Membrane Potential and Ion Channels

Background readings:


Hille *Ionic channels of excitable membranes* 3rd ed.
LIFE - A complex interplay of chemistry and physics
The action potential – Physics in cell biology

1. Interstitial fluid

2. Sodium ions (Na+) entering, potassium ions (K+) exiting

3. Sodium ions (Na+) entering, potassium ions (K+) exiting

4. Sodium ions (Na+) entering, potassium ions (K+) exiting

(a) Membrane potential (mV) vs. time (msec)

The Nobel Prize in Physiology or Medicine 1963

"For their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"
Excitation-contraction coupling
The islet of Langerhans
Glucose-dependent hormone secretion
The Lipid Bilayer is a Selective Barrier

inside

- hydrophobic molecules (anesthetics)
- gases ($O_2$, $CO_2$)
- small uncharged polar molecules
- large uncharged polar molecules
- Ions
- charged polar molecules (amino acids)
- water

outside
Ion Channel Diversity

- Cys-loop (GABA, nACH, glycine, 5HT)
- Ionotropic glutamate
- ENaC/ASIC/P2X
- Voltage-gated cation
- CNG
- IP₃/ryanodine
- TRP
- PKD
- Kᵢᵣ/GIRK
- 2-pore K⁺
- Chloride
- ABCC
Cation channel Subunit Topology
The 'inner core' of cation channel superfamily members
Structure of a Potassium Channel
Selectivity Filter and Space-Filling Model

Doyle et al., 1998
Ion selectivity filter in KcsA
Doyle et al., 1998, Zhou et al., 2002
Selectivity filter diversity

- **Strong K⁺**
  - All ‘potassium channels’

- **Weak K⁺**
  - HCN channels

- **Nonselective cation/Ca²⁺ permeable**
  - TRP channels
  - CNG channels
  - IP3/ryanidine receptors
  - Ionotropic glutamate receptors
  - NaK channels?

- **Strong Ca²⁺**
  - Voltage-gated Ca²⁺ channels

- **Weak Na⁺**
  - Voltage-gated Na⁺ channels
Ion selectivity filter in Na channels

NavMs, NavAb, Kv1.2

Views of the Channel Open and Shut

KcsA $K^+$ channel "closed"

MthK $K^+$ channel "open"

Jiang et al., 2002
Views of the Channel Open and Shut

Nature Communications, Oct 2, 2012. Structure of a bacterial voltage-gated sodium channel pore reveals mechanisms of opening and closing. Emily C McCusker,1,3 Claire Bagnéris,1 Claire E Naylor,1 Ambrose R Cole,1 Nazzareno D’Avanzo,2,4 Colin G Nichols2, & B A Wallace1
Ion gradients and membrane potential
Ion gradients and membrane potential
Ion gradients and membrane potential
Ion gradients and membrane potential

The inside of the cell is at ~ -0.1V with respect to the outside solution.

How does this membrane potential come about?
Ion gradients and membrane potential

How does this membrane potential come about?
Ion gradients and membrane potential

How does this membrane potential come about?
## Movement of Individual $K^+$ ions

<table>
<thead>
<tr>
<th>Ion</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>117</td>
</tr>
<tr>
<td>K</td>
<td>3</td>
</tr>
<tr>
<td>Cl</td>
<td>120</td>
</tr>
<tr>
<td>Anions</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
</tr>
</tbody>
</table>

$[+ \text{ charge}] = [- \text{ charge}]$

$0 \text{ mV}$
Movement of Individual $\text{Cl}^-$ ions

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>117</td>
</tr>
<tr>
<td>K</td>
<td>3</td>
</tr>
<tr>
<td>Cl</td>
<td>120</td>
</tr>
<tr>
<td>Anions</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
</tr>
</tbody>
</table>

$[+\text{ charge}] = [-\text{ charge}]$

$0 \text{ mV}$

$[+\text{ charge}] = [-\text{ charge}]$

$-89 \text{ mV}$
Setting a membrane potential - questions!

- *How many ions must cross the membrane to set up this membrane potential?*

- *What makes it stop at this potential?*
Capacitance

Capacitance $C$  
Coulombs / Volt  
Farads

$C = \frac{Q}{V}$  
$Q = C \times V$  
$I = \frac{dQ}{dt} = C \times \frac{dV}{dt}$

For biological membranes:  
Specific Capacitance = $1 \ \mu F / cm^2$
How many ions?

- How many ions must cross the membrane of a spherical cell 50 µm in diameter (r = 25 µm) to create a membrane potential of -89 mV?

\[ Q \text{ (Coulombs)} = C \text{ (Farads)} \times V \text{ (Volts)} \]

Specific Capacitance = 1.0 \( \mu \)Farad / cm\(^2\)

Surface area = \( 4 \pi r^2 \)

Faraday’s Constant = 9.648 \( \times \) 10\(^4\) Coulombs / mole

Avogadro’s # = 6.022 \( \times \) 10\(^{23}\) ions / mole
Calculations

<table>
<thead>
<tr>
<th>Area ( = 4 \pi r^2 )</th>
<th>Q ( = C \times V )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( = 4 \pi (25 \times 10^{-4} \text{ cm})^2 )</td>
<td>( = 7.85 \times 10^{-5} \text{ cm}^2 )</td>
</tr>
<tr>
<td>( = 78.5 \times 10^{-6} \mu\text{Farads} )</td>
<td>( = 78.5 \times 10^{-12} \text{ Farads} \times 0.089 \text{ Volts} )</td>
</tr>
<tr>
<td>( = 78.5 \times 10^{-12} \text{ Farads} )</td>
<td>( = 7 \times 10^{-12} \text{ Coulombs} )</td>
</tr>
</tbody>
</table>

\(~ 7 \times 10^{-12} \text{ Coulombs} / 9.65 \times 10^4 \text{ Coulombs per mole} ~\)

\(~ 7.3 \times 10^{-17} \text{ moles of ions must cross the membrane} ~\)

\(~ 0.073 \text{ femtmoles} \text{ or } \sim 44 \times 10^6 \text{ ions} \sim 1.1 \mu\text{M change in } [\text{ ]} ~\)
Why does it stop?  
- The Nernst Equation

Calculates the membrane potential at which an ion will be in electrochemical equilibrium.

At this potential: total energy inside = total energy outside

Electrical Energy Term:  \( zFV \)
Chemical Energy Term:  \( RT \ln[\text{Ion}] \)

\( Z \) is the charge, 1 for Na\(^+\) and K\(^+\), 2 for Ca\(^{2+}\) and Mg\(^{2+}\), -1 for Cl\(^-\)

\( F \) is Faraday’s Constant = 9.648 \times 10^4 \text{ Coulombs} / \text{mole}

\( R \) is the gas constant = 8.315 \text{ Joules} / \text{°Kelvin} * \text{mole}

\( T \) is the temperature in °Kelvin
Nernst Equation Derivation

\[ zF \cdot V_{\text{in}} + RT \cdot \ln [K^+]_{\text{in}} = zF \cdot V_{\text{out}} + RT \cdot \ln [K^+]_{\text{out}} \]

\[ zF \left( V_{\text{in}} - V_{\text{out}} \right) = RT \left( \ln [K^+]_{\text{out}} - \ln [K^+]_{\text{in}} \right) \]

\[ E_K = V_{\text{in}} - V_{\text{out}} = \frac{RT}{zF} \cdot \ln \left( \frac{[K^+]_{\text{out}}}{[K^+]_{\text{in}}} \right) \]

\[ E_K = 2.303 \left( \frac{RT}{F} \right) \times \log_{10} \left( \frac{[K^+]_{\text{out}}}{[K^+]_{\text{in}}} \right) \]

In General:

\[ E_{\text{ion}} \sim 60 \times \log \left( \frac{[\text{ion}]_{\text{out}}}{[\text{ion}]_{\text{in}}} \right) \text{ for monovalent cation at } 30^\circ \]
Nernst Potential Calculations

- First K and Cl
  \[ E_K = 60 \text{ mV} \log \left( \frac{3}{90} \right) = 60 \times -1.477 = -89 \text{ mV} \]

  \[ E_{Cl} = (60 \text{ mV} / -1) \log \left( \frac{120}{4} \right) = -60 \times 1.477 = -89 \text{ mV} \]

  Both Cl and K are at electrochemical equilibrium at -89 mV

- Now for Sodium
  \[ E_{Na} = 60 \text{ mV} \log \left( \frac{117}{30} \right) = 60 \times 0.591 = +36 \text{ mV} \]

When Vm = -89 mV, both the concentration gradient and electrical gradient for Na are from outside to inside.
At Electrochemical Equilibrium:

- The concentration gradient for the ion is exactly balanced by the electrical gradient.
- There is no net flux of the ion.
- There is no requirement for any energy-driven pump to maintain the concentration gradient.
Ion Concentrations

Na  117
K   3
Cl  120
Anions 0
Total 240

[+ charge] = [- charge]
0 mV

Na  30
K   90
Cl  4
Anions 116
Total 240

[+ charge] = [- charge]
-89 mV
## Ion Concentrations

Add water - 50% dilution

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>117</td>
</tr>
<tr>
<td>K</td>
<td>3</td>
</tr>
<tr>
<td>Cl</td>
<td>120</td>
</tr>
<tr>
<td>Anions</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>30</td>
</tr>
<tr>
<td>K</td>
<td>90</td>
</tr>
<tr>
<td>Cl</td>
<td>4</td>
</tr>
<tr>
<td>Anions</td>
<td>116</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
</tr>
</tbody>
</table>

\[ (+ \text{ charge}) = (- \text{ charge}) \]

0 mV

\[ (+ \text{ charge}) = (- \text{ charge}) \]

-89 mV

Add water – 50% dilution
Environmental Changes: Dilution

Add water - 50% dilution

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th></th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>58.5</td>
<td>Na</td>
<td>30</td>
</tr>
<tr>
<td>K</td>
<td>1.5</td>
<td>K</td>
<td>90</td>
</tr>
<tr>
<td>Cl</td>
<td>60</td>
<td>Cl</td>
<td>4</td>
</tr>
<tr>
<td>Anions</td>
<td>0</td>
<td>Anions</td>
<td>116</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>Total</td>
<td>240</td>
</tr>
</tbody>
</table>

\[ + \text{charge} = - \text{charge} \]

\[-89 \text{ mV}\]
Environmental Changes: Dilution

Add water - 50% dilution

<table>
<thead>
<tr>
<th>Ion</th>
<th>Original</th>
<th>Diluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>58.5</td>
<td>30</td>
</tr>
<tr>
<td>K</td>
<td>1.5</td>
<td>90</td>
</tr>
<tr>
<td>Cl</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Anions</td>
<td>0</td>
<td>116</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>240</td>
</tr>
</tbody>
</table>

[+ charge] = [- charge]

$E_K = -107$ mV

$E_{Cl} = -71$ mV

$E_{K} = -89$ mV
Environmental Changes: Dilution

Add water - 50% dilution

Na 58.5
K 1.5
Cl 60
Anions 0
Total 120

[+ charge] = [- charge]

$E_K = -107 \text{ mV}$
$E_{Cl} = -71 \text{ mV}$

Na 30
K <90
Cl <4
Anions 116
Total <240

[+ charge] = [- charge]

-89 mV
Resting membrane potentials in real cells deviate from the Nernst equation, particularly at low external potassium concentrations.

The Goldman, Hodgkin, Katz equation provides a better description of membrane potential as a function of potassium concentration in cells.

The Goldman, Hodgkin, Katz equation provides a better description of membrane potential as a function of potassium concentration in cells.
The Goldman Hodgkin Katz Equation

\[
V_m = 60mV \times \log \left( \frac{P_K [K]_{\text{out}} + P_{Na} [Na]_{\text{out}} + P_{Cl} [Cl]_{\text{in}}}{P_K [K]_{\text{in}} + P_{Na} [Na]_{\text{in}} + P_{Cl} [Cl]_{\text{out}}} \right)
\]

- Resting \(V_m\) depends on the concentration gradients and on the relative permeabilities to Na, K and Cl. The Nernst Potential for an ion does not depend on membrane permeability to that ion.

- The GHK equation describes a steady-state condition, not electrochemical equilibrium.

- There is net flux of individual ions, but no net charge movement.

- The cell must supply energy to maintain its ionic gradients.
Suppose $P_K : P_{Na} : P_{Cl} = 1 : 0.1 : 1$

$$V_m = 60 \text{mV} \times \log \left( \frac{3 + 11.7 + 4}{90 + 3 + 120} \right)$$

$$= 60 \text{ mV} \times \log \left( \frac{18.7}{213} \right)$$

$$= 60 \text{ mV} \times -1.06$$

$$= -63 \text{ mV}$$
Competing Batteries Model

**Ohm's Law:** \( V = I \times R \), so \( I_{Na} = V_1 \times g_{Na} \)
\( \) \( I_K = V_2 \times g_K \quad I_{Cl} = V_3 \times g_{Cl} \)

At rest there is no net current

\( I_{Na} + I_K + I_{Cl} = 0 \)

\( \) \( P \) \( V_1 \times g_{Na} + V_2 \times g_K + V_3 \times g_{Cl} = 0 \)

\( V_m = V_1 + E_{Na} = V_2 + E_K = V_3 + E_{Cl} \)

\( \) \( P \) \( V_1 = V_m - E_{Na} \quad \) and \( V_2 = V_m - E_K \quad \) and \( V_3 = V_m - E_{Cl} \)

\( \Rightarrow 0 = (V_m - E_{Na}) \times g_{Na} + (V_m - E_K) \times g_K + (V_m - E_{Cl}) \times g_{Cl} \)

\( V_m = \frac{g_{Na}E_{Na} + g_{K}E_{K} + g_{Cl}E_{Cl}}{g_{Na} + g_K + g_{Cl}} \)

\( I_{ion} = g_{ion} \times (V_m - E_{ion}) \)
Recording Membrane Potential

current

reference

ground 0 mV

voltage

sharp microelectrode

whole-cell "patch" electrode
To here L1
On-Cell Patch Recording (Muscle)
Forming a Tight Seal

[Diagram showing a close-up of a muscle fiber with a pipette attached, labeled ACh. The pipette is connected to an amplifier, and there are traces showing electrical activity with annotations for suction and time scales.]
Whole-Cell and Outside-Out Patch
Channels Exist in at least 2 States

![Graph and illustrations showing the states of a channel: closed and open, with transitions labeled by \( \frac{\beta}{\alpha} \).]
Current \(\sim 3.2 \text{ pA}\) \(= 3.2 \times 10^{-12} \text{ Coulombs/sec}\)

Charge on 1 ion \(\sim 1.6 \times 10^{-19} \text{ Coulombs}\)

Ions per second \(= \frac{3.2}{1.6} \times 10^7\)

\(\sim 20 \text{ million}\)
Exponential Distribution of Lifetimes

mean open time = $\tau_{\text{open}} = 1 / a = 1 / \text{closing rate constant}$
Summary:

I. Cell membranes form an insulating barrier that acts like a parallel plate capacitor (1 \( \mu \text{F/cm}^2 \))

II. Ion channels allow cells to regulate their volume and to generate membrane potentials

III. Only a small number of ions must cross the membrane to create a significant voltage difference
    ~ bulk neutrality of internal and external solution

IV. Permeable ions move toward electrochemical equilibrium
   - \( E_{\text{ion}} = (60 \text{ mV} / z) \times \log ([\text{Ion}]_{\text{out}} / [\text{Ion}]_{\text{in}}) @ 30^\circ\text{C} \)
   - Electrochemical equilibrium does not depend on permeability, only on the concentration gradient
Summary (continued):

v. The Goldman, Hodgkin, Katz equation gives the steady-state membrane potential when Na, K and Cl are permeable

\[ V_m = 60 \text{mV} \times \log \left( \frac{P_K \times [K]_{\text{out}} + P_{Na} \times [Na]_{\text{out}} + P_{Cl} \times [Cl]_{\text{in}}}{P_K \times [K]_{\text{in}} + P_{Na} \times [Na]_{\text{in}} + P_{Cl} \times [Cl]_{\text{out}}} \right) \]

- In this case, \( V_m \) does depend on the relative permeability to each ion and there is steady flux of Na and K.

The cell must supply energy to maintain its ionic gradients.