

Multigate model

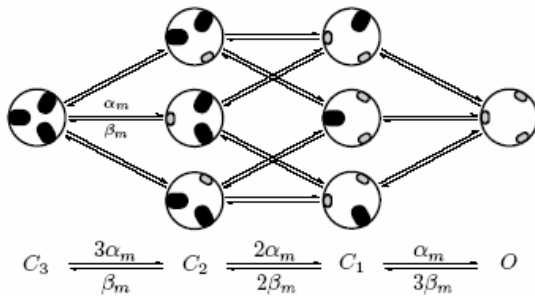
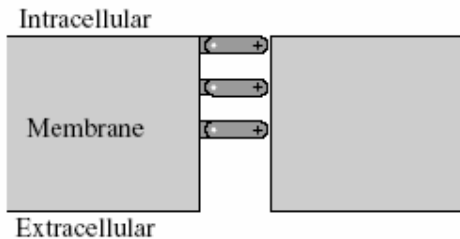
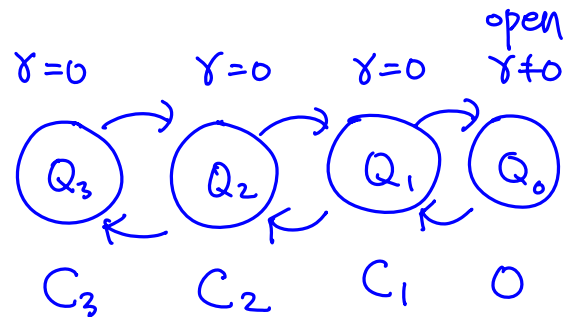
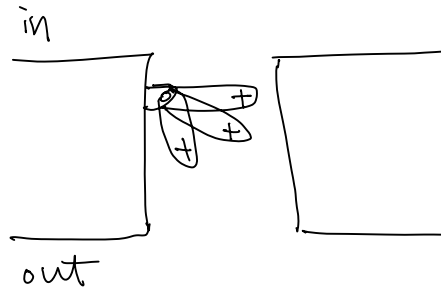


Figure 6.54

Multistate model



Micro

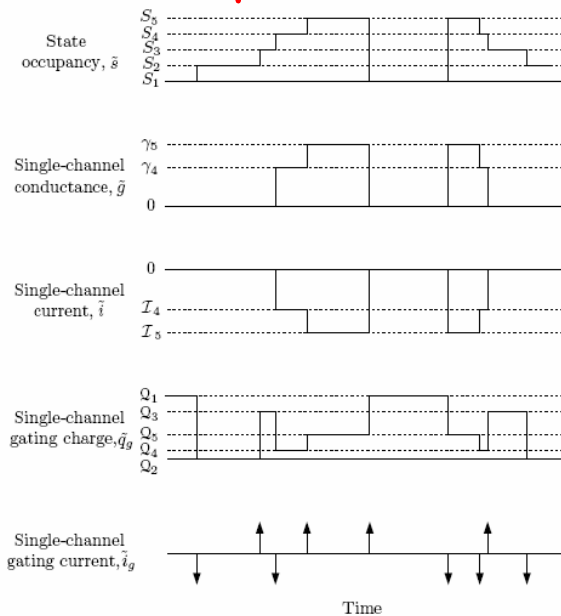


Figure 6.61

Macro

$\chi_1(t), \chi_2(t) \dots$

Average conductance = $\gamma_4 \chi_4(t) + \gamma_5 \chi_5(t)$

Average current = $I_4 \chi_4(t) + I_5 \chi_5(t)$
 current = $(\gamma_4 \chi_4 + \gamma_5 \chi_5) (V_m - V_{Na})$

Av. gating charge = $Q_1 \chi_1 + Q_2 \chi_2 + \dots$

Average gating current = $Q_1 \frac{d\chi_1}{dt} + Q_2 \frac{d\chi_2}{dt} + \dots$

Activation gate: Open state has higher Q
 Inactivation gate: Open state has lower Q

Problem 1. Three three-state voltage-gated channels (channels a, b, and c) have the kinetic diagram and state occupancy probabilities shown in Figure 5. These channels have the same voltage dependent rate constants and the same equilibrium potential which is +40 mV. For the membrane

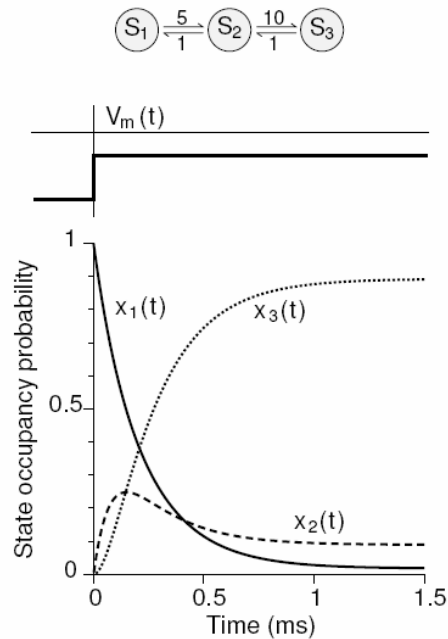
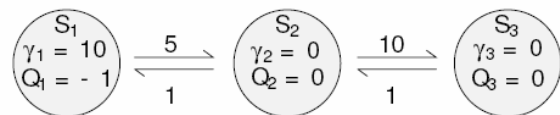


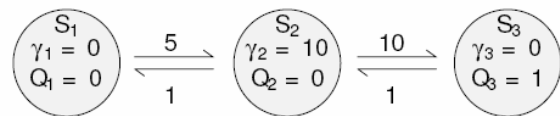
Figure 5: State diagram and occupancy probabilities for a three-state channel. The state occupancy probabilities for states S_1 , S_2 , and S_3 are $x_1(t)$, $x_2(t)$, and $x_3(t)$, respectively.

potential shown, the channels are in state 1 with probability 1 for $t < 0$ and have the indicated rate constants for $t > 0$. The channels differ only in their state conductances and state gating charges as shown in Figure 6. Denote the expected values of the single-channel random variables as follows:

Channel a



Channel b



Channel c

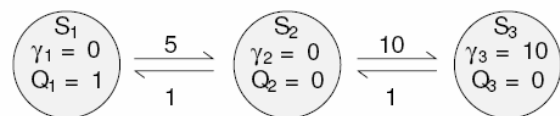


Figure 6: State diagrams of three three-state channel models. The models differ in state conductances and state gating charge but not in rate constants.

the conductance as $g_a(t)$, $g_b(t)$, and $g_c(t)$; the ionic currents as $i_a(t)$, $i_b(t)$, and $i_c(t)$; the gating charges as $q_a(t)$, $q_b(t)$, and $q_c(t)$; the gating currents as $i_{ga}(t)$, $i_{gb}(t)$, and $i_{gc}(t)$.

- Which of the waveforms shown in Figure 7 best represents $g_b(t)$? Explain.
- Which of the waveforms shown in Figure 7 best represents $g_c(t)$? Explain.
- Which of the waveforms shown in Figure 7 best represents $i_{ga}(t)$? Explain.
- Which of the waveforms shown in Figure 7 best represents $i_{gc}(t)$? Explain.
- Which of these channel models exhibits activation followed by inactivation of the ionic current? Explain.
- Which of these channel models exhibits an ionic current that does not inactivate? Explain.
- Which of these channel models represents a channel that closes on depolarization? Explain.

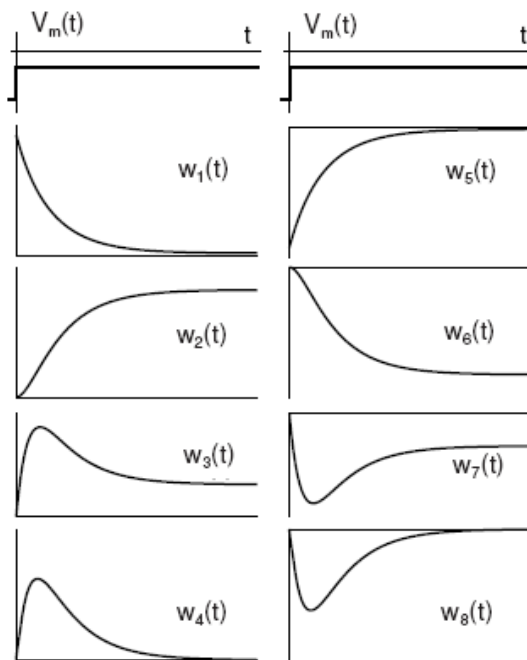
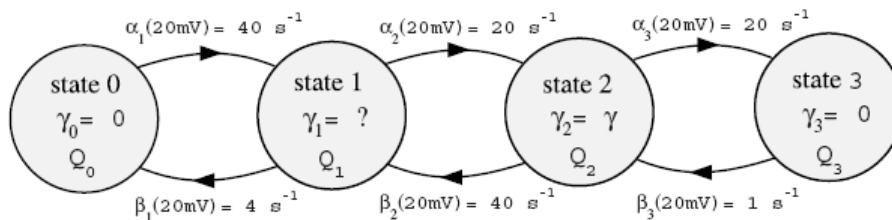


Figure 7: Waveforms of responses. The horizontal axis corresponds to $w(t) = 0$, and the vertical axis to $t = 0$.

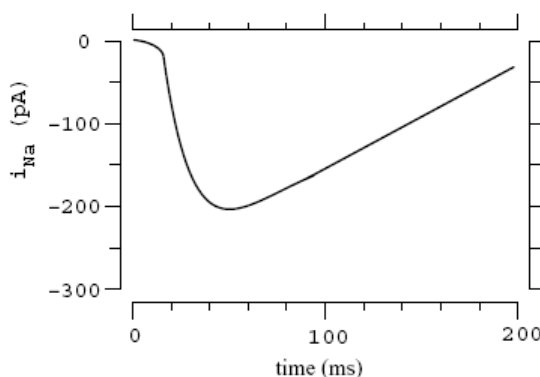
Problem 1.

- a. Only S_2 in channel b is conducting. Therefore, $g_b(t) = 10x_2(t)$ which looks like $w_3(t)$.
- b. Only S_3 in channel c is conducting. Therefore, $g_c(t) = 10x_3(t)$ which looks like $w_2(t)$.
- c. Only S_1 in channel a has a non-zero gating charge. Therefore, $q_a(t) = -x_1(t)$ and $i_{ga} = -dx_1(t)/dt$ which looks like $w_1(t)$.
- d. Only S_1 in channel c has a non-zero gating charge. Therefore, $q_c(t) = x_1(t)$ and $i_{gc} = dx_1(t)/dt$ which looks like $w_5(t)$.
- e. Channel b shows activation followed by inactivation. As indicated in part a, the conductance increases as the channel state progresses from S_1 to S_2 and decreases as the channel state progresses from S_2 to S_3 .
- f. Channel c exhibits no inactivation. As shown in part b, the channel shows an S-shaped activation and no inactivation.
- g. Channel a is open for $t < 0$ and then the channel state progresses to S_2 and to S_3 which are both non-conducting states. Thus, channel a closes on depolarization.

Problem 2. You are studying a membrane sodium channel protein of vertebrate neurons. This channel protein has only two conductance values (zero and γ) from the single ion channel experiment. According to X-ray crystallography results, this particular protein has four stable conformations as shown below, two of which (state 0 and 3) are known to be non-conducting states and state 2 is known to be a conducting state. Gating charges for each state are increasing in the order of state number ($Q_0 < Q_1 < Q_2 < Q_3$). In other words, higher number states are energetically more preferred in a depolarizing condition. Rate constants shown are the values for $V_m = 20mV$.



On the node of myelinated fiber where this protein is found abundantly, sodium current is measured in a voltage clamp experiment (using a gap technique) by increasing the membrane potential abruptly from -60 to $20mV$ at $t = 0$. At the beginning of the experiment, almost all the channels are known to be in the state 0, from an independent measurement. The resulting current profile is shown below.



- For $V_m=20mV$, calculate the steady state occupancy probabilities (x values) for each states.
- Is state 1 conducting or non-conducting? Explain!
- Now a certain enzyme called Galenase is added to the bath, which is known to react with the channel protein so that it does not inactivate. How would you modify the above 4 state model to reflect this modification? Draw the state diagram below, along with the rate constants.
- Your company is studying a drug molecule called EugenoxTM for treating a certain neural disease. This molecule is known to bind the sodium channel protein and decrease the energy barrier for transition between state 0 and 1. The net result would be that α_1 and β_1 becomes much larger (by several orders of magnitudes) than other rate constants. Now both

EugenoxTM and Galenase enzyme is added to the bath, and the original voltage clamp experiment was repeated. Sketch the resulting current profile from this new experiment on top of the original plot below. Describe the features of the new current profile that are different from the original plot.

Problem 2.

Part a. The relevant kinetic equations are the following. In the steady state, all the time derivatives become zero.

$$\frac{dx_0}{dt} = 0 = -\alpha_1 x_0 + \beta_1 x_1 \quad (1)$$

$$\frac{dx_1}{dt} = 0 = \alpha_1 x_0 - \beta_1 x_1 - \alpha_2 x_1 + \beta_2 x_2 \quad (2)$$

$$\frac{dx_2}{dt} = 0 = \alpha_2 x_1 - \beta_2 x_2 - \alpha_3 x_2 + \beta_3 x_3 \quad (3)$$

$$\frac{dx_3}{dt} = 0 = \alpha_3 x_2 - \beta_3 x_3 \quad (4)$$

$$x_0 + x_1 + x_2 + x_3 = 1 \quad (5)$$

Using the equation 4 and 7, one can get $x_0 = x_1/10$ and $x_3 = 20x_2$. Using these for equations 8 and 5 (or 6), one can get,

$$\frac{11}{10}x_1 + 21x_2 = 1 \quad -20x_1 + 40x_2 = 0 \quad (6)$$

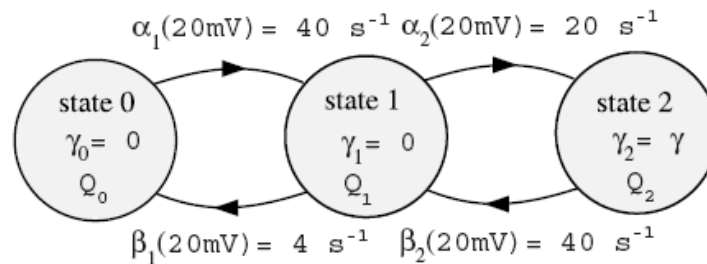
Solving these, one can get $x_0(\infty) = 1/116$, $x_1(\infty) = 10/116$, $x_2(\infty) = 5/116$, and $x_3(\infty) = 100/116$.

Part b. The initial current profile shows nonlinear, delayed offset. This cannot result from state 1 being a conducting state, because then the initial current profile should exhibit an exponential-like behavior. Therefore $\gamma_1 = 0$.

Part c. Enzyme Galenase gets rid of inactivation from sodium channels. In the original 4-state model, the state 3 is most likely to be the inactivated state of the sodium channel, because of the following reason.

- 1) The steady state partitioning between state 2 and 3 ($\alpha_3 x_2 = \beta_3 x_3$) heavily favors the state 3. In other words, any channel protein that are in the state 2 will eventually move onto the state 3.
- 2) The most likely trajectory of a ion channel protein would be starting from state 0 to 1, to 2 (which is conducting state), and then eventually state 3.

Therefore, when the inactivation is removed from sodium channels, it is equivalent to removing the state 3 from the possible state for channel proteins. The resulting state diagrams are shown below.



Part d. When the transition between the state 0 and 1 becomes faster (due to larger α_1 and β_1), the transition between the two state will be quickly reach the steady state (equilibrium) between them. (Remember carrier mediated transport, where binding between the glucose and carrier was assumed to be faster than transport processes.) Therefore, very soon after the beginning of the experiment, state 1 will be populated based on the rule $\alpha_1 x_0 = \beta_1 x_1$. The result is that there will be many channel proteins in the state 1, from the very beginning of the experiment. Therefore, the transition from non-conducting to conducting state will now follow the first order kinetics (between state 1 and 2), which will be an exponential-like increase in the conductances. Also, the addition of enzyme Galenase gets rid of inactivation, so the final sodium current will saturate to a certain nonzero negative value.

