NEUROPHYSIOLOGY: NEURONS AND CIRCUITS

SEMESTER ONE

L1: Introduction to the Cellular Components of the Brain

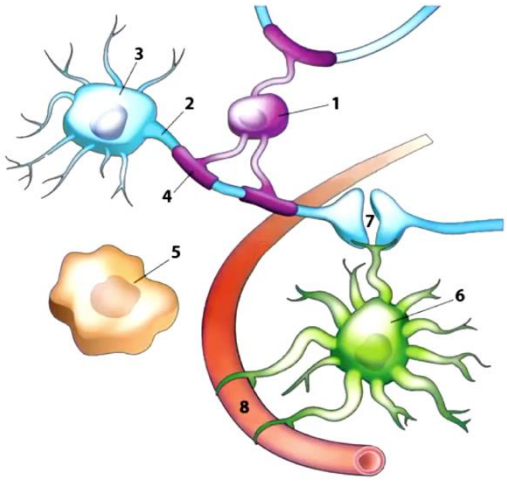
WHAT IS NEUROPHYSIOLOGY?

- Neurophysiology is about the function of the nervous system.
- The nervous system has 3 components:
    - Central, Peripheral and Enteric

NEURONS

- Neurons are excitable cells with a resting membrane potential (lecture 2-5)
    - Differentiated by function and phenotype (chemical, functional, structural).

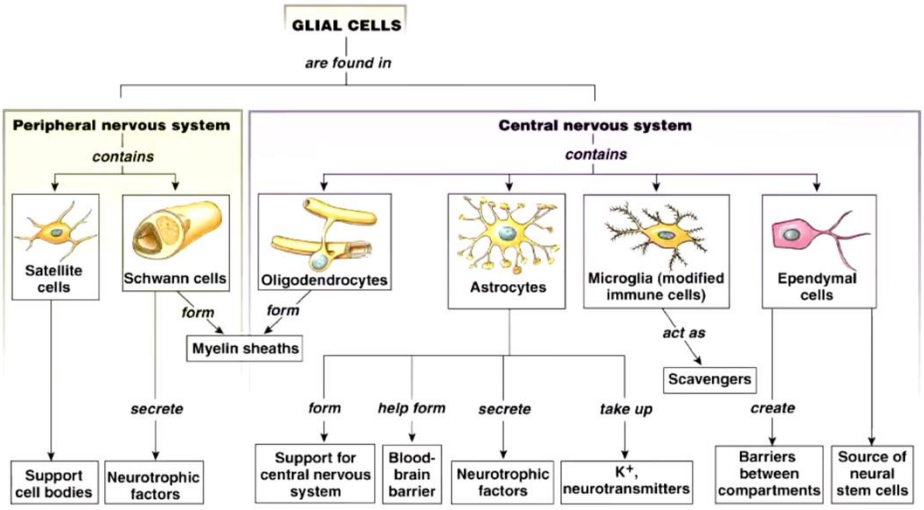
CELL TYPES



- The brain is not just composed of neurons but also different cell types and structures:

1. Oligodendrocyte → Myelination in CNS.
    a. Schwann cells → Myelination in PNS.
2. Axon initial segment
3. Soma of neuron (cell body)
4. Myelin sheath
5. Microglia → Immune cells that help to defend the CNS.
    a. Their stem cell progenitors are immune cells.
6. Astrocyte → Comes in various star shapes:
    a. In the image, you can see some of their legs contacting a synapse and blood vessel → Has roles in synaptic transmission, blood flow and many more.
7. Synapse
8. Blood vessel
9. Ependymal cells → Important for the Blood-Brain Barrier (BBB) – lines the ventricles.
10. Radial Glia → Important for neural migration (developmental).

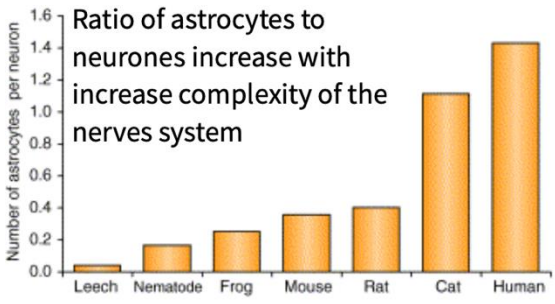
GLIAL CELLS



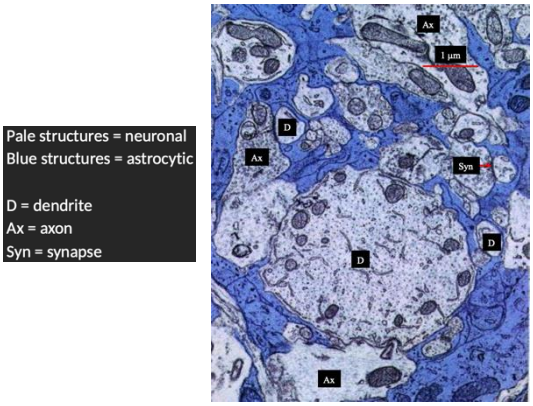
- Glial means 'glue' → Glial cells were initially thought to function as glues that hold the neurons together and by doing so, provide support for the CNS (e.g. regulate rubbish and breaking excess proteins etc.)
    - Now we know that they do a lot more than to support and regulate the CNS (see image).

ASTROCYTES

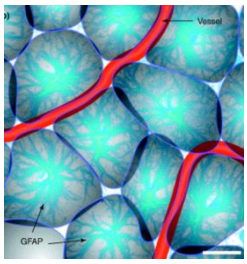
- Astrocytes have been identified for as long as neurons have been identified and has multiple functions:
    - Form support for CNS → Localised to brain and spine.
    - Help form Blood-Brain-Barrier.
    - Secrete neurotrophic factors → Stimulate migration of neurons, axon growth and degeneration.
    - Take up K^+ and neurotransmitters.
- If you initially thought that astrocytes were just support cells, you might think that each neuron requires more than one astrocyte to support it because neuronal axons are very long.
    - I.e. Have astrocytes along the axons, not just on the cell body – e.g. 10 astrocytes per neuron.
- If astrocytes existed purely for the supporting role, you should be able to predict a ratio (e.g. 10:1).
    - **If** that was the case, as the total number of neurons change (different species), the proportion of astrocytes to neurons shouldn't change.

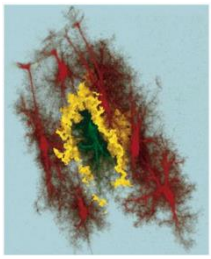


- **However**, experiments showed that the ratio of astrocytes to neurons <u>increased</u> with increasing complexity of the nervous system.
    - E.g. Humans have more astrocytes <u>per</u> neuron than a mouse or a leech.
    - There is no clear explanation to this result.



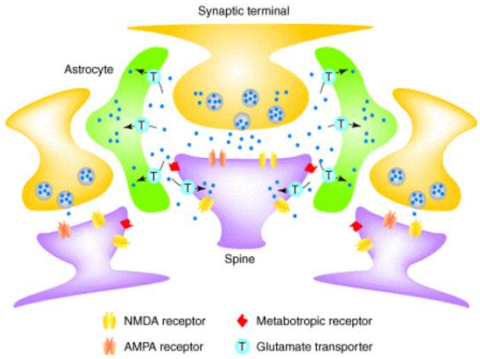
- From this micrograph, you can see that everything is either a neuron or an astrocyte.
    - I.e. Astrocytes are packed around neurons.
- Astrocytes are also not static → Calcium levels within the astrocytes are changing dynamically (likely due to the changes in the neuronal environment).





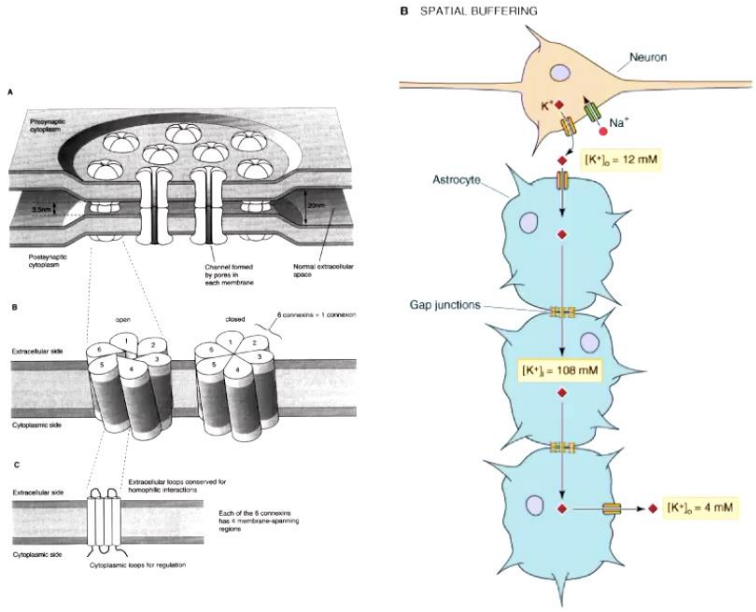
- Right image shows astrocytes in red and green, and any overlap is shown in yellow → Shows very little overlap between adjacent astrocytes:
    - Each astrocyte has a sphere of influence and it overlaps with other astrocytes' sphere of influence (left image shows this well) → One astrocyte control one finite space.
- Astrocytes are also critically important in regulating and modulating blood flow to local areas of the brain.
    - Important in PET scans → Measures index of activity by looking at the blood flow in the brain.

FIDELITY OF NEUROTRANSMISSION

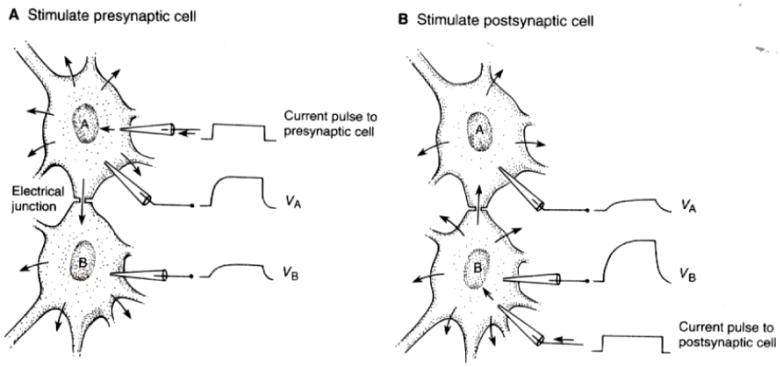


- In amongst the synaptic terminal are the processes of astrocytes (green):
    - When there's ionic movement in relation to the action potential generated, repolarization of neurons tends to raise K^+ concentration in the ECF → Synaptic activity results in extravasation of K^+.
        - Significant $[K^+]$ rise can interfere with neuronal signalling by depolarizing neurons.
    - Astrocytes take up excess release of material from neurons → Important for ECF ion homeostasis.

SPATIAL BUFFERING



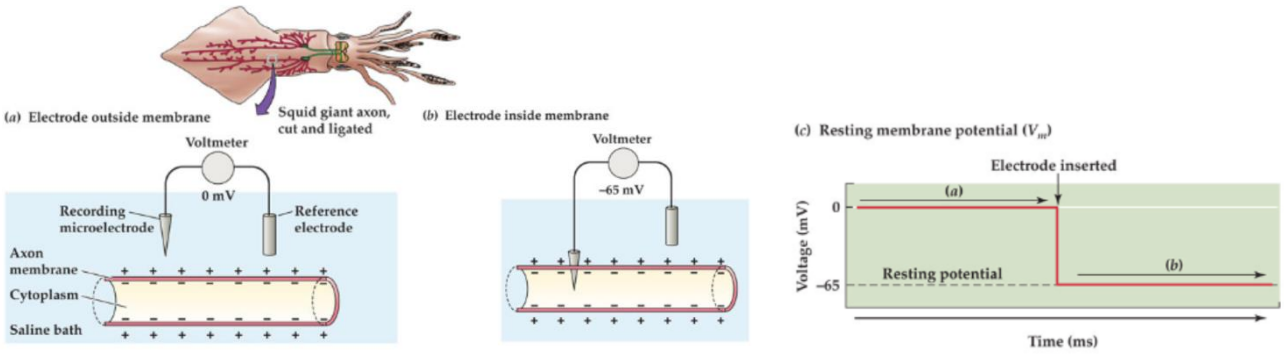
- The aforementioned mechanism (ECF ion homeostasis) is referred to as Spatial Buffering.
    - The right image shows a neuron with increasing ECF K^+ concentration and astrocytes.
        - Astrocytes have receptors and channels that respond to the environment (later lectures).
- Remember that each astrocyte has their own sphere of influence:
    - When there's a significant [K^+] rise due to many action potentials in the area, K^+ is taken up at one region of the astrocyte and then distributed throughout the cytoplasm of the cell, and further to its neighbours via Gap Junctions (formed by 6 connexins → 1 connexon)
        - This keeps ECF [K^+] at levels that prevent interference with normal propagation of an action potential.
        - Gap junctions allow the leakage of excess ICF K^+ from one astrocyte to the next → Allows continual uptake of K^+ by preventing saturation and maintaining the concentration gradient in astrocytes.
        - Gap junctions of electrical synapses are also composed of connexins (4TM domains each).
        - Gap junctions can also be regulated → In response to certain signals, they can open and close.



- The existence of gap junctions is proven by experiments with electrodes:
    - When a current is applied from the uppermost electrode (I.e. depolarise the cell); the next electrode is able to detect a current change because it poked into the same cell.
    - However, an electrode poked into an adjacent astrocyte is also able to detect the current change, which implies that current is leaking from the first astrocyte to the next via gap junctions.
- Note that gap junctions have no selectivity pore → Most small molecules can move through them (relatively non-selective).

L2: Membrane Potential

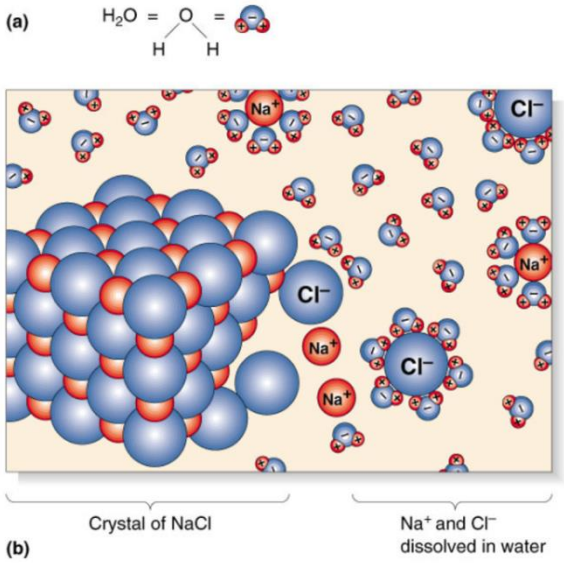
RESTING MEMBRANE POTENTIAL



- How do we measure resting membrane potential? What is actually being conveyed when you see the lines at – 65mV?

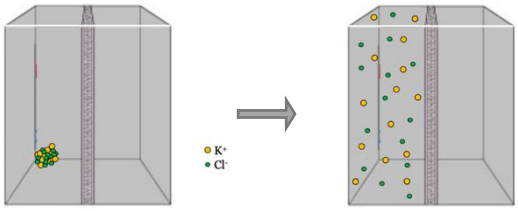
    - Much of what we know about action potentials and the function of nervous system actually comes from the studies in invertebrates – in particular, the squids and those with escape reflexes (for reasons that will become clear later) because they have very long axons.
        - Their axons can be up to 1mm wide → Pin electrodes in them.
            - The cell is approximately -65mV relative to the ECF.
        - If our optic nerve conducted at the speed it does and used the invertebrate's principle of increasing size as a mean to increase conduction velocity, our optic nerve, instead of being 2-3mm wide, will be 30cm wide.
            - We use different processes to increase the speed of action potentials – myelination.

POLAR MOLECULES



- Water is a polar molecule that is arranged such that there's a positive and negative side.

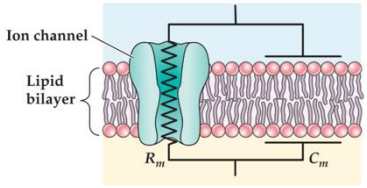
    - This polarity means that an ion like Na^+ or Cl^- in solution will get surrounded by water to balance the charges.
    - This is how salts dissolve in solution.



- If you put a crystal of salt in solution, it will dissolve to the point where those molecules become evenly distributed in the compartment.

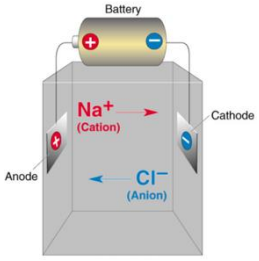
    - Principle 1: When allowed to move freely within a solution, ions will move along their concentration gradient until equally distributed (diffusion).

- An uneven distribution of ions leads to the development of a concentration gradient.



- The lipid bilayer represents a barrier to diffusion of polar molecules:

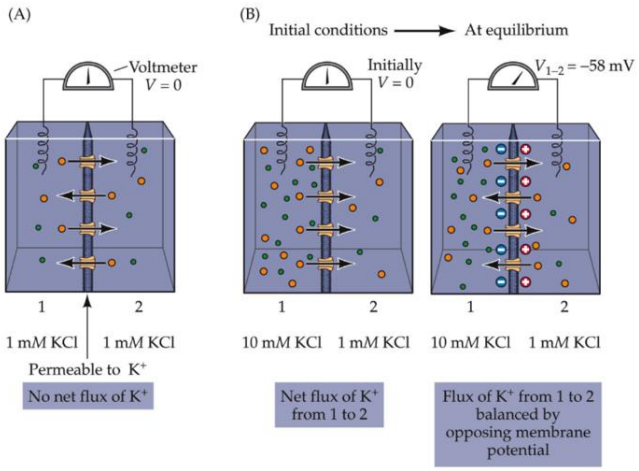
    - Proteins like channels or carriers embedded in the membranes can open or close to modulate the movement of ions across the membrane → They represent resistance to flow across the membrane.
    - The other characteristic that is represented in the diagram is capacitance – the membrane of the cell is so thin that there's still an ability for ions to interact across the membrane.
        - Hence, even if there's a barrier to movement, an electrostatic interaction can occur between positively charged or negatively charged ion across the membrane.
        - So, when you have uneven distribution of charge on one side versus the other, ions of different charges will line up across the membrane.
            - Capacitators are present in all sorts of electronic equipment, and they are sort of like batteries – they line up the charges and if you can discharge it, you get movement of current.
    - So, ions concentrated on one side of the membrane but not on the other, wishes to move across the membrane, and that wish to move, is potential or power.



- Principle 2: Being charged, ions will also move along an electrical potential gradient.

    - Movement of a charge is called current (I) and current is measured in amps.
    - Conventionally, positive current is in the direction of positive current flow.
    - Current is dependent upon:
        - Potential Difference: The difference in charge between the positive and negative poles.
            - Measured in volts (V).
        - Ability of charge to move in a particular substance – conductance (g).
            - Measured in Siemens.

- Now, there are 2 processes that are going to influence the movement of ions (diffusion and electrical charge), and these are critical factors for understanding resting membrane potential, and much of signalling of neurons.



- This image shows chambers with KCl solution and membrane that's permeable to only K^+:
    - Image A shows no potential or concentration difference between side 1 and 2.
        - So, there's no drive for K^+ to move to either sides.
    - Image B shows increased concentration of KCL on side 1:
        - Since the membrane is only permeable to K^+, K^+ move along its concentration gradient to side 2, while Cl^- will stay on side 1 (membrane is impermeable to Cl^-).
        - Initially, there is no potential difference between the two sides; but at equilibrium, side 1 will have excess Cl^- and this is shown on the voltmeter as a negative potential.
        - **Note**: Each time the K^+ moves across, the concentration gradient decreases; but it won't get to the point where the $[K^+]$ is even on either side because electrical interaction interferes with the diffusion process. Each time the K^+ leaves side 1 through diffusion, side 1 becomes more negative, which is attractive to the positively charged K^+ ions and they will be drawn back against their concentration gradient → Balance between concentration and electrical gradient → Equilibrium Potential for K^+.

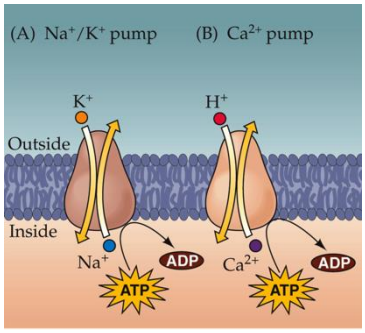
- Principle 3: Equilibrium is the point at which the concentration and electrical gradients balance, such that there is no net movement of an ion.

	Concentration (mM)	
Ion	Intracellular	Extracellular
Squid neuron		
Potassium (K^+)	400	20
Sodium (Na^+)	50	440
Chloride (Cl^-)	40–150	560
Calcium (Ca^{2+})	0.0001	10
Mammalian neuron		
Potassium (K^+)	140	5
Sodium (Na^+)	5–15	145
Chloride (Cl^-)	4–30	110
Calcium (Ca^{2+})	0.0001	1–2

- This is the case for neurons:
    - Neurons have ionic concentration gradients across the membrane as shown above.
        - An exam will not ask questions like "what is the concentration of sodium in the squid axon?"; but what's important in a diagram like this is that you understand the main points.
    - For the purpose of this subject, we will focus on mammalian neurons:
        - Ca^{2+} is rapidly taken up by the sarcoplasmic reticulum and various intracellular organelles and this is why the ICF $[Ca^{2+}]$ is vanishingly small (will learn why in later lectures).
        - This is **all** you need to know about this table.

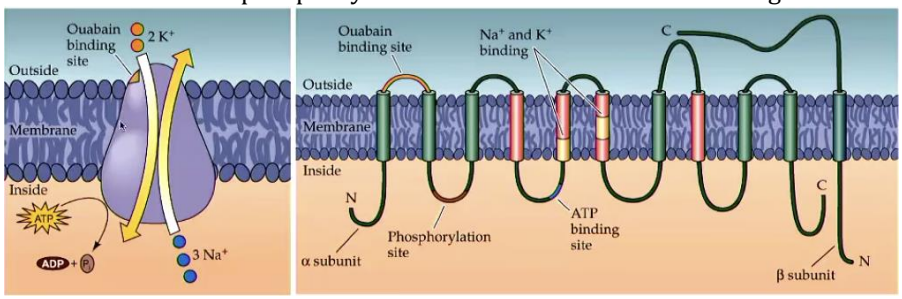
ION TRANSPORTERS

- There are **2 classes** of molecules that are critical for maintaining and generating these concentrations:

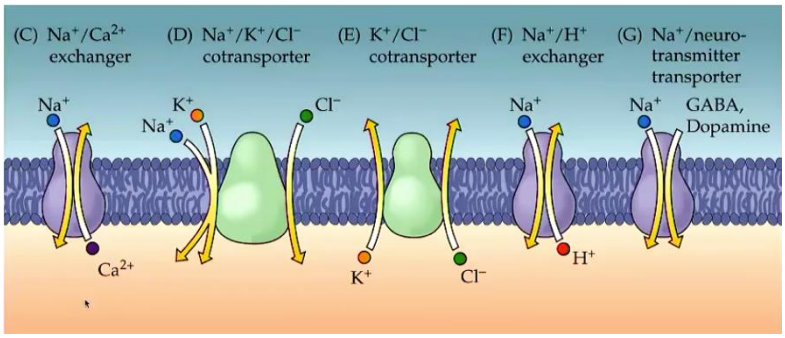


1. ATPase Pumps: **Actively** move ions against concentration gradient to create concentration gradients.

   - Na^+/K^+ ATPase Pump → About 70% of brain's energy is spent on setting up the Na^+/K^+ gradient.
     - 3 Na^+ binds to the intracellular side of the ATPase → ATP phosphorylates the ATPase → Conformational change leads to the release of Na^+ → 2 K^+ binds to the extracellular side of the ATPase → Dephosphorylation induced conformational change leads to K^+ release.



     - It has a binding site for an inhibitory molecule called ouabain → Various plant toxins can bind to this site and kill you.
     - It's a transmembrane molecule with an α (main part) and β (trafficking) subunit.
     - It's electrogenic: 3 Na^+ out and 2 K^+ in → Makes cell's interior more negative.
   - Ca^{2+}/H^+ ATPase Pump → Sets up intracellular ion concentrations for acidity/pH.



2. Ion Exchangers: Uses the gradients set-up by ion transporters to move other ions against their concentration gradient.

   - **Passive** but uses the energy generated by ion concentration gradients.
     - E.g. Na^+/Ca^{2+} Exchanger: Since ECF $[Na^+]$ > ICF $[Na^+]$ → Na^+ can move down its concentration gradient, and this is the energy used to extrude Ca^{2+} against the concentration gradient.
   - Ion exchangers exist for the majority of major molecules and some neurotransmitters.

- Principle 4: Energy dependent pumps maintain ion concentration gradients across the cell membrane.

GOLDMAN EQUATION

$$V_m = \frac{RT}{zF} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + \cdots}{P_K[K^+]_i + P_{Na}[Na^+]_i + \cdots}$$

R = Gas Constant
T = Absolute Temperature
z = Valence (charge)
F= Faraday's constant
$[X]_o$ = Outside concentration of X
$[X]_i$ = Inside concentration of X

- Goldman Equation predicts what the membrane potential will be, taking into account, the permeability (P_x) and concentration for each ion.

NERNST EQUATION

- Nernst Equation is essentially the same as the Goldman Equation, but simplified for each ion.
- IF:
  - $P_K = 1$ → Membrane represents no barrier to K^+ movement (channels are open).
  - $P_{Na} = 0$ → Membrane is a perfect barrier to Na^+ movement (channels are closed).

- Then, the permeability constant cancels out and the Goldman Equation simplifies to:

$$V_m = \frac{RT}{zF} \ln \frac{[K^+]_o}{[K^+]_i}$$

- This allows us look at the influence of an ion's concentration gradient on membrane potential (equilibrium potential).
- At room temperature (temperature for invertebrates):

$$V_m = \frac{58}{z} \log_{10} \frac{[K^+]_o}{[K^+]_i}$$

$$V_m = 58 \log_{10} \frac{5}{100}$$

$$V_m = 58 \times -1.301$$

$$V_m = -75.5\ mV$$

  - This is the membrane potential of a cell that is perfectly permeable to K^+ with known intra- and extra-cellular K^+ concentrations → Equilibrium Potential of K^+ at room temperature at this concentration gradient is -75.5 mV.
  - **Note**: Use 61 instead of 58 in the equation for mammals (body temperature = 37°C)

- What if we close the K^+ channels and open the Na^+ channels?

$$V_m = \frac{58}{z} \log_{10} \frac{[Na^+]_o}{[Na^+]_i}$$

$$V_m = 58 \log_{10} \frac{150}{15}$$

$$V_m = 58 \times 1$$

$$V_m = +58\ mV$$

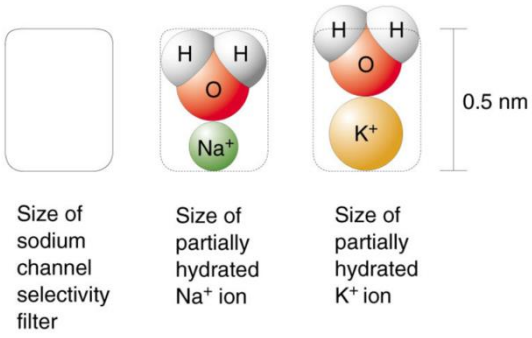
  - This is the membrane potential of a cell that is perfectly permeable to Na^+ with known intra- and extra-cellular K^+ concentrations → Equilibrium Potential of Na^+ at room temperature and this concentration gradient is +58 mV.

- In a normal neuron, the permeability of K^+ is actually not 1 (there's resistance to K^+ movement) and there are different types of K^+ channels that can be opened to influence the membrane potential.

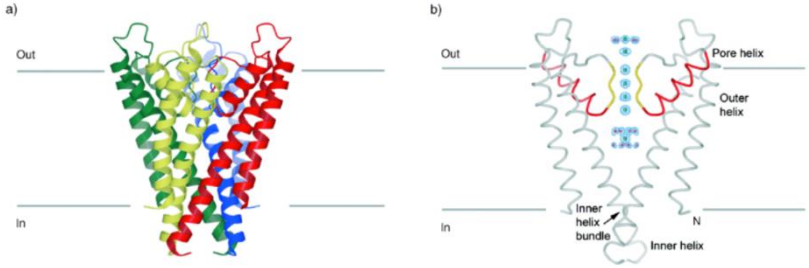
  - The permeability of other ions is also not 0 (e.g. there will be leaks of Na^+ and Cl^-)

- Hence, Goldman equation is more accurate when calculating the membrane potential and Nernst equation is good to get a rough idea of the perfect situation.

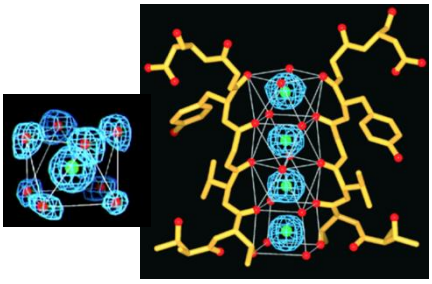
ION SELECTIVITY



- How do you generate something that is able to differentiate between two molecules that aren't much different in size, have the same charge and surrounded by water?



  - Here is a K^+ channel and with its 4 subunits shown in different colours.
    - It has an outside and inside orientation.
  - There are transmembrane helical organisations and loops which converge at the selectivity pore within the membrane.
    - The loops near the extracellular side of the membrane is essentially the selectivity filter for the ion.
    - Ions exist outside, surrounded by water molecules, and in the process of going through the ion selectivity filter of the channel, they lose the water molecule and line up at the selectivity pore to work their way towards the other side when the channel opens.
    - Similar to the Newton's Cradle toy, when an ion enters the channel, it knocks the ion at the end of the line through the channel when the channel opens.



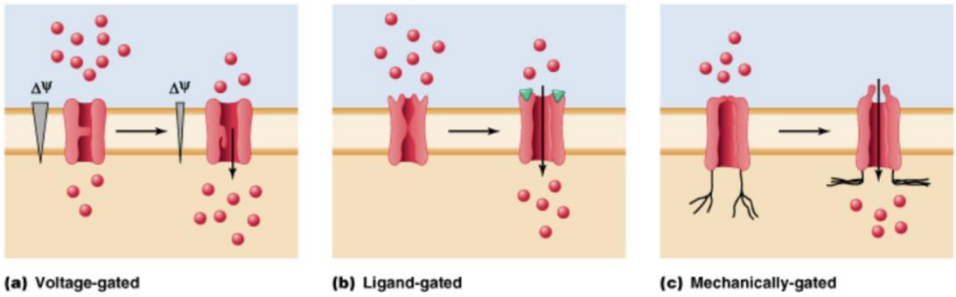
- What gives the selectivity pore an ability to differentiate between different ions?

  - Green dot represents K^+ ions and blue spheres represent the size of the electron cloud.
    - K^+ ions in solution are surrounded by oxygen atoms → Most relaxed state within solution.
  - The selectivity pore of K^+ channel is composed of amino acids with oxygen side chains to exactly mimic the relaxed state of the hydrated K^+ ion in solution.
  - Na^+ is smaller than K^+, and its most relaxed state in solution differs to that of K^+ → Na^+ cannot enter the K^+ channel as it doesn't offer the most relaxed state to Na^+.

- Hence, different ions channels have different selectivity pores to only allow the entry of specific ions.

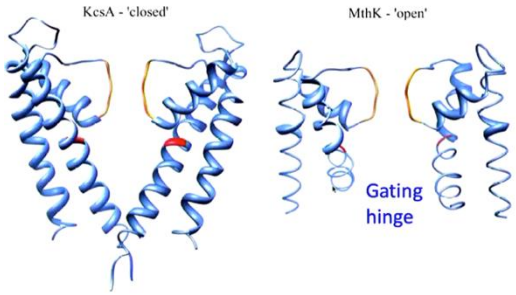
  - Some channels are cation sensitive/selective → Permits the entry of all cations.
  - Some channels are non-selective → Permits the entry of all ions.
  - I.e. There's a vast array of ion channels with varying levels of selectivity to enable the movement of specific ions at particular times.

GATING



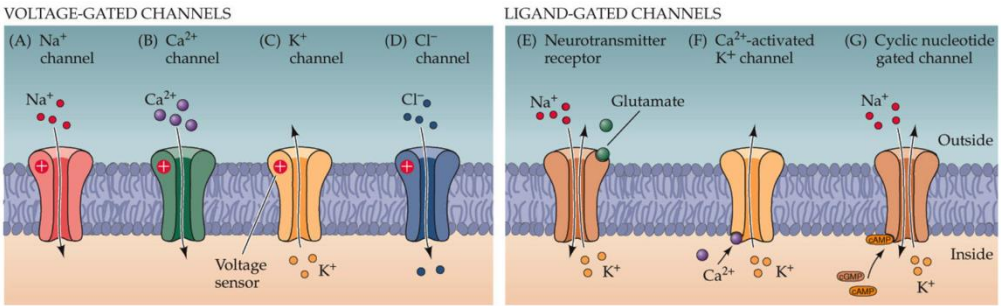
- Channels can open and close in response to various stimuli.
    - Voltage gated: Channels open in response to a change in voltage or potential across the membrane.
        - These are critical for generating action potentials.
    - Ligand gated: Channels open in response to the binding of a ligand.
    - Mechanically gated: Opens in response to a physical distortion of the cell membrane.
        - You can feel something on your finger because mechanically gated ion channels open by the depression of the object touching the finger → Transmitted into an electrical language by the neuron.

- So, how does a channel open or close?

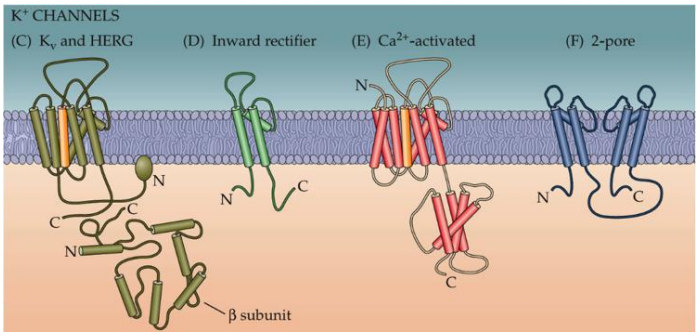


    - This is a voltage-gated K^+ channel in its closed state, and in response to a voltage change, it opens via the gating hinge.
        - The channel has a portion with a gating hinge (red), and the part below the hinge moves away, so the ions can move across the selectivity pore.

- There's a vast array of ion channels with different characteristics:



    - For instance, there are at least 8 different voltage-gated Na^+ channels, and these have slightly different characteristics:
        - One might be more sensitive to open more quickly.
        - One might have more effective pore to allow the ions to move through quickly.
        - One might close more quickly than others.
    - These little differences mean that, with a vast array of channels, you can have a vast array of different effects to give the complexity of the nervous system.



- Voltage gated channels exist for most flavours of ions; but there are also ligand-gated channels for neurotransmitters, intracellular ions and also for cyclic nucleotides (cAMP):

    - **4 families** of K^+ channels exist based on structure (6, 2, 6+, 4 transmembrane domains); and within these families are variants with minor differences (opening and closing, trafficking patterns, exist in different places in neuron) that bring different characteristics:
        - Some are good at switching off AP firing, some can restore AP to rest faster → High firing frequency.
        - These variances in the intricate detail of ion channels allow different information to be transmitted by neurons.