Electrical Properties of Neurons

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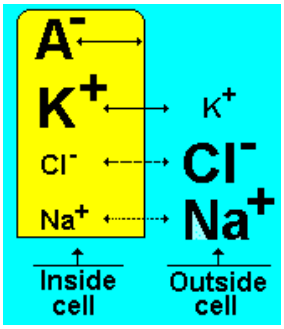
History of Electric Brain

- After discovery of electricity, scientists discovered that conduction from brain to muscle was mediated by flow of electricity
- In 1939, Hodgkin and Huxley discovered that axons are electrically negative at rest

Electrical signals carried by ions

The concentration of ions within the cell is different than the concentration outside of the cell

- Some ions have higher concentration inside
  - K^+
- Some ions have higher concentrations outside:
  - Na^+
  - Cl^-
  - Ca^{++}



	many	few
K^+	400	20
Cl^-	40	560
Na^+	50	440

Steady State - Equilibrium

- Electrical signals
  - Are departure from steady state (equilibrium)
  - Are caused by net changes in ion movement
  - Underlie information processing in neurons
- The resting membrane potential of a cell
  - Membrane potential when cell is at steady state

Steady State - Equilibrium

- Charge balance
- No **net** movement of water
  - No change in the volume of the cell
  - No dilution of concentration gradients
- No **net** change in ion movement
  - For every K^+ moving inward, there is a K^+ moving outward
  - No change in concentration gradients

Charge Balance

Charge in each compartment are approximately balanced

- Outside the cell, sum of anions = sum of cations
  - $[Na^+] + 2*[Ca^{++}] + [K^+] = [Cl^-]$
- Inside the cell, sum of anions = sum of cations
  - $[Na^+] + 2*[Ca^{++}] + [K^+] = [Cl^-] + [A^-]$
  - A^- are other anions, which are mostly proteins
  - Anions are impermeant to the membrane

Osmolarity Balance

- Water balance = Osmolarity balance
- Osmolarity inside cell is equal to osmolarity outside cell
- $[Na^+]_i + [Ca^{++}]_i + [K^+]_i + [Cl^-]_i + [A^-]_i = [Na^+]_o + [Ca^{++}]_o + [K^+]_o + [Cl^-]_o$
- Membrane is permeable to water. If osmolarity is different, water will flow to equalize osmolarity.

Ion Movement

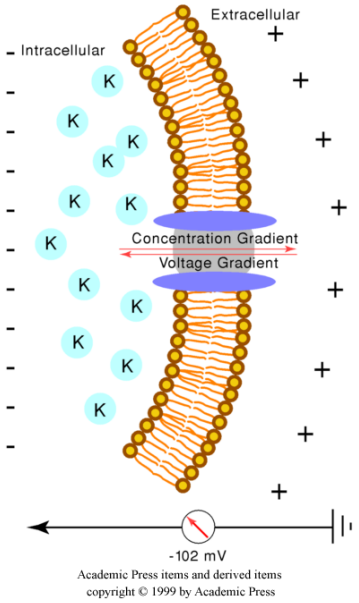
- Concentration gradient produces tendency for ions to move from high concentration to low concentration
  - Mechanism is diffusion
- Ions move through ionic channels
  - Protein pores in membrane
- At rest, pores for sodium and calcium are closed
  - Membrane is selectively permeable to potassium

Ion Movement

- How is equilibrium maintained if K^+ ions can move down concentration gradient?
- Movement of potassium from inside to outside causes slight imbalance in charge
  - Recall that anions are impermeable and can't move with potassium
  - Excess of K^+ outside
  - Excess of A^- inside

Ion Movement Forces

- Concentration gradient is balanced by voltage gradient



Ion Movement

- Charge distribution creates an electrical field.
  - Produces a potential difference between inside and outside
- Potential difference permitted by special property of membrane
  - Capacitance
  - Farad = Coulomb per Volt
  - Quantity of charge producing a 1 volt potential.

Ion Movement

- Potential difference produces force of attraction
  - Negative potential of cell attracts potassium ions
  - As potential decreases, the force that draws potassium ions inside the cell increases
- At some potential, electrostatic forces pulling K^+ in equals diffusive tendency for K^+ to move out.
  - At that potential and concentration gradient, no net flow of K^+ occurs.

Resting Potential is called Equilibrium potential

Nernst Equation

- Equilibrium potential is determined by
  - Concentration outside, C_{out}
  - Concentration inside, C_{in}
  - Temperature of solution in Kelvin, T
  - Valence of ion, z
  - Work required to separate charge, R

Nernst Equation

$$E_R = \frac{RT}{zF} \ln\left(\frac{C_{out}}{C_{in}}\right)$$

- R is the ideal gas constant
  - 8.32 joules/Kelvin/mole
- F is Faraday's constant
  - 96,485 Coulombs per mole

Squid Axon

Ion	Conc in	Conc out	Equilibrium Potential
Na+	50	440	55
K+	400	20	-76
Cl-	40	560	-66
Ca++	0.4	10	145

Concentration in millimoles,
potential in millivolts.

Mammalian Neuron

Ion	Conc in	Conc out	Equilibrium Potential
Na+	18	145	56
K+	135	3	-102
Cl-	7	20	-76
Ca++	0.0001	1.2	125

Concentration in millimoles,
potential in millivolts.
Calcium is heavily buffered; thus total internal calcium is higher

Reversal Potential

Equilibrium potential also called reversal potential, E_R

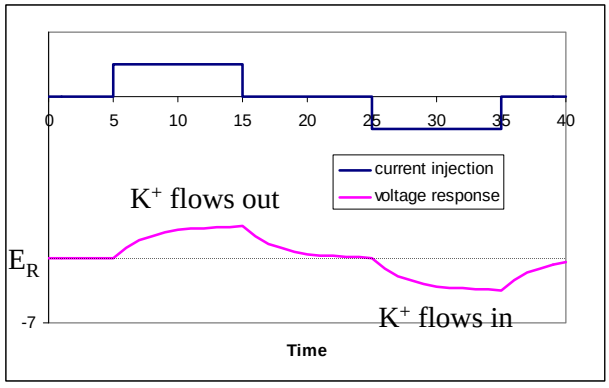
- If membrane potential (V_M) is greater than E_R, then potassium ions flow out
- If V_M is lower than E_R, then potassium ions flow in
- If $V_M = E_R$, then forces balance, no net flow

In other words:

- If $V_M - E_R > 0$, then positive ions flow out
  - Outward current
- If $V_M - E_R < 0$, then positive ions flow in
  - Inward current
- If $V_M - E_R = 0$, no current flow

Ion Movement controlled by V_M

Depolarize cell with current injection
Higher V_M implies insufficient charge to attract K^+
K+ moves down concentration gradient



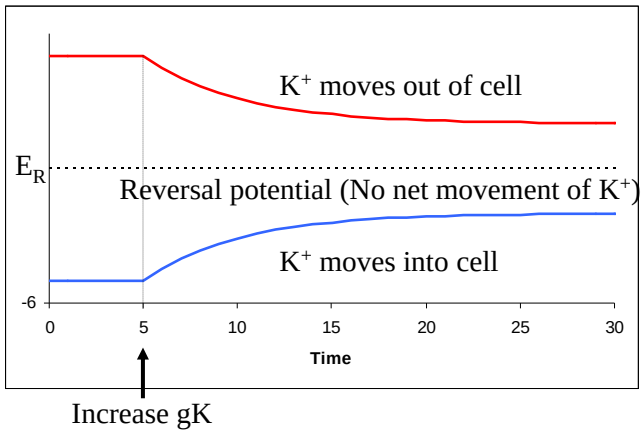
V_M controlled by Ion Movement

Tends to return membrane potential to equilibrium

1. Begin above E_R

Membrane Potential

2. Begin below E_R



Resting Potential

- **If membrane is permeable to K^+, then ions flow until $V_M = E_K$**
- Neuron membranes are permeable to multiple ions
  - Cl^-
  - Na^+
- Permeability is less than K^+
- Permeability varies between neuronal types

Resting Potential

- Represents a steady state
  - No net ion fluxes
  - No net water movement (osmotic balance)
  - Charge balance
- Not all neurons have steady state
  - Spontaneous activity in absence of input
  - In live brain, are any neurons in steady state?
- Varies between neuron types
  - Photoreceptors rest at -40 mV
  - Thalamic cells rest at -70 mV during sleep, -55 mV during waking
  - Spiny projection neurons alternative between -80 mV and -55 mV
  - Cortical and hippocampal neurons rest near -75 mV

Goldman-Hodgkin-Katz Equation

- Resting potential depends on concentration of all ions to which membrane is permeable
- Relative contribution of each ion depends on
  - Concentration gradient
  - Permeability (relative to potassium)

$$V_M = \frac{RT}{F} \ln\left(\frac{p_K \cdot K_{out} + p_{Na} \cdot Na_{out} + p_{Cl} \cdot Cl_{in}}{p_K \cdot K_{in} + p_{Na} \cdot Na_{\text{int}} + p_{Cl} \cdot Cl_{out}} \right)$$

Squid Axon

Ion	Conc in	Conc out	Equilibrium Potential
Na+	50	440	55
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Ca++	0.4	10	145

Concentration in millimoles,
potential in millivolts.
pK : pNa : pCl = 1.0 : 0.04 : 0.45; T=20 C
Calculate resting potential: ?

Based on Goldman-Hodgkin-Katz Equation:
If pNa = pCl = 0, GHK equation reduces to Nernst Equation
In squid, pK : pNa : pCl = 1.0 : 0.04 : 0.45
At 20☜ C, Vm = -62 mV
In mammals, pCl is lower, pNa is lower,
Thus Vm is lower, -80 to –90 mV

Concepts in Electricity

	Water Flow	**Electricity**	**Neurons**
Driving force	pressure (lb/area)	Electrical potential (Volts)	Electrical Potential (Volts)
source	Gravity/pump	Battery	Concentration Gradient
flow	Water molecules: gallons/sec	Electrons: charge/sec (Amperes)	Ions: charge/sec (Amperes)
resistance	narrow pipes	resistors	Membrane Channels

Ionic Currents

- Rate of flow of ions depends on
  - Concentration gradient (Nernst Equation)
  - Membrane potential
  - Conductance of ion channels
    - Ease of ion moving through channels
    - Conductance is inverse of resistance
    - Analogous to permeability
    - Think of water moving through hose – wide hose can carry more water than narrow hose
- Current = rate of flow of electrons
  - Each ion has either
    - Extra proton (missing an electron)
    - Extra electron
  - Flow of ions creates flow of electrons
    - Rate of flow of electrons ☞ rate of flow of ions
      - Exactly equal for Na^+, K^+, Cl^-
      - Double for Ca^{2+}

Ionic Currents

Relation between membrane potential, concentration gradient, conductance

- Larger conductance = larger current
- Larger difference between V_M and E_R = larger current
- If $V_M - E_R > 0$, Outward current
- If $V_M - E_R < 0$, Inward current

- Examples $I = Gm (Vm – Er)$

Ionic Currents at Equilibrium

- I is total current flowing across membrane
  - Sum of currents due to each ion is the total current
- In equilibrium, total current is zero
  - Some current positive, some currents negative

$$I_{tot} = I_{Na} + I_K + I_{Cl} =$$

$$G_{Na}(V_M - E_{Na}) + G_K(V_M - E_K) + G_{Cl}(V_M - E_{Cl})$$

Ionic Currents at Equilibrium

- Can solve for V_M algebraically
- V_M is weighted sum of reversal potentials:

$$V_M = \frac{G_{Na} E_{Na} + G_K E_K + G_{Cl} E_{Cl}}{G_{Na} + G_K + G_{Cl}}$$

- Thus, V_M can be calculated from *permeabilities* using GHK, or from *conductances* using above equation

Active Transport

- How are concentration gradients maintained?
- Active Transport
  - Ion carriers are large proteins
  - Directly or indirectly use ATP molecules
  - Ions are moved "uphill"
  - Distinguished from channels on kinetic basis
- 40% of energy in brain used for ion carriers

Active Transport

Classified by the following characteristics

1. Type of ions transported
2. Stoichiometry
3. Direct vs. indirect use of ATP
4. Charge transfer (depends on 1 and 2)
5. Affinity for transported ions
6. Location of pump (which membrane surface)

Active Transport

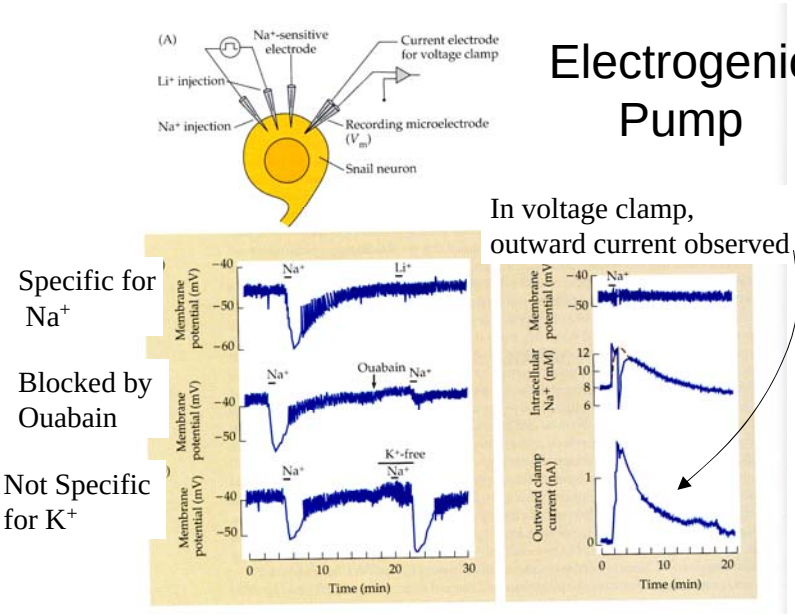
Types of Pumps

- Na^+-K^+ pump
- Na^+-Ca^{++} exchange
- Ca^{++} pump
- Cl^--HCO_3^- (bicarbonate) pump
- Na^+-H^+ exchange (proton pump)
- K^+-Cl^- Co-transporter

Na^+-K^+ pump

- Stoichiometry
  - Extrudes 3 Na^+ for each 2 K^+ brought in
- Charge transfer
  - Unequal => electrogenic
  - One proton flows out for each transport cycle
  - Small current produces small hyperpolarization
- Hydrolyzes one ATP for each cycle

Electrogenic Pump



Na^+-K^+ pump Structure

Hetero Tetramer

- Two of each of two subunits: α and β

α: 100 kDa

- Responsible for enzymatic activity
- 6 hydrophobic regions form transmembrane helices

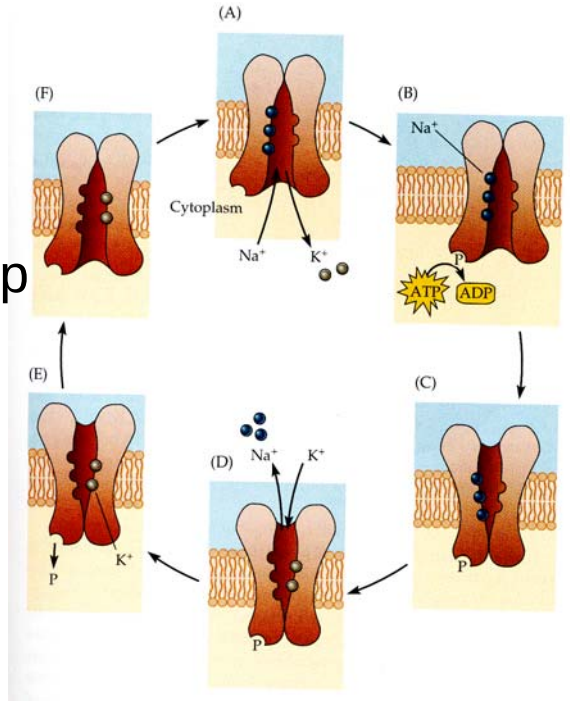
β: 38 kDa

- 1 hydrophobic/membrane spanning segment

Na^+-K^+ pump Operation

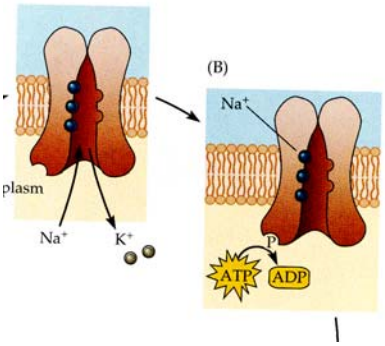
- Cation binding sites have variable specificity
  - Will only bind sodium Intracellularly
  - Will bind potassium, lithium, cesium, ammonium, rubidium extracellularly
- Sodium and potassium binding sites are exposed alternately to intracellular and extracellular solutions
  - Conformation changes driven by phosphorylation and dephosphorylation reactions

Na+-K+ Pump Operation



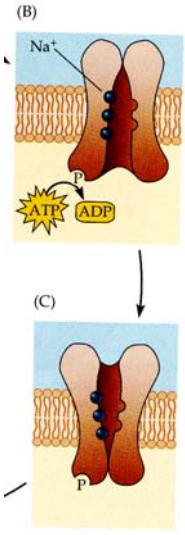
Na^+-K^+ Pump Operation

A. Inward facing sites have low affinity for K+ and high affinity for Na+
B. Binding of 3 Na+ causes small conformation change



Na^+-K^+ Pump Operation

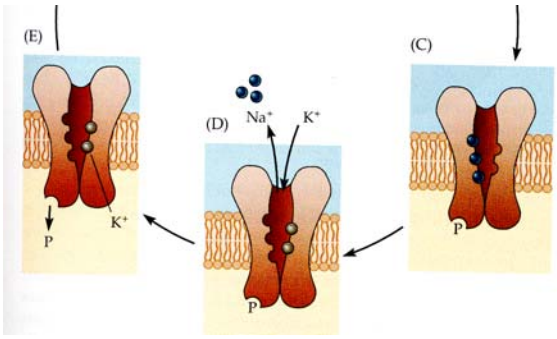
C. Conformational change leads to ATP binding and phosphorylation of pump
D. Phosphorylation produces further conformational change to expose Na+ ions extracellularly



Na^+-K^+ Pump Operation

E. Outward facing sites have low Na+ and high K+ affinities

F. Na+ ions unbind, K+ ions bind

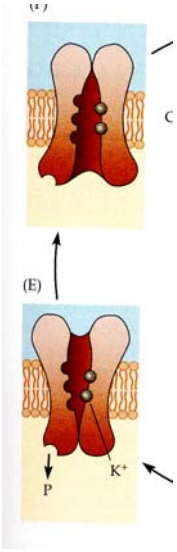


Na^+-K^+ Pump Operation

G. K+ binding leads to dephosphorylation

H. Dephosphorylation leads to conformational change to expose K+ Intracellularly

I. K+ leaves



Ca2+ Pumps

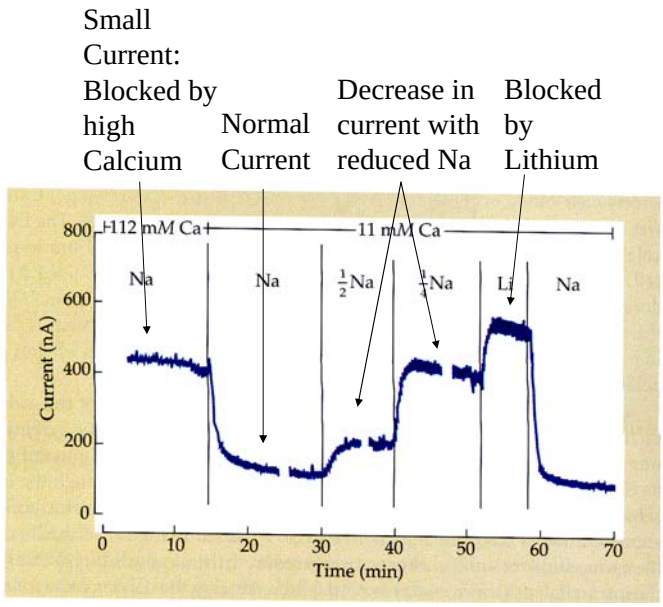
- Calcium is highly regulated because it influences many other processes
- Thus, there are many calcium regulatory mechanisms
  - Buffers
  - Several pumps and exchangers
  - Calcium is stored within mitochondria and ER

Ca2+ Pumps

- Calcium-magnesium ATPase pumps
  - Plasma membrane (PMCA)
    - Extrudes calcium to extracellular space
    - Binds one calcium ion each cycle
    - Affinity ~300 -600 nM
  - Smooth Endoplasmic Reticulum (SERCA)
    - Sequesters calcium in SER
    - Binds two calcium ions each cycle
    - Affinity ~100 nM

Na+/Ca2+ Exchanger (NCX)

- Stoichiometry
  - 3 sodium exchanged for 1 calcium
  - Charge transfer
  - Unequal => electrogenic
  - One proton flows in for each transport cycle
  - Small current produces small depolarization

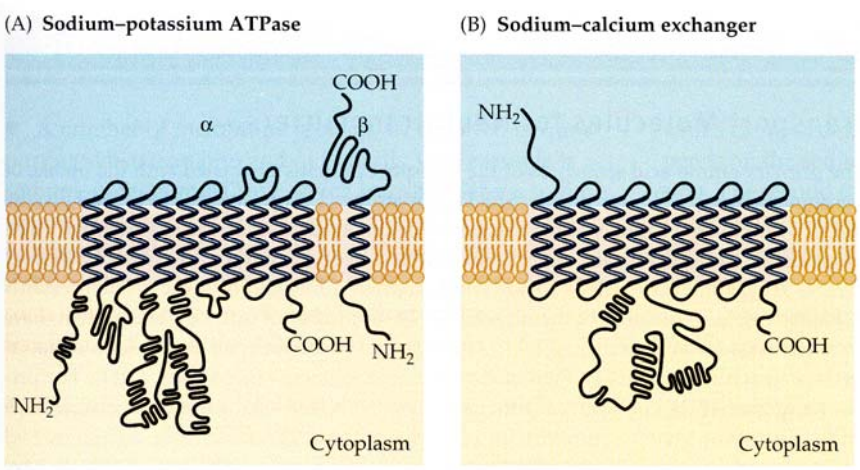


Sodium Calcium Exchange

- Does not hydrolyze ATP
- Driven by sodium concentration gradient
  - Inward sodium removed by Na-K pump
  - *Indirectly* uses ATP
- Affinity for calcium ~ 1.0 μM
- Plasma membrane location only

Na+/Ca2+ Exchanger

- Theoretical capacity ~50x greater than PMCA
- Actual capacity depends on membrane potential
  - Depolarization may reverse pump direction
  - Reduction in concentration gradient will decrease activity and may even reverse direction
    - Increase in intracellular sodium, or
    - Decrease in extracellular sodium, or
    - Decrease in intracellular calcium, or
    - Increase in extracellular calcium
- Structure
  - 11 transmembrane segments
  - Large intracellular loop between segments 5 and 6
    - Contains regulatory domain
  - 120 kDa
  - Single subunit: 970 amino acids



Na+/Ca2+ Exchanger

- Potassium is co-factor in some neurons
  - Retinal rods
- Stoichiometry
  - 4 sodium : 1 potassium : 1 calcium
  - Additional energy from potassium gradient
- Unlikely to reverse

Sodium Bicarbonate Exchange

- Stoichiometry
  - 1 Na^+ and 2 HCO_3^- flow in, 1 Cl^- pumped out
- Charge Transfer
  - Electrically neutral
- Does not hydrolyze ATP
  - Driven by Na gradient
  - Indirectly uses ATP of Na-K pump
- Regulates intracellular pH

K+/Cl- Co-transporter (KCC)

- Several isoforms exist
  - KCC1-4
  - KCC2 is neuron specific
  - KCC4 found in peripheral neurons
- Increased expression during development causes a decrease in resting potential
- Regulated by kinases and phosphatases

K+/Cl- Cotransporter

- Electroneutral
  - Extrudes one K^+ and one Cl^- per cycle
- Plays a role in volume regulation
  - Activated by swelling
  - Water accompanies KCl
- Regulates chloride gradient and reversal potential

Other Pumps

- Na^+/H^+
  - Electrically neutral
  - Directly passive (driven by $[Na^+]$ gradient)
  - Regulates intracellular pH
- Inward Chloride transport
  - Depends on sodium and potassium concentrations
  - Tubular cells of kidneys
  - Blocked by furosemide (lasix)

Summary

- Resting potential = Equilibrium potential, determined by
  - Concentration gradients
    - Maintained by active transport
  - Ionic permeability
- Resting potential calculated from
  - Goldman-Hodgkin-Katz equation
  - Weighted sum of reversal potentials
- Reversal potential calculated from Nernst equation
  - Depends on concentration gradients

Summary - Equilibrium

- A cell is in equilibrium if
  - Osmolarity is in balance (inside = outside)
  - No net flow of water (implied by above)
  - Charge is in balance (anions = cations)
  - No net flow of ions
    - Flow of ions to inside equals flow of ions to outside
- All signals considered with respect to resting potential
  - Action potentials
  - Synaptic potentials

Summary - Electricity

- Resistance is opposite of conductance :

  $R = 1/G$

  – High resistance to flow = low conductance and low permeability
- Driving force = potential difference
  – Difference between membrane potential and reversal potential: $\Delta V = V_M - E_R$
- Each current has simple relation (Ohm's Law) to Driving force (or potential difference):
  – $I = \Delta V/R$ or $I = \Delta V*G$