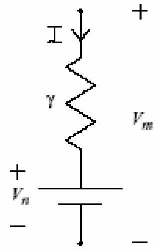
Passive electrodiffusive model of permeation

$\mathcal{I}$: single channel current

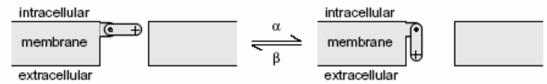


Open channel conductance: γ

V_n: Equilibrium Potential

(Similar to Nernst Potential)

First-order reversible reaction



Assume $\mathcal{N}$ channels per unit area, of which $n(t)$ are open.

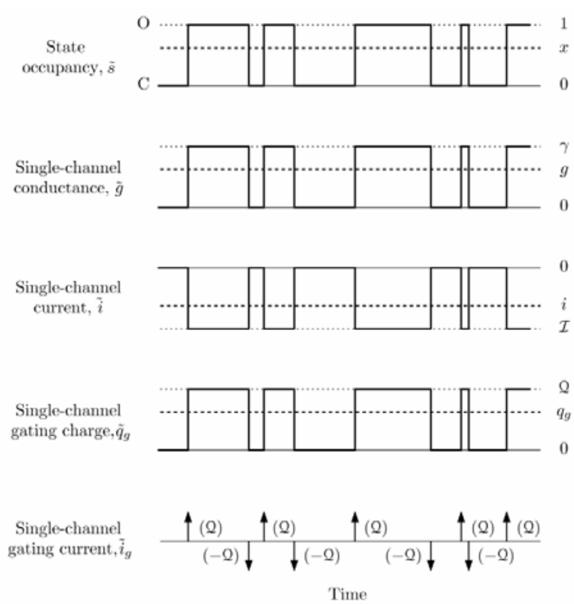
$$\frac{dn(t)}{dt} = \alpha(\mathcal{N} - n(t)) - \beta n(t)$$

$$n(t) = n_\infty + (n(0) - n_\infty)\, e^{-t/\tau_x}\,; \quad n_\infty = \frac{\alpha}{\alpha+\beta}\,\mathcal{N}\,, \quad \tau_x = \frac{1}{\alpha+\beta}$$

Assume $\mathcal{N}$ is large.

$$x(t) = \text{probability gate is open} \approx \frac{n(t)}{\mathcal{N}}$$

$$x(t) = x_\infty + (x(0) - x_\infty)\, e^{-t/\tau_x}\,; \quad x_\infty = \frac{\alpha}{\alpha+\beta}\,, \quad \tau_x = \frac{1}{\alpha+\beta}$$



$$x = E_x[\tilde{s}]$$

$$g = E_x[\tilde{g}] = \gamma x$$

$$\tilde{g} \rightarrow \gamma \tilde{s}$$

$$i = E_x[\tilde{i}] = E_x[\tilde{g}(V_m - V_n)] = \gamma x (V_m - V_n)$$

$$\tilde{q}_g = Q\tilde{s} \qquad q_g = E_x[Q\tilde{s}] = Qx$$

$$\tilde{i}_g = \frac{d}{dt}(\tilde{q}_g) \qquad i_g = Q\frac{dx}{dt}$$

m, h, n = probabilities of gate opening

- **Follows first order kinetics**
- **Activation gate**
- **Inactivation gate**

Problem 1. A single channel contains one two-state activation gate whose kinetic diagram is

$$C \underset{\beta(V_m)}{\overset{\alpha(V_m)}{\rightleftharpoons}} O,$$

where C is the closed state and O is the open state. $\alpha(V_m)$ and $\beta(V_m)$ are the voltage-dependent forward and reverse rate constants, respectively. The channel is permeable to sodium ions only. The current through the open channel is

$$\mathcal{I} = \gamma(V_m - V_{Na}),$$

where V_{Na} is the Nernst equilibrium potential for sodium.

Figure 1 shows single channel ionic current variables in response to a voltage step. Row 1 shows the membrane potential. In the remaining rows, the right panels show single-channel ionic current random variables and the left panels show average single-channel currents. Row 2 illustrates results for a default set of parameters. Rows A-E show results when one or two parameter values are changed. Determine which of rows A-E corresponds to each of the following changes and give a brief reason for your choice.

a) The single-open-channel conductance γ was increased.

b) The final value of the membrane potential V_m^f was increased.

c) The extracellular concentration of sodium was decreased.

d) Both $\alpha(V_m)$ and $\beta(V_m)$ were decreased without changing the ratio $\alpha(V_m)/\beta(V_m)$.

e) Only $\alpha(V_m)$ was decreased.

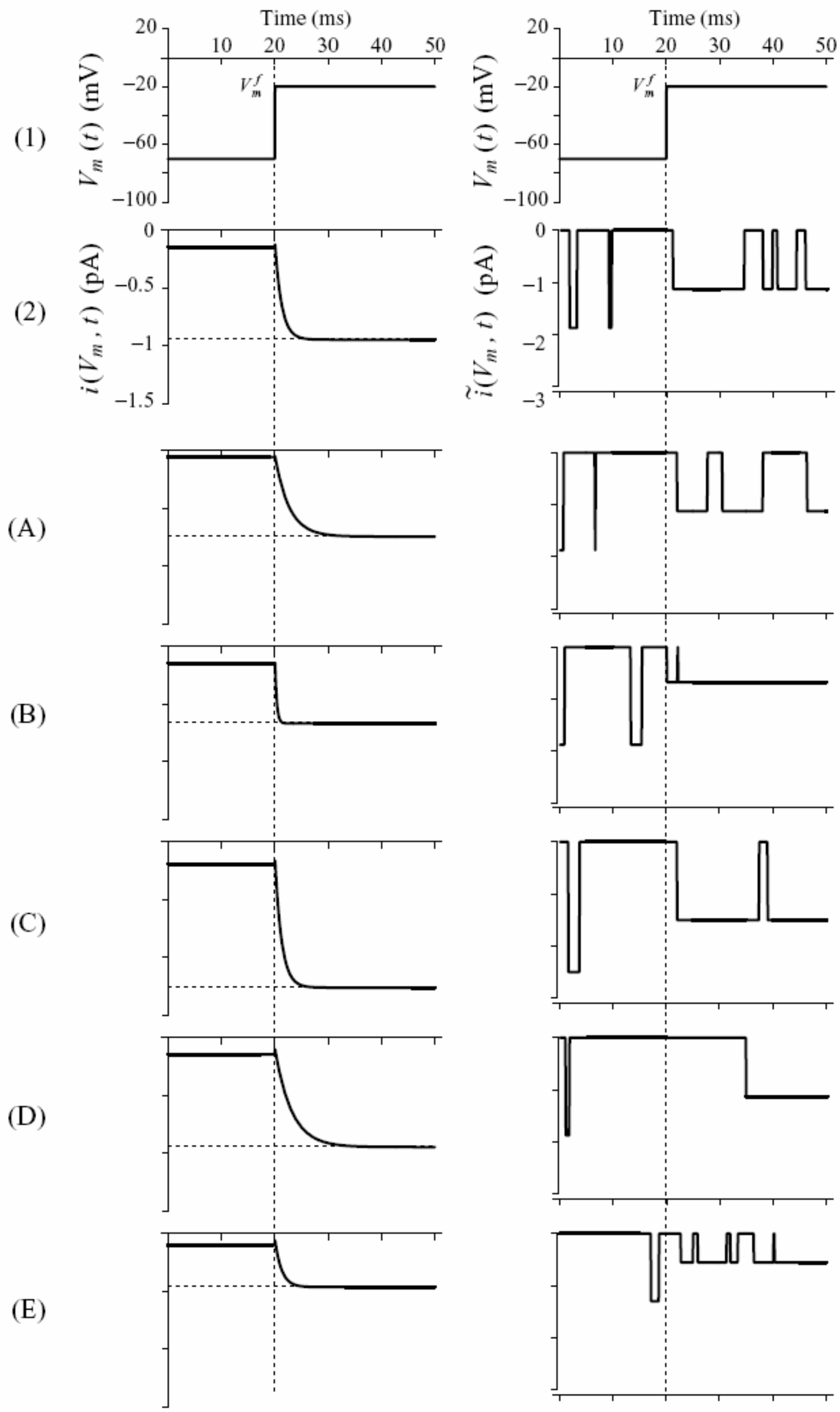


Figure 1: Single channel currents of a channel with one two-state activation gate. The scales for the results shown in rows A-E are the same as for row 2.

Problem 1. The relations between single-channel random variable currents and average single-channel currents are summarized as follows. The average single channel current is

$$i(V_m, t) = \mathcal{I}x(V_m, t),$$

where

$$\mathcal{I} = \gamma(V_m - V_{Na}).$$

	Average current ($i(V_m,t)$)			Random-variable current ($\tilde{i}(V_m,t)$)			
	Steady-state value		Time constant	Open current ($\mathcal{I}$)		Prob. open	
	$t < 20$ ms	$t > 20$ ms		$t < 20$ ms	$t > 20$ ms	$t < 20$ ms	$t > 20$ ms
A	⇑	⇑	⇑	NC	NC	?	⇓
B	NC	⇑	⇓	NC	⇑	?	⇑
C	⇓	⇓	NC	⇓	⇓	?	?
D	NC	NC	⇑	NC	NC	?	NC
E	⇑	⇑	NC	⇑	⇑	?	?

Table 1: The table indicates the change in the indicated quantity from its value for the default parameters — "NC" means there was no change, "⇑" means there was an increase in the algebraic value, "⇓" means there was a decrease in the algebraic value, and "?" indicates that it is difficult to determine whether or not there was a change. Changes in the average single-channel current and in the single open-channel current amplitude are relatively easy to judge from such brief records. Estimates of a change in the probability that the channel is open are not accurately judged with such short records. Hence, those results will not be weighed as heavily in our assessment.

The probability that the channel is open $x(V_m, t)$ is governed by the differential equation

$$\frac{dx(V_m,t)}{dt} = \alpha(V_m)\Big(1 - x(V_m,t)\Big) - \beta(V_m)x(V_m,t).$$

The probability of a transition from the closed to the open state in the interval $(t, t + \Delta t)$ is $\alpha(V_m)\Delta t$, and the probability of a transition from the open to the closed state in that interval is $\beta(V_m)\Delta t$.

In order to decide which records go with which change in parameter, the results are first summarized in Table 1.

a. An increase in γ, decreases the steady-state value of the average current in both time intervals, but does not affect the time constant. The single-open channel current also decreases in both time intervals, but the probability that the channel is open does not change. Therefore, the answer is C.

b. A increase in V_m^f will not affect the average or single-channel currents for $t < 20$ ms. However for $t > 20$ ms, this increase will increase the probability that the channel is open, and will increase the single channel current. Hence for $t > 20$ ms, the average current will increase. Therefore, the answer is B.

c. A decrease in the extracellular concentration of sodium, will decrease the Nernst equilibrium potential for sodium. This decrease will not affect the probability that the channel is open nor any of the rate constants. However, the driving voltage on the channel will increase. Hence, the single-channel current will increase for both time intervals. The steady-state value of the average current will also increase. Therefore, the answer is E.

d. A decrease in both $\alpha(V_m)$ and $\beta(V_m)$ without changing the ratio, will affect neither the probability that the channel is open nor the single-open channel current. Therefore, the steady-state values of the average current will be the same. However, the time constant will increase. The rates of transitions between states will also decrease. Therefore, the answer is D.

e. If $\alpha(V_m)$ is decreased, the time constant will increase and the probability that the channel is open will decrease. However, the values of the single-open channel currents will not be affected. Therefore, steady-state values of the average current will increase. Therefore, the answer is A.

Problem 2. Figure 2 shows a model of a voltage-gated ion channel with one three-state gate plus representative single-channel ionic and gating current records.

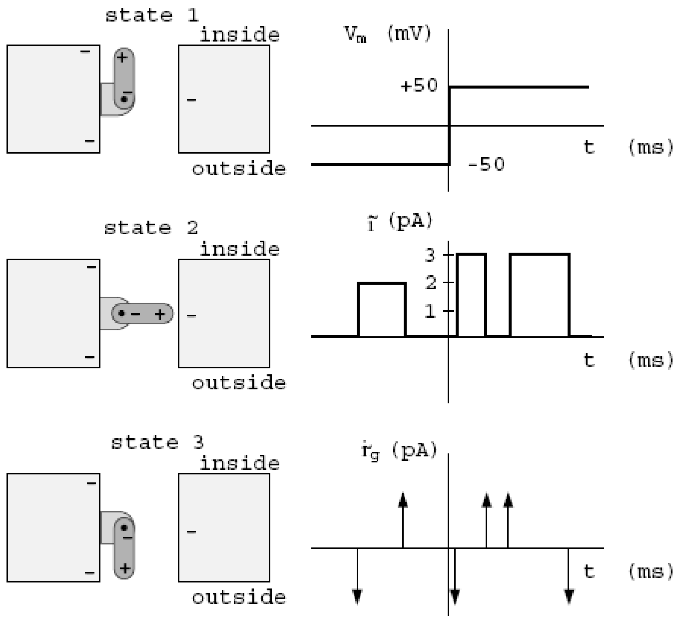


Figure 2: Channel with one three-state gate. The left panels illustrate the three states: states 1 and 3 are open states, state 2 is a closed state. The right panels illustrate the responses of the channel to a step in membrane potential $V_m(t)$ at time $t = 0$ (top right) which gives rise to the ionic current $\tilde{i}(t)$ and gating current $\tilde{i}_g(t)$ illustrated in the middle right and lower right panels, respectively.

a) Assume that the voltage-current characteristic of the channel is the same for states 1 and 3 and is linear. Determine the open channel conductance and equilibrium (reversal) potential for this channel.

b) The ionic current trace shown in Figure 2 has three non-zero segments. Determine which state the gate is in during each non-zero segment. Explain your reasoning.

c) Figure 3 illustrates the dependence of the steady-state probability that the channel will be in each of its three states on the membrane potential. Let i_{ss} represent the average value of the ionic current that results after steady-state conditions are reached in a voltage clamp experiment in which V_m is held constant. Assume that the experiment is repeated for a number of different values of membrane potential V_m. Plot the relation between i_{ss} and V_m. Describe the important features of your plot.

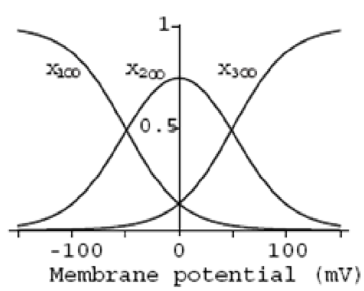


Figure 3: Steady-state probabilities for a channel with one three-state gate. $x_{1\infty}$, $x_{2\infty}$, and $x_{3\infty}$ represent the steady-state probabilities of being in state 1, state 2, and state 3, respectively, as a function of membrane potential.

Problem 2.

a. The open-state single-channel current $\mathcal{I} = \gamma(V_m - V_n)$, where γ is the single-channel open-state conductance and V_n is the Nernst equilibrium potential. The problem statement indicates that $\mathcal{I}$=2 pA when $V_m = -50$ mV, and that $\mathcal{I}$=3 pA when $V_m = +50$ mV. These two conditions can be used to obtain a solution,

$$\gamma = 10 \text{ pS and } V_n = -250 \text{ mV}.$$

b. The first and second conducting segments are preceded by negative gating currents. Negative gating currents represent inward motion of positive charge, starting from the closed state. Therefore, the first two conducting segments are in state 1. By similar reasoning, the last conducting state is state 3.

c. The steady-state value of the average ionic current is $i_{ss} = \sum_i x_{i\infty}\mathcal{I}$. Since only states 1 and 3 are conducting and since they have identical permeation characteristics,

$$i_{ss} = \sum_i x_{i\infty}\mathcal{I} = (x_{1\infty} + x_{3\infty})\mathcal{I} = (x_{1\infty} + x_{3\infty})\gamma(V_m - V_n).$$

$x_{1\infty}$ is nearly 1 for very negative values of V_m and $x_{3\infty}$ is nearly 1 for very positive values of V_m. The sum $x_{1\infty} + x_{3\infty}$ is therefore small only near $V_m = 0$. The solid line in Figure 4 shows the relation between i_{ss} and V_m. The dashed line shows the open-channel current

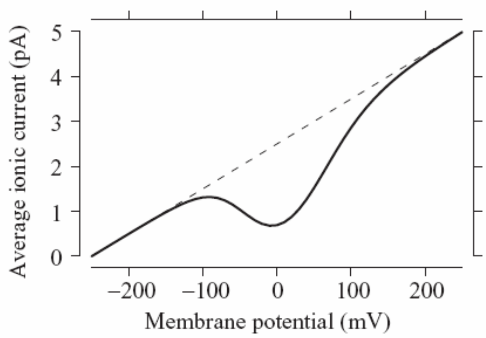
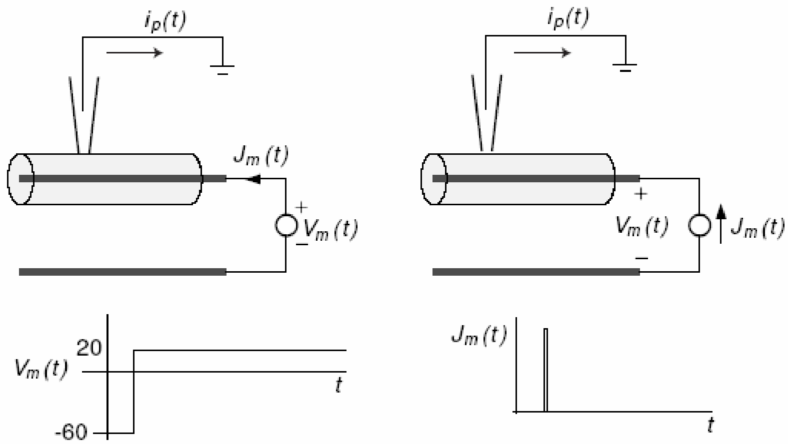


Figure 4: Average open channel current.

$\gamma(V_m - V_n)$ for the same range of membrane potential. The important point is that the average current approaches the open-channel current for very negative and for very positive values of V_m. However, the average current is smaller than the open-channel current for V_m near zero because the gate is open less frequently.

Problem 3. A squid giant axon is immersed in sea water at the default temperature (6°C) and other conditions described by the Hodgkin-Huxley model. In addition, a patch electrode is used to clamp a single channel protein within the patch, and measures the single ion channel current $i_p(t)$. Two experiments are done on this axon: A voltage clamp experiment to study the cell response to the step of membrane potential (left panel of following figure); A current clamp experiment to study the cell response to a brief, suprathreshold pulse of membrane current density (right panel).



The following eight (A-H) current traces in the next page are possible results from the experiments.

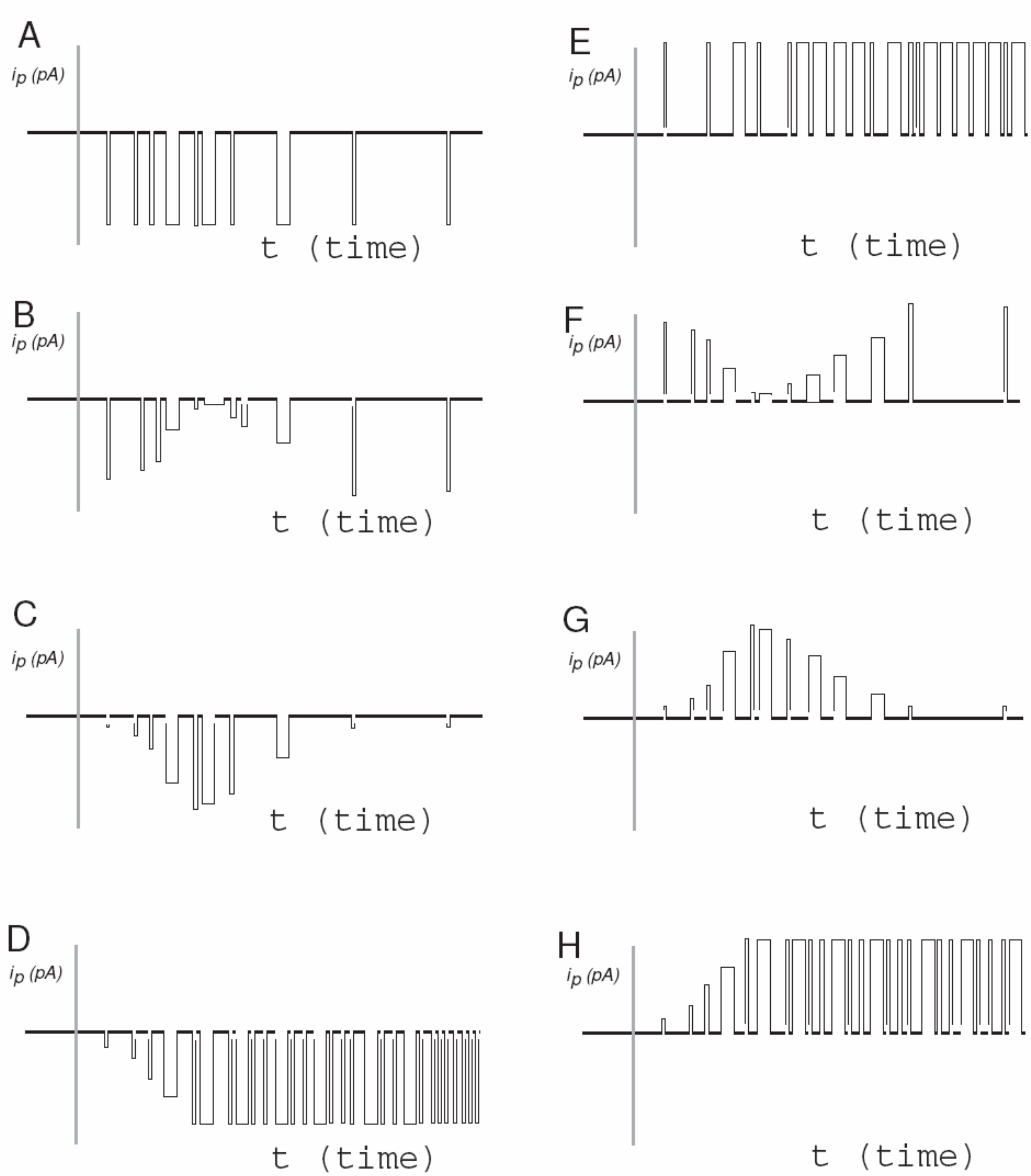
a) Which of the eight data is from the current-clamp experiment with a single sodium channel isolated in the patch? If there is no good match, sketch a correct current trace that is likely from this experiment. Explain!

b) Which of the eight data is from the voltage-clamp experiment with a single sodium channel isolated in the patch? If there is no good match, sketch a correct current trace that is likely from this experiment. Explain!

c) Which of the eight data is from the current-clamp experiment with a single potassium channel isolated in the patch? If there is no good match, sketch a correct current trace that is likely from this experiment. Explain!

d) Which of the eight data is from the voltage-clamp experiment with a single potassium channel isolated in the patch? If there is no good match, sketch a correct current trace that is likely from this experiment. Explain!

e) Which of the eight data is from the voltage-clamp experiment in a bath containing enzyme pronase, with a single sodium channel isolated in the patch? If there is no good match, sketch a correct current trace that is likely from this experiment. Explain!


A
i_p (pA)
t (time)
B
i_p (pA)
t (time)
C
i_p (pA)
t (time)
D
i_p (pA)
t (time)
E
i_p (pA)
t (time)
F
i_p (pA)
t (time)
G
i_p (pA)
t (time)
H
i_p (pA)
t (time)

Problem 3. In the current clamp experiment, potential is allowed to change. This means the step height of the single channel current trace can be changing after $t > 0$. However, in the voltage clamp experiment, the membrane potential is controlled, therefore the step height of the single channel current trace should be constant after $t > 0$. Also, for sodium channels current is always negative ($V_m(t) < V_{Na}$), while for potassium channels current is always positive ($V_m(t) > V_K$).

Part a. ANSWER: B In the current clamp experiment, action potential is generated and the sodium channel is initially inactivated, then gets activated and then becomes inactivated again. Also, the current step height gets smaller at the peak, since V_m goes near V_{Na}. However, the probability that the channel to be in open state becomes larger at the peak.

Part b. ANSWER: A Similar to **Part a**, the sodium channel gets activated and then inactivated in the voltage clamp experiment, but the step height in the current profile is constant.

Part c. ANSWER: G In the current clamp experiment, potassium channels initially gets activated and then inactivated afterwards, due to the fall of V_m back to the resting value. Also, the step height of the current trace should be smaller initially and during the later period, because at those times $V_m \approx V_K$.

Part d. ANSWER: E In the voltage clamp experiment, potassium channels gets activated but never gets inactivated. Also, the step height should be constant.

Part e. ANSWER: No correct answer When enzyme pronase is added to the bath, it removes inactivation from the sodium channel. Therefore, in the voltage clamp experiment the sodium channel should get activated but not inactivated. Data E seems right, but the current should be negative for sodium channel current. Therefore, the right trace would be the negative of E.