Cellular Electrodynamics

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Website:

Reference/Acknowledgement:
- TF Weiss (Cellular Biophysics)
- D Freeman
Summary: HH Equations

\[
\frac{1}{2\pi a(r_o + r_i)} \frac{\partial^2 V_m}{\partial z^2} = C_m \frac{\partial V_m}{\partial t} + G_K(V_m, t) (V_m - V_K) \\
+ G_{Na}(V_m, t) (V_m - V_{Na}) + G_L(V_m - V_L)
\]

\[
G_K(V_m, t) = \bar{G}_K n^4(V_m, t) \\
G_{Na}(V_m, t) = \bar{G}_{Na} m^3(V_m, t) h(V_m, t) \\
n(V_m, t) + \tau_n(V_m) \frac{dn(V_m, t)}{dt} = n_\infty(V_m) \\
m(V_m, t) + \tau_m(V_m) \frac{dm(V_m, t)}{dt} = m_\infty(V_m) \\
h(V_m, t) + \tau_h(V_m) \frac{dh(V_m, t)}{dt} = h_\infty(V_m)
\]

**Question:**
So what do \(m\), \(h\), and \(n\) physically represent?
→ Notion of an *ion channel*
Hollow superpower

Putin, Syria and the propaganda machine
Susumu Tonegawa used a technique known as optogenetics, which activates clusters of neurons by shining light on them. As they report in Nature, the researchers prepared seven month-old Alzheimer's mice by injecting a harmless virus into the rodents' dentate gyrus, a part of the hippocampus that helps to store fearful memories. The virus contains a gene for channelrhodopsin-2, a light-sensitive protein which forms pores in the cell membranes of neurons infected with the virus. These pores are closed in the dark, but open in response to blue light, flooding neurons with positively charged ions. The resulting pulse of current makes the neurons fire. During their experiments, the researchers were able to illuminate the infected neurons of the mice using optical fibres implanted in their brains.
“The virus contains a gene for channelrhodopsin-2, a light-sensitive protein which forms pores in the cell membranes of neurons infected with the virus. These pores are closed in the dark, but open in response to blue light, flooding neurons with positively charged ions. The resulting pulse of current makes the neurons fire.”

http://web.stanford.edu/group/dlab/optogenetics/
Macroscopic Ionic Currents: HH Methodology

Voltage clamp

'subtraction'

NOTE: Other methods besides subtraction (e.g., TTX to block Na\(^+\) current, replace K\(^+\) with Cs\(^+\), etc...)
Ion channels ‘prefer’ certain ions, but are not necessarily exclusive.
Microscopic Current Mechanism

Scaled version of macroscopic current?  
Discrete on/off current?

Macroscopic sodium current

Single-channel sodium current candidates

Figure 6.27
Patch Clamp

Figure 6.1

Refinement of voltage-clamp
Goal is to isolate a single ion channel
Patch Clamp

Hamill et al. (1981)
Patch Clamp

→ Current through a single channel!

Hamill et al. (1981)
→ Single ion channel current appears ‘gated’ (i.e., on/off)
Macroscopic sodium current

Single-channel sodium current candidates

Figure 6.27
Random nature of channels

Voltage-gated channels more likely to be open when magnitude of potential increased

Note change in current (both cases) with respect to holding potential
Current types:
1. Ionic
2. Gating/capacitive
Current types

- **f, i** – Ionic currents (due to charge “flow” across membrane)

- **a-e, g, h, j** – Capacitive currents (due to charge “displacement” or redistribution along/inside membrane)
Gating current

- Component \((i_g)\) of the capacitive current
- Due to channel (molecule with non-uniform charge distribution) moving open/closed
Separating Out the Gating Current

Figure 6.22
Ion Channel Model: Two Parts

1. Two-state gate model of kinetics

For a gate that is either closed or open, conductance is equal to $[0, \gamma]$ respectively.

2. Passive electrodiffusive model of permeation

$\gamma = \text{single open-channel conductance}$

For a gate that is either closed or open, conductance is equal to $[0, \gamma]$ respectively.
Model: Voltage-Gated Two-State Molecular Gate

Note: The interplay between micro- & macro-scopic descriptions requires a transition into the domain of probability & expectation values

\[ x = \text{state occupancy probability} \]

\[ g = \text{average single-channel conductance} \]

\[ i = \text{average single-channel current} \]

\[ \rightarrow \text{Note stochastic nature for an individual channel} \]
Why would the state of an individual channel be “stochastic” (i.e., randomly fluctuating)?

→ Molecular size + thermodynamics
Model: Voltage-Gated Two-State Molecular Gate *(Expected Values)*

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.

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

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

$n(t)$ is average # of open channels
Microscopic model (+ law of large numbers) gives rise to macroscopic behavior
Biophysically, this figure encapsulates numerous key ideas....
\[ G_K(V_m, t) = \overline{G}_K n^4(V_m, t) \]
\[ G_{Na}(V_m, t) = \overline{G}_{Na} m^3(V_m, t) h(V_m, t) \]
\[ n(V_m, t) + \tau_n(V_m) \frac{dn(V_m, t)}{dt} = n_\infty(V_m) \]
\[ m(V_m, t) + \tau_m(V_m) \frac{dm(V_m, t)}{dt} = m_\infty(V_m) \]
\[ h(V_m, t) + \tau_h(V_m) \frac{dh(V_m, t)}{dt} = h_\infty(V_m) \]
Question:
So what do $m$, $h$, and $n$ physically represent?

\[
G_K(V_m, t) = \overline{G}_K n^4(V_m, t)
\]
\[
G_{Na}(V_m, t) = \overline{G}_{Na} m^3(V_m, t) h(V_m, t)
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n(V_m, t) + \tau_n(V_m) \frac{dn(V_m, t)}{dt} = n_\infty(V_m)
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m(V_m, t) + \tau_m(V_m) \frac{dm(V_m, t)}{dt} = m_\infty(V_m)
\]
\[
h(V_m, t) + \tau_h(V_m) \frac{dh(V_m, t)}{dt} = h_\infty(V_m)
\]
State whether each of the following is true or false, and give a reason for your answer.

a. Tetrodotoxin blocks the flow of potassium through the sodium channel.

b. The macroscopic sodium current recorded by an electrode in a cell is a sum of the single-channel sodium currents that flow through single sodium channels.

c. The macroscopic sodium current recorded by an electrode in a cell is the average of the single-channel sodium currents that flow through single sodium channels.

d. Ionic and gating currents give identical information about channel kinetic properties.
Exercises

Figure 6.72 shows two putative records of membrane currents recorded from two membrane patches, each of which contains a single channel, in response to a step of depolarizing membrane potential. Each of these channels has a linear voltage-current characteristic when the channel is open.

\[ V_m(t) \]

\[ \begin{array}{c}
\text{(1)} \\
\text{(2)}
\end{array} \]

\[ t \]

Figure 6.72  Two putative single-channel currents in response to a voltage step (Exercise 6.5).

a. Which, if any, of these records could be from a single voltage-gated channel? Explain.

b. Which, if any, of these records could be from a single channel that is not voltage gated? Explain.
Exercises (SOL)

State whether each of the following is true or false, and give a reason for your answer.

a. Tetrodotoxin blocks the flow of potassium through the sodium channel.

b. The macroscopic sodium current recorded by an electrode in a cell is a sum of the single-channel sodium currents that flow through single sodium channels.

c. The macroscopic sodium current recorded by an electrode in a cell is the average of the single-channel sodium currents that flow through single sodium channels.

d. Ionic and gating currents give identical information about channel kinetic properties.

Exercise 6.4

a. **True.** Tetrodotoxin blocks the sodium channel. Hence, it blocks the flow of any ion that can pass through the channel including potassium.

b. **True.**

c. **False.** See part b.

d. **False.** Gating currents give information about charge movements in the membrane between any states — conducting and non-conducting states — whereas ionic current give information about the conducting states only.
Exercises (SOL)

**Exercise 6.5** Trace 1 shows a single-channel current with two states of conduction: one current is zero and the other is negative. The negative current represents ion flow when the channel is open. The magnitude of that current is not changed by the step change in membrane potential. This is inconsistent with the assumption that the open-channel voltage-current relation is linear. Therefore trace 1 cannot result from an ion channel: neither from a voltage-gated ion channel nor any other ion channel.

The open-channel currents in trace 2 are different before and after the step change in membrane potential. This is expected if the open-channel voltage-current relation is linear. From this short segment of data, one cannot conclude that the probability that the channel is open has or has not changed during the step change in membrane potential. Therefore, the current in trace 2 could be from a voltage-gated ion channel or any other ion channel.

a. Trace 2 only.

b. Trace 2 only.

---

![Figure 6.72](image)

Figure 6.72 Two putative single-channel currents in response to a voltage step (Exercise 6.5).

a. Which, if any, of these records could be from a single voltage-gated channel? Explain.

b. Which, if any, of these records could be from a single channel that is not voltage gated? Explain.
Figure 12.14: (Schematic; optical micrograph.) (a) A small patch of membrane containing only a single voltage-gated sodium channel (or a few) is electrically isolated from the rest of the cell by a patch electrode. The current entering the cell through these channels is recorded by a monitor connected to the patch electrode. (b) Patch-clamp manipulation of a single, live photoreceptor cell from the retina of a salamander. The cell is secured by partially sucking it into a glass micropipette (bottom), while the patch-clamp electrode (upper left) is sealed against a small patch of the cell’s plasma membrane. [Digital image kindly supplied by T. D. Lamb; see Lamb et al., 1986.]
**Model:** Voltage-Gated Two-State Molecular Gate

**Note:** The interplay between micro- & macroscopic descriptions requires a transition into the domain of probability & expectation values.
Microscopic model (+ law of large numbers) gives rise to macroscopic behavior

Question(s): How big must $N$ be? How “local” does it need to be (i.e., as a channel density)?
Model: Voltage-Gated Two-State Molecular Gate

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

\[
\frac{dn(t)}{dt} = \alpha(N - n(t)) - \beta n(t)
\]

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

First-order kinetics!!

Assume $N$ is large.

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

\[x(t) = x_\infty + (x(0) - x_\infty) e^{-t/\tau_x} ; \quad x_\infty = \frac{\alpha}{\alpha + \beta}, \quad \tau_x = \frac{1}{\alpha + \beta}
\]
Model: Voltage-Gated Two-State Molecular Gate

The potential energy of an ion channel includes mechanical, chemical, and electrical contributions, each of which can be different in different conformations. Electrical potential energy depends on both the distribution of charge in the gate and on transmembrane potential. Therefore, $E_B$, $E_O$, and $E_C$ depend on $V_m$.

First-order kinetics variables

$$\frac{dx}{dt} = \alpha_x (1-x) - \beta_x x$$

$\rightarrow$ Potential modifies energy configuration

$$\alpha = A e^{(E_C - E_B)/kT}$$
$$\beta = A e^{(E_O - E_B)/kT}$$

[Weiss vol.1 ch.6]
\[ E_B = \frac{1}{2} Q V_B; \quad E_C = \frac{1}{2} Q V_m; \quad E_O = -\frac{1}{2} Q V_m \]

\[ \alpha = A e^{\frac{1}{2} Q (V_m - V_B)/kT}; \quad \beta = A e^{-\frac{1}{2} Q (V_m + V_B)/kT} \]

\[ x_\infty = \frac{\alpha}{\alpha + \beta} = \frac{1}{1 + \beta/\alpha} = \frac{1}{1 + e^{-QV_m/kT}} \]

\[ \tau_x = \frac{1}{\alpha + \beta} = \frac{1}{A(e^{\frac{1}{2} Q (V_m - V_B)/kT} + e^{-\frac{1}{2} Q (V_m + V_B)/kT})} \]

\[ = \frac{1}{Ae^{-\frac{1}{2} Q V_B/kT}(e^{\frac{1}{2} Q V_m/kT} + e^{-\frac{1}{2} Q V_m/kT})} \]
Separating Out the Gating Current

Two components (why?)
- Linear
- Nonlinear

$J_c = J_{cl} + J_{cn}$

Figure 6.20

Figure 6.21
Separating Out the Gating Current

Sign of gating current can inform about structure/charge distribution of the channel

![Graphs showing current density and membrane potential over time](Figure 6.22)
Reversibility
(i.e., $Q_{on} = Q_{off}$ implies charge is conserved)

Saturation
(i.e., finite number of channels)

Question: If we know the single channel conductance, can we estimate the total # of contributing channels?
Hodgkin Huxley model

\[ G_K(V_m, t) = \overline{G}_K n^4(V_m, t) \]
\[ G_{Na}(V_m, t) = \overline{G}_{Na} m^3(V_m, t) h(V_m, t) \]

\[ n(V_m, t) + \tau_n(V_m) \frac{dn(V_m, t)}{dt} = n_\infty(V_m) \]
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\( \overline{G}_{Na} = 120, \overline{G}_K = 36, \) and \( G_L = 0.3 \text{ mS/cm}^2; \)
\( c_{Na}^0 = 491, c_{Na}^i = 50, c_K^0 = 20.11, c_K^i = 400 \text{ mmol/L;} \)
\( C_m = 1 \mu\text{F/cm}^2; V_L = -49 \text{ mV}; \) temperature is 6.3°C.
Two-state model $\Rightarrow$ First-order kinetics

\[
\tau_x \frac{dx}{dt} + x = x_\infty \quad \frac{dx}{dt} = \alpha x (1 - x) - \beta x x
\]

\[
x_\infty = \frac{\alpha x}{(\alpha x + \beta x)} \text{ and } \tau_x = \frac{1}{\alpha x + \beta x}
\]

First-order, reversible reaction

\[
R \xrightarrow{\frac{\alpha}{\beta}} P
\]

$\Rightarrow$ Single two-state model too ‘simple’

\[
G_K(V_m, t) = \overline{G}_K n_1^4(V_m, t)
\]
\[
G_{Na}(V_m, t) = \overline{G}_{Na} m^3(V_m, t) h(V_m, t)
\]
Figure 6.54
Conductance voltage-dependence consistent w/ HH if there are three independent activation gates (i.e., $m^3$).

HH predicts values for the various time constants...

... that are inconsistent with data ("tail currents").

→ Four independent two-state models still too simple. Multi-state channels?
Multistate Channel Models

Figure 6.60

Figure 6.61
s-shape stems from “lag incurred by the state occupancy having to traverse earlier stages”
Molecular Underpinnings

**Figure 6.68**

**Figure 6.69**
Figure 6.70


Figure 12.16: (Schematic; sketch.) (a) Conceptual model of a voltage-gated ion channel. A spring normally holds a valve closed. An electric field pointing upward lifts the positively charged valve, letting water flow downward. (b) Sketch of the sodium channel. Left: In the resting state, positive charges in the channel protein’s four “sensing” alpha helices are pulled downward, toward the negative cell interior. The sensing helices in turn pull the channel into its closed conformation. Right: Upon depolarization, the sensing helices are pulled upward. The channel now relaxes toward a new equilibrium, in which it spends most of its time in the open state. The lower blob depicts schematically the channel-inactivating segment. This attached object can move into the channel, blocking ion passage even though the channel itself is in its open conformation. [After Armstrong & Hille, 1998.]