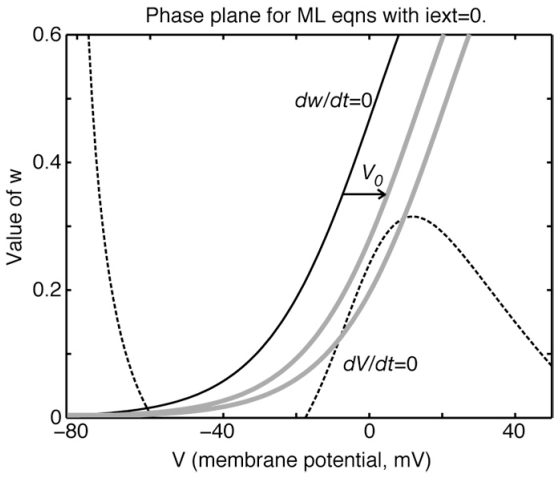
580.439/639 Midterm Exam Solutions, 2012

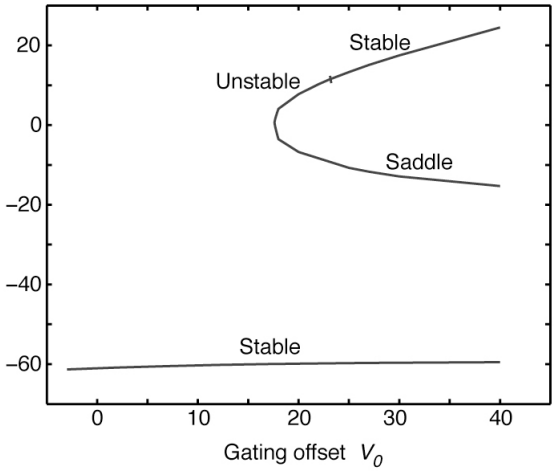
Problem 1

Part a) i) The $\dot{w} = 0$ nullcline is just $w_\infty(V)$, so it will shift to the right by V_0. The $\dot{V} = 0$ nullcline is unaffected by the w_∞ function and is not changed. Thus the nullclines will look like the gray lines at right for two values of V_0.



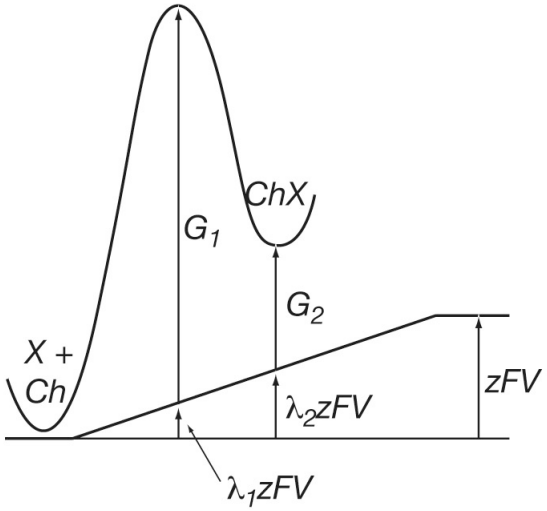
ii) The original equilibrium point will move to higher membrane potentials following the nullcline. At a sufficiently high V_0, two additional equilibrium points will appear, as shown for the right-hand nullcline in the figure above. However the equilibrium point near -60 mV will remain for all V_0.

iii) The bifurcation diagram is shown at right. The stability of the various points are shown, although these are not part of the required answer. Note that because of the shape of the nullclines near -60 mV, the stable equilibrium point at low voltages never meets the saddle node.



Part b) The sketch should show the selectivity filter on the outside of the membrane with the four rings of negative dipoles (from carbonyl oxygens) that define the four locations for ions in the selectivity filter. This precise organization is not seen in sodium channels, where the selectivity filter is a larger opening without the dipoles defining locations within it; this filter allows a sodium ion with one hydration molecule to pass through. The sketch should also show the central cavity of the channel and point out that the gate is formed by a change in the size of the opening to the intracellular space produced by moving S6 domains through the coupling of the S5 domains to the charged S4 domains that move in the membrane. The sketch could be something like Fig. 5 in the notes on ion channels and permeation.

Part c) The barrier model is drawn at right. The blocker moves from solution to the channel by moving between solution *S* and channel *Ch* in the diagram (these could also be labeled *X+Ch* and *ChX* or some such). The parameters requested are shown on the plot.



kinetics – the rate constants are determined by the barrier height G_1 with respect to the reference state *S* and the channel-bound state *Ch* with energy G_2.

***V* dependence –** the membrane potential *V* affects the binding to the extent that the blocker moves through a portion λ_2 of the membrane potential in binding to the channel. The contribution of membrane potential to the barriers is indicated by the fractions λ_1 and λ_2 of membrane potential at the peak of the barrier and bound the channel. The parameter *z* is the charge on the blocker *X*.

½ block concentration – this is determined by the parameters defined above.

The differential equation for the binding reaction is

$$\frac{dChX}{dt} = k_1 X\, Ch - k_{-1} ChX = k_1 Q\, X - (k_1 X + k_{-1}) ChX \quad ,$$

where $Q = Ch + ChX$ is the total amount of channel present. In the steady state, $dChX/dt = 0$ so

$$ChX = \frac{k_1 Q\, X}{(k_1 X + k_{-1})} \quad .$$

The criterion for half-saturation is that $ChX = Q/2$, or

$$\frac{k_1 Q\, X_{1/2}}{(k_1 X_{1/2} + k_{-1})} = \frac{Q}{2} \quad \text{or} \quad X_{1/2} = \frac{k_{-1}}{k_1} \quad .$$

The rate constants are given by

$$k_1 = (const) e^{-(G_1 + \lambda_1 zFV)/RT} \quad \text{and}$$
$$k_{-1} = (const) e^{-(G_1 + \lambda_1 zFV - G_2 - \lambda_2 zFV)/RT} = (const) e^{-(G_1 - G_2 - (\lambda_2 - \lambda_1) zFV)/RT} \quad ,$$

so (recalling that the constants in the rate constants above are different)

$$X_{1/2} = \frac{k_{-1}}{k\ 1} = \frac{(const) e^{-(G_1 - G_2 - (\lambda_2 - \lambda_1) zFV)/RT}}{(const) e^{-(G_1 + \lambda_1 zFV)/RT}} = (const) e^{(G_2 + \lambda_2 zFV)/RT} \quad .$$

Problem 2

Part a) Writing the free energies of the constituents before and after one cycle of the reaction and setting them equal (the condition for equilibrium) give

$$3RT\ln Na_i + 3zFV + 2RT\ln K_o + RT\ln ATP = 3RT\ln Na_o + 2RT\ln K_i + 2zFV + RT\ln ADP + RT\ln P \quad .$$

The electrical potential outside the cell is 0 and the potential inside the cell is V, the transmembrane potential. Note that the stoichiometry of the process has been kept. Solving for V with z=1 gives

$$V = \frac{RT}{F}\ln\frac{Na_o^3 K_i^2\, ADP\, P}{Na_i^3 K_o^2\, ATP} \quad .$$

Part b) Converting from flux J to current I_{ATPase} gives

$$I_{ATPase} = zFJ \quad ,$$

where z is the number of charges moved for each ATP consumed. From the pump reaction, this number is 1 in the outward direction, the direction in which I_{ATPase} is positive. J is by definition positive.

Part c) Steady state requires that there is no net transfer of K ions, Na ions, or charge through the membrane. For the ions (using the definitions in the problem statement),

$$I_{Kv} + f_K I_L = 2I_{ATPase} = 2FJ \quad \text{and} \quad I_{Nav} + (1 - f_K)I_L = -3I_{ATPase} = -3FJ \quad ,$$

where f_K is the fraction of the leakage current that is potassium and I_L=$G_L(V$-$E_L)$. If there is no ionic transport through the membrane, then the zero-charge-transfer condition is met without further equations. However, <u>because the time scale of charge-transfer steady state is much faster than ionic steady state,</u> the system can be in charge-transfer steady state and not be in ionic steady state. For charge-transfer steady state

$$I_{Kv} + I_{Nav} + I_L + I_{ATPase} = 0$$

With a current-voltage model for I_{Kv} and I_{Nav}, these equations are sufficient to solve for the membrane potential, but that was not necessary here.

If the ATPase is at equilibrium then I_{ATPase} = 0. This is possible in steady state only if the passive ionic current flows (I_{Kv} + $f_K I_L$ and similar for sodium) are zero. However these are not possible unless the voltage-gated ionic channel currents are zero and the membrane potential equals E_L (because the equilibrium potentials for sodium and potassium are different). However, even if that can be arranged, there is no guarantee that $I_{ATPase}(E_L)$ = 0. So the answer is “no” except in some unlikely special condition.

Part d) This is a condition in which the membrane potential adjusts to make the net charge transfer zero while the ionic concentrations are not in steady-state. Because the ATPase produces an

outward current, removing the ATPase is like an inward current and the membrane potential will depolarize.

Part e) This is a charge-transfer steady state, as required for the GHK membrane potential equation, so that one possibility for the membrane potential is

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o}{P_K K_i + P_{Na} Na_i} \quad , \qquad (*)$$

where the permeabilities are the sums of the values for the voltage-gated channels and the leakage channels. This assumes that flux of potassium and sodium meet the conditions for independence and the fluxes through the voltage-gated and leakage channels follow similar equations. One could also write the equation in electrical circuit form

$$V = \frac{(G_L f_K + G_{Kv}) E_K + (G_L (1 - f_K) + G_{Nav}) E_{Na}}{G_L f_K + G_{Kv} + G_L (1 - f_K) + G_{Nav}}$$

which sweeps all the details of the transport under the rug.

Part f) With no active transport, there will be a steady flux of potassium out of the cell, down its electrochemical gradient, through both K_V and leakage channels and a similar flux of sodium into the cell. Over a long enough time period, the concentration gradients will disappear (equal concentrations on both sides of the membrane) and the potential will be 0. In between, the potential will decline steadily to 0.

Part g) 1) The hyperpolarization observed during the spiking and after as a hyperpolarization of the resting potential reflects an increase rate of pumping of the Na-K ATPase. Action potentials by there nature take the cell out of steady state. During an action potential, potassium flows out of the cell and sodium in, slightly changing the intracellular concentrations. The pump rate of the ATP-ase increases because it is working against a lower electrochemical gradient (a smaller potassium and sodium concentration gradient). Because I_{ATPase} is an outward current (part b) above), an increase in $I_{ATPazse}$ hyperpolarizes the cell. After the spiking stops, the resting potential is hyperpolarized because of pump has not restored the resting steady-state concentrations of potassium and sodium inside the cell.

2) The post-spiking steady state is a charge-transfer steady state, but not an ionic concentration steady state. The hyperpolarization declines as the concentration gradients are restored and the pump rate decreases.

3) With a blocking concentration of ouabain, the ATPase current goes to zero. As explained in part d) above, removal of this outward current depolarizes the membrane potential, as observed in the gray traces. The changes discussed in 1) during the spiking are not seen because there is no ATPase current.

4) The small depolarization of the minimum potential during spiking seen in the gray traces is caused by the decrease of K_i and the increase of Na_i. Such a change is predicted by Eq. (*) above, for example.