned}$$

The first equation follows from the fact that the system is linear so that superposition of responses from different inputs holds. Now the change in  $V_S$  with application of current in the absence of the synapse ( $g_{syn} = 0$ ) is  $R_T I_S$ , so the input resistance in the absence of the synapse is  $R_T$ , as in part a). In the presence of the synapse:

$$\begin{aligned}
I_S = 0: \quad V_S &= \frac{g_{syn} K_{iS} E_{syn}}{1 + g_{syn} K_{ii}} \quad \text{and} \quad I_S \neq 0: \quad V_S = R_T I_S + \frac{g_{syn} K_{iS} E_{syn}}{1 + g_{syn} K_{ii}} - \frac{g_{syn} K_{iS}^2 I_S}{1 + g_{syn} K_{ii}} \\
\frac{\Delta V_S}{I_S} &= R_{in} = R_T - \frac{g_{syn} K_{iS}^2}{1 + g_{syn} K_{ii}} \quad \text{and} \quad \Delta R = - \frac{g_{syn} K_{iS}^2}{1 + g_{syn} K_{ii}}
\end{aligned}$$

The conductance change can be computed as  $1/R_{in} - 1/R_T$  but it is not particularly instructive. Dendritic sites further from the soma have smaller  $K_{iS}$  and larger  $K_{ii}$ , so will show smaller resistance (and conductance) changes with synaptic activation. This means that they will be less “visible” from the soma.

The argument above is that given by Koch et al. in their paper on this subject. However, one could argue that  $K_{iS} \neq K_{Si}$  in this case because the synaptic conductance changes the termination impedance at the synapse for  $K_{Si}$  compared to  $K_{iS}$ . If you follow the arguments through, this changes the  $K_{iS}^2$  term in the final answer to  $K_{iS} K_{Si}$ .

**Part c)** This is mainly a problem in algebra. The input impedance defined in the problem statement is the inverse of the admittance  $Y_0$  plus the input admittance of the cable.

$$\begin{aligned}
K_{00} &= \frac{1}{Y_0 + G_{\infty}q \frac{Y_1/G_{\infty}q + \tanh qL}{1 + Y_1/G_{\infty}q \tanh qL}} \\
&= \frac{1 + Y_1/G_{\infty}q \tanh qL}{Y_0(1 + Y_1/G_{\infty}q \tanh qL) + G_{\infty}q(Y_1/G_{\infty}q + \tanh qL)} \\
&= \frac{\cosh qL + Y_1/G_{\infty}q \sinh qL}{(Y_0 + Y_1)\cosh qL + (Y_0 Y_1/G_{\infty}q + G_{\infty}q)\sinh qL}
\end{aligned}$$

In the last equation, use has been made of the fact that  $\tanh x = \sinh x / \cosh x$ . For the transfer impedance

$$K_{01} = \frac{1}{(Y_0 + Y_1)\cosh qL + (Y_0 Y_1/G_{\infty}q + G_{\infty}q)\sinh qL}$$

The denominators of these two equations are the same. Considering the numerator of the expression for  $K_{00}$ , for real arguments,  $\cosh x > 1$  and the second term is positive, so it must be that  $K_{00} > K_{01}$ . Because both  $\cosh$  and  $\sinh$  are monotone increasing for positive arguments,  $K_{00}$  is increasingly larger than  $K_{01}$  as the cable gets longer, all other things being the same.

### Problem 3

**Part a)** At -65 mV, the T-type calcium channels are deactivated ( $h_{CaT} \sim 0$ ) and so the membrane produces only sodium spikes in response to individual current pulses. At -80 mV, the T-type calcium channels are active and the membrane produces a calcium action potential (the pedestal) with sodium spikes bursting in response to the depolarization. The data in B support this hypothesis: blocking sodium spikes leaves the pedestal, the calcium spike.

**Part b)** The calcium transients are associated with the long-duration calcium spikes, whether or not there are sodium spikes present. The somatic action potentials in Fig. 2A invade the dendrites, producing significant depolarization there. The T-type calcium channels admit significant calcium, producing the increases in calcium concentration. L-type calcium channels open during sodium action potentials (inferred from the  $m_{CaL}$  function shown in Fig. 1, but not shown in the data), but apparently do not admit significant calcium. Thus there is no calcium signal for cases ii) and iii) in Fig. 2A.

**Part c)** The differential equation is below:

$$\text{Vol} \frac{dCa}{dt} = -\frac{AI_{Ca}}{2F} - PACa$$

where  $\text{Vol} = \pi a^2 l =$  volume of the compartment ( $\text{m}^3$ ),  $Ca$  is calcium concentration in moles/ $\text{m}^3$ ,  $A = 2\pi a l =$  surface area of the compartment,  $I_{Ca}$  is the outward calcium current in coul/ $\text{sm}^2$ ,  $F$  is the number of coulombs in a mole of ions, and  $P$  is the pumping rate per area of membrane in m/s. Each term has units moles/s.

Rewriting the equation by dividing through by volume gives

$$\frac{dCa}{dt} = -\frac{A}{Vol} \frac{I_{Ca}}{2F} - \frac{PA}{Vol} Ca = -\frac{1}{aF} I_{Ca} - \frac{P}{a} Ca \quad (***)$$

The first term  $-I_{Ca}/aF$  is the driving force raising  $Ca$  in the cytoplasm when the membrane is depolarized. The second term produces the decay of calcium in the absence of a calcium current.

There are three assumption necessary to interpret Eqn. (\*\*\*): 1) the membrane is identical everywhere in the dendrites, meaning the same calcium current density  $I_{Ca}$  and same pumping rate  $P$ ; and 2) the membrane depolarization due to the backpropagating action potential is the same everywhere producing equal drive on the voltage-gated calcium channels. With these assumptions, Eqn. (\*\*\*) describes the change in calcium concentration (which is what is sensed in the experiments) produced by a pulse of calcium current. The transients produced by Eqn. (\*\*\*) will look like those in the data as long as the calcium current is a short impulse to produce the leading edge. The trailing decay should be exponential with time constant  $a/P$ . The third assumption is that the calcium current injected by  $I_{Ca}$  should be independent of the calcium concentration. Increased calcium concentration decreases  $I_{Ca}$  by two mechanisms: decreasing the flow of  $Ca$  through the membrane and closing  $Ca$  channels by inactivating them. These should be small effects, since the  $Ca$  concentration is very small even at the peak of the transient and T-type channels to not show  $Ca$  inactivation.

This discussion ignores calcium buffering.

With these assumptions, smaller dendrites will have larger calcium concentration transients because of the  $1/a$  multiplier of the calcium current and will also have faster recovery time constants.

**Part d)** The rising and falling edges of the transients are determined by the first and second terms in the differential equation, respectively. The fall has time constant  $a/P$ , which presumably could be slow. As stated in the problem, it is likely that buffering slows the fall of calcium concentration as well.

**Part e)** In this case the synapse depolarizes the cell and induces an action potential which produces the calcium transient as above (red plot). However, NMDA channels admit significant calcium, so the effect of CPP is probably to reduce the NMDA calcium flux (blue plot). Usually most of the synaptic activation in cells is through AMPA channels, not blocked by CPP. DNQX blocks AMPA channels so with both blockers present (green line), the synapse doesn't activate and so the cell is not depolarized.